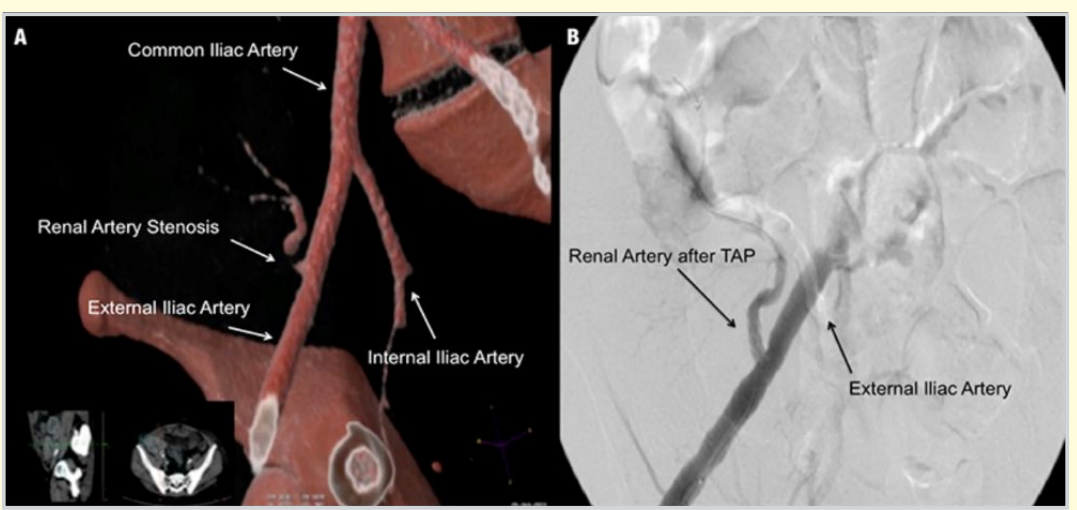
ISSN 1677-5538

INTERNATIONAL BRAZ J UROL

OFFICIAL JOURNAL OF THE BRAZILIAN SOCIETY OF UROLOGY
VOLUME 45, NUMBER 5, SEPTEMBER - OCTOBER, 2019



Three-dimensional reconstruction of arteriography showing TRAS due to kinking (A). Arteriography evidenciating successful outcome after stent placement at the kinking site (B).

XXXVII Brazilian Congress of Urology
2021 - Brasilia - DF - Brazil

INTERNATIONAL

BRAZ J UROL

OFFICIAL JOURNAL OF THE BRAZILIAN SOCIETY OF UROLOGY - SBU

INTERNATIONAL
BRAZ J UROL

CONSULTING EDITORS

INTERNATIONAL

BRAZ J UROL

INTERNATIONAL

BRAZ J UROL

Eletronic Version: Full text with fully searchable articles on-line:

http://www.intbrazjurol.com.br/

Correspondence and Editorial Address:

Rua Real Grandeza, 108 - conj. 101 - 22281-034 – Rio de Janeiro – RJ – Brazil
Tel.: + 55 21 2246-4003; E-mail: brazjurol@brazjurol.com.br

The paper on which the International Braz J Urol is printed meets the requirements of ANSI/NISO Z39, 48-1992 (Permanence of Paper). Printed on acid-free paper.

The International Braz J Urol is partially supported by the Ministry of Science and Technology. National Council for Scientific and Technological Development. Editorial and Graphic Composition

CNPq Conselho Nacional de Desenvolvimento Científico e Tecnológico

CAPES

The International Braz J Urol, ISSN: 1677-5538 (printed version) and ISSN: 1677-6119 (electronic version) is the Official Journal of the Brazilian Society of Urology– SBU, is published 6 times a year (bimonthly, starting in January - February).

The International Braz J Urol is indexed by: EMBASE/Excerpta Medica; SciELO, Lilacs/Latin America Index; Free Medical Journals; MD-Linx; Catálogo Latindex; SCImago, Index Medicus - NLM, PubMed/MEDLINE, PubMed/Central, ISI - Current Contents / Clinical Medicine and Science Citation Index Expanded.

ONLINE manuscript submission: **www.intbrazjurol.com.br**

DISCLAIMER

CONTENTS

Volume 45 | number 5 | September . October, 2019 | INT BRAZ J UROL

SURGICAL TECHNIQUE

CHALLENGING CLINICAL CASES

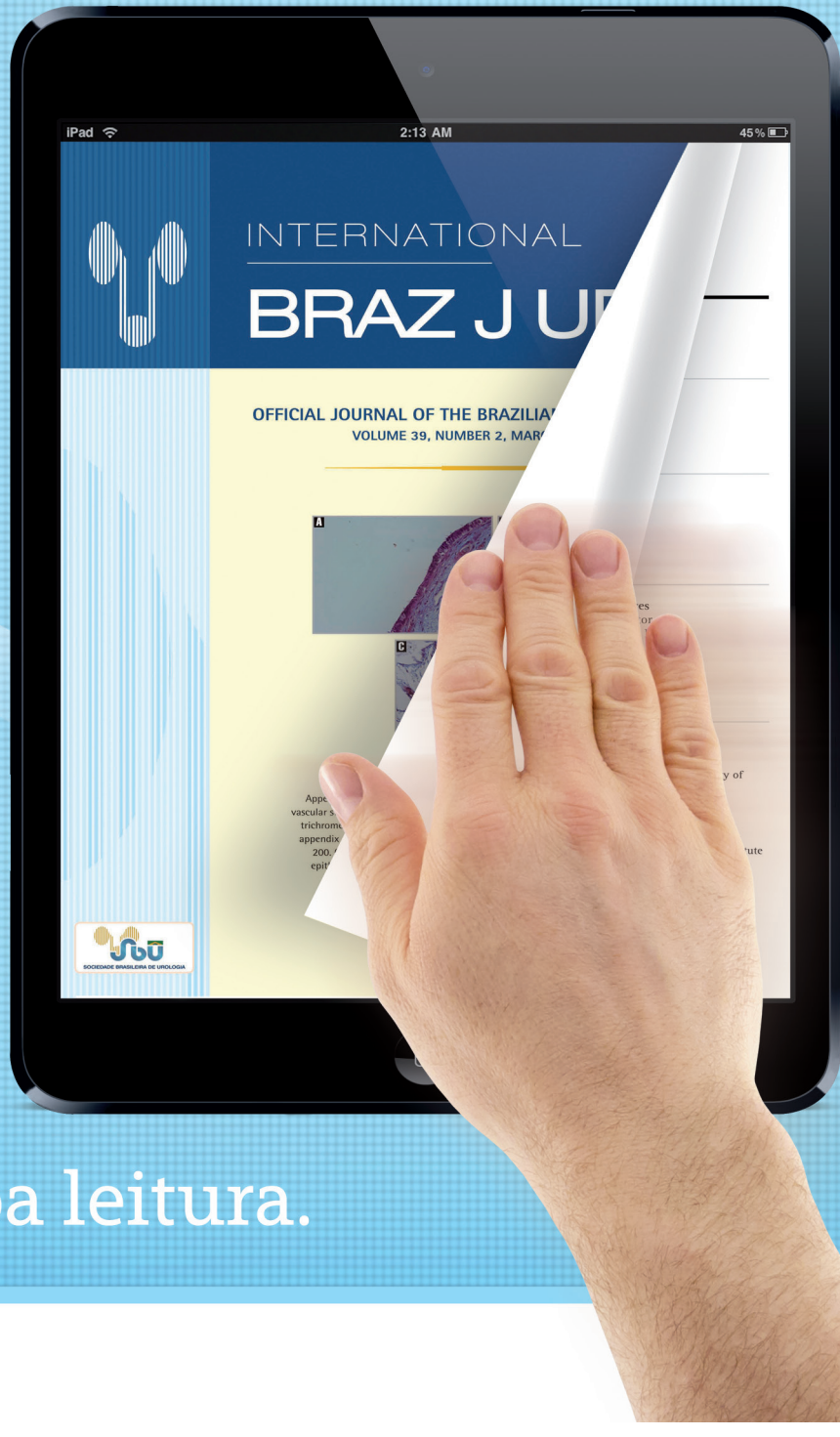
VIDEO SECTION

INT BRAZ J UROL

Acesse agora as edições do seu iPad.

DOWNLOAD iPad VERSION

ACCESS WEB VERSION



Boa leitura.

EDITORIAL
IN THIS ISSUE

Vol. 45 (5): 871-872, September - October, 2019
doi: 10.1590/S1677-5538.IBJU.2019.05.01

Bulbar urethral stricture: penile skin flap may be a good option?

The September-October 2019 issue of the International Brazilian Journal of Urology presents original contributions with a lot of interesting papers in different fields: Infertility, Bladder Cancer, Prostate Cancer, Renal Cell Carcinoma, Partial nephrectomy, Kidney stones, Nocturnal Enuresis, Basic Research, Urinary Incontinence, Transplantation, UPJ Obstruction, Pelvic Organ Prolapse, Hypogonadism, Vasectomy, Herbal Medicine in Fertility and Urethral Strictures. The papers come from many different countries such as Italy, Brazil, USA, Turkey, China, France, Iran, Lebanon, Singapore, Colombia, Tunisia, India and Spain, and as usual the editor´s comment highlights some papers. We decided to comment the paper about a very interesting topic: Penile skin flap for anterior urethral sctricture (1).

Doctor Hmida and collegues from the Sahloul Hospital Sousse, Tunisia, performed on page 1057 an interesting study about the Penile skin flap for anterior urethral stricture. They studied 77 patients underwent substitution urethroplasty using dorsal penile skin flap for bulbar urethral strictures. The mean stricture length was 5cm (3-10 cm) and the mean flap length was 6cm. The mean follow-up was 60 months (6-120). The overall success rate was 88%. The authors concluded that urethroplasty using penile skin flap appear to be a safe and efficient technique for the treatment of a long and complex anterior urethral stricture.

There are several options for the treatment of anterior urethral stricture (2-4). The patient's stricture position, length and complexity are important factors to decide the surgical technique (5-7). For long bulbar strictures the buccal mucosa graft (BMG) as gold-standard material due to its histological characteristics and very good long term results (8-12). However, there are multiple situations whereby BMG is inadequate (prior buccal harvest) or inappropriate for utilization (heavy oral radiation). The fascio--cutaneous flaps could be a good option in these situations. The penile skin flap is easy to perform, do not need urethral mobilization and the present paper shows a success rate of 88%, a very significant number. We congratulate the authors for this very important contribution.

REFERENCES

1. Hmida W, Othmen MB, Bako A, Jaidane M, Mosbah F. Penile skin flap: a versatile substitute for anterior urethral stricture. Int Braz J Urol. 2019;45:1057-63.
2. Singh O, Gupta SS, Arvind NK. Anterior urethral strictures: a brief review of the current surgical treatment. Urol Int. 2011;86:1-10.
3. Prakash G, Singh BP, Sinha RJ, Jhanwar A, Sankhwar S. Is circumferential urethral mobilisation an overdo? A prospective outcome analysis of dorsal onlay and dorso - lateral onlay BMGU for anterior urethral strictures. Int Braz J Urol. 2018;44:323-9.

Volume 45 | number 5| September . October, 2019 | INT BRAZ J UROL

4. Favorito LA, Conte PP, Sobrinho UG, Martins RG, Accioly T. Double inlay plus ventral onlay buccal mucosa graft for simultaneous penile and bulbar urethral stricture. Int Braz J Urol. 2018;44:838-9.
5. Alsagheer GA, Fathi A, Abdel-Kader MS, Hasan AM, Mohamed O, Mahmoud O, et al. Management of long segment anterior urethral stricture (≥ 8cm) using buccal mucosal (BM) graft and penile skin (PS) flap: outcome and predictors of failure. Int Braz J Urol. 2018;44:163-71.
6. Urkmez A, Yuksel OH, Ozsoy E, Topaktas R, Sahin A, Koca O, et al. The effect of urethroplasty surgery on erectile and orgasmic functions: a prospective study. Int Braz J Urol. 2019;45:118-26.
7. Wessells H. Ventral onlay graft techniques for urethroplasty. Urol Clin North Am. 2002;29:381-7, vii.
8. Barbagli G, Palminteri E, Guazzoni G, Montorsi F, Turini D, Lazzeri M. Bulbar urethroplasty using buccal mucosa grafts placed on the ventral, dorsal or lateral surface of the urethra: are results affected by the surgical technique? J Urol. 2005;174:955-7; discussion 957-8.
9. Palminteri E, Manzoni G, Berdondini E, Di Fiore F, Testa G, Poluzzi M, et al. Combined dorsal plus ventral double buccal mucosa graft in bulbar urethral reconstruction. Eur Urol. 2008;53:81-9.
10. Barbagli G, Selli C, Tosto A, Palminteri E. Dorsal free graft urethroplasty. J Urol. 1996;155:123-6.
11. Andrich DE, Mundy AR. Substitution urethroplasty with buccal mucosal-free grafts. J Urol. 2001;165:1131-3; discussion 1133-4.
12. Kane CJ, Tarman GJ, Summerton DJ, Buchmann CE, Ward JF, O'Reilly KJ, et al. Multi-institutional experience with buccal mucosa onlay urethroplasty for bulbar urethral reconstruction. J Urol. 2002;167:1314-7.

Luciano A. Favorito, MD, PhD

Professor Associado da Unidade de Pesquisa Urogenital da
Universidade do Estado de Rio de Janeiro
Urologista do Hospital da Lagoa Federal, Rio de Janeiro
Editor Associado da International Braz J Urol

Vol. 45 (5): 873-876, September - October, 2019
doi: 10.1590/S1677-5538.IBJU.2019.05.02

EDITORIAL

Focal therapy for prostate cancer – index lesion treatment vs. hemiablation. A matter of definition

Armando Stabile [1], Marco Moschini [2], Francesco Montorsi [1], Xavier Cathelineau [3], Rafael Sanchez-Salas [3]

[1] Department of Urology and Division of Experimental Oncology, URI, Urological Research Institute, Vita-Salute San Raffaele University, IRCCS San Raffaele Scientific Institute, Milan, Italy; [2] Klinik für Urologie, Luzerner Kantonsspital, Lucerne, Switzerland; [3] Department of Urology, Institut Mutualiste Montsouris and Université Paris Descartes, Paris, France

Current standard of care for localized prostate cancer (PCa) include active surveillance and radical therapy. Tissue-sparing approaches such as focal therapy (FT) has recently emerged to cover that middle ground between active surveillance and whole gland therapies in order to provide cancer control while reducing morbidities and side-effects. Evidence from a systematic review including thirty-seven studies reporting on 3230 patients receiving FT through different energy sources reported a rate of significant disease (csPCa) at follow-up biopsy ranging between 0% and 13% within a median follow-up ranging from 4 to 61 months. Leak-free continence and potency rate were 83.3-100% and 81.5-100%, respectively (1). The largest series (n=1032) of men receiving FT using high-intensity focused ultrasound (HIFU) reported a freedom from csPCa at follow-up biopsy of 54% at 8 years from treatment, with 46% of men being free from any additional treatment at the same time point (2). Yap et al, reported functional results of 180 patients submitted to tissue-preserving therapy, describing no significant changes in International Index of Erectile Function (IIEF) from preoperative to 12-months values (3). Nonetheless, due to the lack of consistent and long-term results and the presence of several difficulties in providing standardized treatment and follow-up strategies, FT has been proposed by the European Association of Urology as an investigational modality (4). This statement raised several arguments supporting the potential useful role of FT (5,6), underlining that other innovative therapeutic strategies has been recommended in the past before the availability of long-term outcomes or data from randomized controlled trials (e.g partial nephrectomy for kidney cancer, tumorectomy for breast carcinoma). The trade-offs that patients are willing to make in terms of ratio between rate of treatment failure and rate of side-effects is key. We must avoid to deny our patients a potential further therapeutic strategy, that many would accept as an alternative.

The most controversial topic against FT is the multifocal nature of PCa. Indeed, PCa is multifocal in up to 75% of cases which is in contrast with the possibility of achieving cancer control with a focal treatment of the prostate gland. Nonetheless, in the last few years, several efforts have been made in order to demonstrate the "index lesion theory" according to which the largest and most aggressive tumour focus within the prostate drives the natural history of the disease (7). Given the fact that in up to 25% of cases PCa is a true unifocal disease, there are some evidences demonstrating that PCa metastasis have often a monoclonal origin, and that in a non-negligible proportion of cases these metastatic cells derive

from the index lesion (IL) (8,9). In a recent survey study including 425 urologist showed as only 45% of the participants believed in the "index lesion theory", with a higher incidence of believer coming from academic centres (10). Even though this topic remains one of the most controversial in the field of PCa biological nature, evidences supporting the possibility to achieve acceptable cancer control through FT, at least at mid-term follow-up, are increasing (2).

During the treatment planning process of FT is therefore considered pivotal aiming at accurately covering the so called IL, regardless of the energy used.

The introduction of multiparametric MRI of the prostate together with the possibility to perform targeted biopsies, has provided a cornerstone tool in the patient selection process when talking about FT, helping to identify the IL and rule out the presence of surrounding csPCa foci (11). Although the combination of MRI and targeted biopsies represent the gold standard for FT patient selection, the diagnostic accuracy of this strategy is still unclear. Nassiri et al. demonstrated as eligibility for FT was confirmed in 75% of men considered eligible with the use of MRI targeted biopsies (12). On the other hand Jonson et al. showed as, using the same diagnostic technique, up to 48% men would have been incorrectly identified as having unilateral PCa (13). Being that said, the use of MRI and targeted biopsies is suggested as the probably the most efficient method to provide an acceptable mapping of the prostatic gland.

Identifying the IL and achieving a good treatment coverage of it during FT is crucial. Based on the characteristic of the disease, particularly the volume and the extension within the prostate, different treatment strategies, with different energy sources, have been proposed: I) "index lesion ablation or focal-ablation" when the treatment is limited to the IL, plus safety margins; II) "hemi-ablation" consists in the treatment of the half of the prostate containing the tumour; III) "sub-total ablations" when the ablation volume is greater than half the prostate, for example hokey-stick ablation (14,15). Each treatment should include safety margins around the area containing the tumour, which usually account for 5mm up to 9mm of normal tissue (2,16). Nowadays the most common used FT strategy is represented by the hemi-ablation (1). Evidences comparing the efficacy of focal- vs hemi-ablation are so far scarce. To the best of our knowledge, Stabile et al. reported the first comparison between focal- and hemi-ablation in terms of the rate of any additional treatment after FU in a population of over one-thousand men receiving primary FT for PCa using high-intensity focused ultrasound (HIFU), reporting no differences between the two strategies (2). Nonetheless, regardless the pre-operative treatment plan, in an intra-operative setting is often challenging discerning between focal- and hemi-ablation, considering safety margins and the different prostate volumes. The difference between focal- and hemi-ablation has been maintained over the years in order to better describe the amount of prostatic tissue spared during FT. Indeed, the more extensive the treatment, the more likely the functional outcomes will get close to those of whole gland therapies (i.e. radical prostatectomy, radiotherapy). Therefore it becomes of great importance, also considering patient counselling, the possibility to give an idea regarding the extension of FT treatment. However, given the extreme variability of IL and prostates characteristics, concerning volume, extension and shape, pushing FT treatments into the aforementioned definitions (i.e. focal-, hemi- and subtotal-ablation) might be not completely accurate for patients counselling as well as for outcomes reporting. Focal therapy represents indeed a continuum of treatment extensions and strategies which is adapted according to each clinical case. Sivaraman et al. two years ago, proposed an "À la carte" approach when delivering FT, that was then officially recognized and validated by the European Society of Urotechnology (ESUT) (17,18). Particularly the authors showed the possibility of choosing different energy modalities according to the intraprostatic cancer location, more specifically using HIFU for posterior tumours and preferring cryotherapy for anterior tumours (17). With the growing use of MRI and targeted biopsies and its introduction in the FT patients selection pathway, the location and extension of the IL has become clear and quite reliable as well as exploitable by the urologist. The concept of tailo-

ring FT treatment on cancer characteristics, particularly the intraprostatic extension, is routinely performed, for every type of FT energy sources. For these reasons we might assert that specifying the difference between focal- and hemi-ablation could be, in the next future, considered obsolete. Indeed, there is a call for further improvements in the field of intraprostatic mapping and refinement of FT devices and interest is growing around the study of prostate tumour microenvironment and its modulation after specific treatments (19). Further developments in the possibility to better define the prostatic area to treat and to identify prostatic tissue eventually treated will make a step forward towards the standardization of FT treatments and consistency of provided oncologic and functional results.

According the results of this experimental study during a partial nephrectomy, the en bloc clamping for warm ischemia should be favored over only the renal artery clamping to minimize renal injury after partial nephrectomies, but more studies will be necessary in the future to confirm these results.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Valerio M, Cerantola Y, Eggener SE, Lepor H, Polascik TJ, Villers A, et al. New and Established Technology in Focal Ablation of the Prostate: A Systematic Review. Eur Urol. 2017;71:17-34.
2. Stabile A, Orczyk C, Hosking-Jervis F, Giganti F, Arya M, Hindley RG, et al. Medium-term oncological outcomes in a large cohort of men treated with either focal or hemi-ablation using high-intensity focused ultrasonography for primary localized prostate cancer. BJU Int. 2019;124:431-40.
3. Yap T, Ahmed HU, Hindley RG, Guillaumier S, McCartan N, Dickinson L, et al. The Effects of Focal Therapy for Prostate Cancer on Sexual Function: A Combined Analysis of Three Prospective Trials. Eur Urol. 2016;69:844-51.
4. van der Poel HG, van den Bergh RCN, Briers E, Cornford P, Govorov A, Henry AM et al. Focal Therapy in Primary Localised Prostate Cancer: The European Association of Urology Position in 2018. Eur Urol. 2018;74:84-91.
5. Valerio M, Emberton M, Ahmed HU. Re: Henk G. van der Poel, Roderick C.N. van den Bergh, Erik Briers, et al. Focal Therapy in Primary Localised Prostate Cancer: The European Association of Urology Position in 2018. Eur Urol 2018;74:84-91. Eur Urol. 2019;75:e21-e22.
6. Carneiro A, Sanchez-Salas R. Re: Focal Therapy in Primary Localised Prostate Cancer: The European Association of Urology Position in 2018. Eur Urol. 2018;74:234.
7. Ahmed HU, Ch B. The Index Lesion and the Origin of Prostate Cancer. N Engl J Med. 2014;1704–6.
8. Liu W, Laitinen S, Khan S, Vihinen M, Kowalski J, Yu G, et al. Copy number analysis indicates monoclonal origin of lethal metastatic prostate cancer. Nat Med. 2009;15:559-65. Erratum in: Nat Med. 2009;15:819.
9. Kneppers J, Krijgsman O, Melis M, de Jong J, Peeper DS, Bekers E, et al. Frequent clonal relations between metastases and non-index prostate cancer lesions. JCI Insight. 2019;4.
10. Jain AL, Sidana A, Maruf M, Sugano D, Calio B, Wood BJ, et al. Analyzing the current practice patterns and views among urologists regarding focal therapy for prostate cancer. Urol Oncol. 2019;37:182.e1-182.e8.
11. Tay KJ, Scheltema MJ, Ahmed HU, Barret E, Coleman JA, Dominguez-Escrig J, et al. Patient selection for prostate focal therapy in the era of active surveillance: an International Delphi Consensus Project. Prostate Cancer Prostatic Dis. 2017;20:294-9.
12. Nassiri N, Chang E, Lieu P, Priester AM, Margolis DJA, Huang J, et al. Focal Therapy Eligibility Determined by Magnetic Resonance Imaging/Ultrasound Fusion Biopsy. J Urol. 2018;199:453-8.
13. Johnson DC, Yang JJ, Kwan L, Barsa DE, Mirak SA, Pooli A, et al. Do contemporary imaging and biopsy techniques reliably identify unilateral prostate cancer? Implications for hemiablation patient selection. Cancer. 2019;125:2955-64.
14. Postema AW, De Reijke TM, Ukimura O, Van den Bos W, Azzouzi AR, Barret E, et al. Standardization of definitions in focal therapy of prostate cancer: report from a Delphi consensus project. World J Urol. 2016;34:1373-82.
15. Nahar B, Parekh DJ. Focal therapy for localized prostate cancer: Where do we stand? Eur Urol Focus. 2019. [Epub ahead of print]
16. Le Nobin J, Rosenkrantz AB, Villers A, Orczyk C, Deng FM, Melamed J, et al. Image Guided Focal Therapy for Magnetic Resonance Imaging Visible Prostate Cancer: Defining a 3-Dimensional Treatment Margin Based on Magnetic Resonance Imaging Histology Co-Registration Analysis. J Urol. 2015;194:364-70.

17. Sivaraman A, Barret E. Focal Therapy for Prostate Cancer: An "À la Carte" Approach. Eur Urol. 2016;69:973-5.
18. Ganzer R, Arthanareeswaran VKA, Ahmed HU, Cestari A, Rischmann P, Salomon G, et al. Which technology to select for primary focal treatment of prostate cancer?-European Section of Urotechnology (ESUT) position statement. Prostate Cancer Prostatic Dis. 2018;21:175-86.
19. Tourinho-Barbosa RR, de la Rosette J, Sanchez-Salas R. Prostate cancer multifocality, the index lesion, and the microenvironment. Curr Opin Urol. 2018;28:499-505.

Rafael Sanchez-Salas, MD

Department of Urology,
L'Institut Mutualiste Montsouris,
Université Paris Descartes, Paris, France
E-mail: raersas@gmail.com

ARTICLE INFO

Rafael Sanchez-Salas
http://orcid.org/0000-0002-7809-3580

DIFFERENCE OF OPINION

Vol. 45 (5): 877-881, September - October, 2019
doi: 10.1590/S1677-5538.IBJU.2019.05.03

Social Media in the Urology Practice | *Opinion: YES*

Mateus Cosentino Bellote [1], Hegel Trujillo Santamaria [2], Marcela Pelayo-Nieto [3], Heman Prasad ES [4], Nariman Gadzhiev [5], Kalyan Gudaru [6]

[1] Departamento de Urologia, Universidade Federal do Paraná - UFPR, Curitiba, PR, Brasil; [2] Instituto Mexicano del Seguro Social. Centro Medico Nacional, Unidad Medica De Alta especialidade No.14 "Lic. Adolfo Ruiz Cortines" Veracruz, México; [3] Centro Médico Puerta de Hierro, Zapopan, Jalisco, Mexico; [4] CIHSR Referral Hospital, Nagaland, India; [5] Departament of Endourological, Pavlov First Saint Petersburg Medical University, Russia; [6] Department of Urology, Sri Venkateswara Institute Of Medical Sciences, Tirupati, Andhra Pradesh, India

Keywords: Social Media; Urology; Review [Publication Type]

INTRODUCTION

Social media (SoMe) are changing the way people communicate, interact, and exchange information. Medical and scientific communities are increasingly utilising of these emerging communication tools. Knowledge and scientific content are now broadly available in multiple platforms that allow providers to interact with peers around the world.

These platforms are now facilitating medical education and helping phisicians to increase their network (1). Nowadays, Twitter has played a key role in the medical comunity. It has been shown that Twitter activity can predict articles that will be highly cited (2).

Over 80% of physicians across specialities have some form of social media presence. The current and upcoming generations of physicians entering work force with an innate and natural drive to communicate, this trend is only going to increase (3).

SCIENTIFIC ASPECTS OF SOCIAL MEDIA

Much has been said about the virtues of social media on popular platforms like Twitter, Facebook, Instagram, etc. Social Media has become a resourceful technology among professionals for the advancement of career as well as staying up-to-date with the latest literature. The internet has brought countless tools to our phones – at the speed of thought. Data has become the most powerful resource and new ideas are the currency of the present. Data can buy robotics and machines and hence, information is to the digital economy what natural oil was to the industrial economy. Scientific analysis of the information pattern shared on social media platforms is overdue -pertaining to quantum of physician usage, reliability of information, index, effect of social media on the impact factor of a journal, etc.

Loeb et al. reported that the most commonly used social media platforms by urologists were Facebook (93%), followed by LinkedIn (46%), Twitter (36%) and Google+ (26%). Physicians less than 40 years was an important predictor of higher social media use (83% vs 56%), with greater uptake among residents/fellows compared to attendings (86% vs

66%). Only 28% of respondents used social media partly or entirely for professional purposes (4). Juan Gomez in his commentary in 2016 quoted that "Twitter is perhaps the social media platform with the most dissemination in healthcare consisting of the broadest possible opportunities for interesting news, knowledge sharing, and networking amongst health professionals" (5, 6). Twitter has 326 million users with 500 million tweets every day. Apart from Twitter, urologys is also the first specialty to use the VineTM platform as a medical education tool and the PeriscopeTM for streaming sessions from a medical conference.

A study on the effect of social media on impact factor of pediatric urology journals using Filtered Journal Citation Reports over a period of 4 years revealed some interesting findings. The presence of a Twitter feed was statistically significant for an increase in impact factor over 4 years (P = 0.017), demonstrating that presence of an article in social media is associated with a rise in the journal impact factor (7).

A study examining 33 prominent urological journals revealed that eight journals had Twitter profiles with a mean of 1845 followers each, ultimately translating into a higher journal impact factor. Journals with a Facebook page were also found to be more likely to have a twitter page (8). In an analysis of 710 articles being shared, and their associated tweets over a six-month period, it was noted that out of the 710 articles 21% were Level 1 evidence-based articles, 14% were level 2, 39% were level 3 and level 25% were level 4.. This adequately gives bearing to the fact that social media can be scientifically accurate in propagation and adsorption of knowledge.

It is pertinent to note that social media articles in urology with a significant number of citations will influence and produce noticeable improvement of the journal impact factor, about 65% of citations were from non-urological literature (8).

PHYSICIAN ASPECTS

In the current era, social media has evolved into a digital space for networking and learning. This inclination towards networking helps us to participate in conversations with diverse groups of individuals, scientists and intellectuals. It can help provide professionals with reliable and relevant information more effectively than traditional methods like emails and journals. The current technology also gives us an important advantage in connecting with our colleagues and peers from other disciplines on social media. Complex cases can be discussed online and this activity can further promote clinical competence in the management of such cases. The use of social media in conferences has also enriched this interactive aspect. It has allowed vibrant exchange of ideas and information during conferences. It is a unique platform that allows interactions between people regardless of geographical restrictions.

The virtual engagement among physicians also leads to exchange of ideas and collaborations. In the current age of multi institutional studies and collaborative clinical trials it is worthwhile to note that social media has brought about breakthroughs in study recruitment, delivery of interventions and data collection (9). Some aspects of clinical activity in studies designs which were done traditionally in the past with face to face approaches are currently being replaced by social media. A prime example of physicians leveraging social media for research is the REMOTE trial for overactive bladder (10). In this study, the authors Orri et al utilized social media, forums and online websites to recruit and manage participants. This trial has shown the advantages of adopting social media with, improved efficiency and lowered costs in this example. Not only does it help in crowd sourcing, the use of social media in clinical trials also helped to achieve higher follow-up rates (11).

One of the forerunners that brought about this vibrant exchange on social media are the international social media consortiums such as journal clubs and groups (12). These social media platforms have demonstrated value to the surgical community. Labelling of content with "hashtags" used on these platforms allows users to further filter data according to their needs. The concerns of the doctors such as ease of content access is now a thing of the past. Most importantly,

these discussions on social media have acted as a podium in seeking timely advise o as well as discussing professional challenges in dealing with clinical and non clinical aspects of healthcare (13).

Education and knowledge update is also an aspect of social media usage, and surgeons are utilizing social media for professional development. One of the foremost modern theories that supports this platform for physician training is the Eraut learning cycle. Social media learning accounts for the informal learning cycle of Eraut. It is the only technology that contributes to implicit, reactive and deliberative learning of the cycle. With duty hours and heavy clinical work, we have fewer opportunities for traditional ward-based learning. Healthcare professionals are increasingly using online educational resources and open access formats available to engage in educational activities. Availability of surgical content online has taken the learning experience one step further and this has even been extended to online learning of surgical skills.

The main reasons for physicians to join social media are well documented. In addition to networking, engaging in medical education, physician branding also plays an important role. Having an online presence has been shown to directly impact clinical practice. With the current set of guidance and amount of work that went into online professionalism, it should not be seen as a challenge but rather an opportunity to both improve and accommodate the traditional values of medicine to the characteristics of social media (14). We should recognize that in this time of translational medicine we need physicians who can connect with stakeholders, improve policy, change clinical practice and social media is one of the best platforms to accomplish this.

PATIENTS ASPECTS

The use of social media has become very instinctive to many and it has become part of a daily routine. Enhanced communication, liberated expressions of oneself, keeping updated with all the trends and news, marketing and promotion are only some of the reasons why people use social media. Physicians are in the "get people better" business and therefore it is important to develop a strategy on how to use social media to engage and connect with patients. Doing this, it is likely that it will improve the chances of providing a better health care experience.

Patients use social media intended to meet an unfulfilled need. The most common reason for social media use by patients health is for social and emotional support. Social support is defined as "the process of interaction in relationships which is intended to improve coping, esteem, belonging, and competence through actual or perceived exchanges of psychosocial resources".

A type of social media use by patients is emotional support, that is defined as "communication that meets an individual's emotional or affective needs". It refers to support gained through expressions of care and concern, which serve to improve an individual's mood. Emotional support helps patients to meet their emotional or affective needs. Examples of emotional support are "sharing of emotional difficulties", "encountering support that feels like a warm blanket wrapped around you", and "share emotions with other people who are coping with similar problems" (15).

Also, esteem support is a social media use for patients, that refers to "communication that bolsters an individual's self-esteem or beliefs in their ability to handle a problem or perform a needed task". The aim of this type of support is to encourage individuals to take the actions needed to successfully live with their condition. Examples of esteem support include "getting support from other patient's encouragement", "share experiences about a new treatment to find encouragement before starting it", and "rituals of confirming each other' s endeavors to follow health instructions".

Another patient's use of use social media is for information gathering. Patients who

are newly diagnose with certain conditions are in a need for information about their health conditions and treatment options. These can be found on health care related websites but also on support groups from patients who have already dealt with the particular condition.

CONCLUSIONS

Social media (SoMe) is a communication revolution and has a great potential to improve health outcomes, although careful and thoughtful utilization is needed to do this effectively. With the continuous increase of social medial use by health care organizations and patients in the daily practice, the risks and benefits of such use needs to be more broadly discussed. Further research is needed to evaluate how twe can incorporate social media into health care in order to improve patients outcomes in the future.

ACKNOWLEDGEMENTS

Rodrigo Donalisio da Silva for your collaboration in developing this article

On Behalf of #UroSoMe working Group

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Loeb S, Catto J, Kutikov A. Social media offers unprecedented opportunities for vibrant exchange of professional ideas across continents. Eur Urol. 2014;66:118-9.
2. Eysenbach G. Can tweets predict citations? Metrics of social impact based on Twitter and correlation with traditional metrics of scientific impact. J Med Internet Res. 2011;13:e123. Erratum in: doi:10.2196/jmir.2041.
3. Klee D, Covey C, Zhong L. Social media beliefs and usage among family medicine residents and practicing family physicians. Fam Med. 2015;47:222-6.
4. Loeb S, Bayne CE, Frey C, Davies BJ, Averch TD, Woo HH, et al. American Urological Association Social Media Work Group. Use of social media in urology: data from the American Urological Association (AUA). BJU Int. 2014;113:993-8.
5. Rouprêt M, Morgan TM, Bostrom PJ, Cooperberg MR, Kutikov A, Linton KD, et al. European Association of Urology (@Uroweb) recommendations on the appropriate use of social media. Eur Urol. 2014;66:628-32.
6. Rivas JG, Socarrás MR, Blanco LT. Social Media in Urology: opportunities, applications, appropriate use and new horizons. Cent European J Urol. 2016;69:293-8.
7. Borgmann H, DeWitt S, Tsaur I, Haferkamp A, Loeb S. Novel survey disseminated through Twitter supports its utility for networking, disseminating research, advocacy, clinical practice and other professional goals. Can Urol Assoc J. 2015;9:E713-7.
8. Matta R, Doiron C, Leveridge MJ. The dramatic increase in social media in urology. J Urol. 2014;192:494-8.
9. Rosa C, Campbell AN, Miele GM, Brunner M, Winstanley EL. Using e-technologies in clinical trials. Contemp Clin Trials. 2015;45(Pt A):41-54.
10. Orri M, Lipset CH, Jacobs BP, Costello AJ, Cummings SR. Web-based trial to evaluate the efficacy and safety of tolterodine ER 4 mg in participants with overactive bladder: REMOTE trial. Contemp Clin Trials. 2014;38:190-7.

11. Mitchell SG, Schwartz RP, Alvanzo AA, Weisman MS, Kyle TL, Turrigiano EM, et al. The Use of Technology in Participant Tracking and Study Retention: Lessons Learned From a Clinical Trials Network Study. Subst Abus. 2015;36:420-6.
12. Gudaru K, Blanco LT, Castellani D, Santamaria HT, Pelayo-Nieto M, Linden-Castro E, et al. Connecting the Urological Community : The #UroSoMe Experience. J Endoluminal Endourol. 2019;2:e20-9.
13. Ventola CL. Social media and health care professionals: benefits, risks, and best practices. P T. 2014;39:491-520.
14. Gholami-Kordkheili F, Wild V, Strech D. The impact of social media on medical professionalism: a systematic qualitative review of challenges and opportunities. J Med Internet Res. 2013;15:e184.
15. Barreto JE, Whitehair CL. Social Media and Web Presence for Patients and Professionals: Evolving Trends and Implications for Practice. PM R. 2017;9:S98-S105.

Correspondence address:
Mateus Cosentino Bellote, MD
Departamento de Urologia
Universidade Federal do Paraná, Curitiba, PR, Brasil
Rua General Carneiro, 181 - Alto da Glória
Curitiba, PR, 80060-900
Telephone: + 55 41 9 9155-4205
E-mail: mcbellote@gmail.com

ARTICLE INFO

Mateus Bellote
https://orcid.org/0000-0003-4238-3452
Int Braz J Urol. 2019; 45: 877-81

Submitted for publication:
July 20, 2019

Accepted after revision:
August 05, 2019

Published as Ahead of Print:
September 15, 2019

DIFFERENCE OF OPINION

Vol. 45 (5): 882-888, September - October, 2019
doi: 10.1590/S1677-5538.IBJU.2019.05.04

Social Media in the Urology Practice | *Opinion: NO*

Rodrigo Donalisio Da Silva [1, 2], Jeffrey J. Leow [3, 4], Zainal Adwin Abidin [5], Edgar Linden-Castro [6], Edgar Iván Bravo Castro [7], Leonardo Tortolero Blanco [8], Jeremy Yuen-Chun Teoh [9], Pablo Nicolas Contreras [10], Marcelo Langer Wroclawski [11, 12]

[1] Division of Urology, Department of Surgery, University of Colorado School of Medicine, Denver, CO, USA; [2] Department of Surgery, Division of Urology, Denver Health Medical Center, Denver, CO, USA; [3] Department of Urology, Tan Tock Seng Hospital, LKC School of Medicine, Nanyang Technological University, Singapore; [4] Division of Urologic Surgery and Center for Surgery and Public Health, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA; [5] Department of Surgery, Universiti Teknologi MARA, Malaysia; [6] Centro Médico Puerta de Hierro, Zapopan Jalisco, Mexico; [7] Servicio de Urologia, Hospital Central Militar, Mexico; [8] Servicio de Urologia, Hospital Imed Levante, Alicante, Spain; [9] S. H. Ho Urology Centre, Department of Surgery, The Chinese University of Hong Kong, Hong Kong; [10] Servicio de Urologia del Hospital Alemán. Buenos Aires, Argentina; [11] Hospital Israelita Albert Einstein. São Paulo, SP, Brasil; [12] Hospital Beneficência Brasileira de São Paulo, São Paulo, SP, Brasil

Keywords: Social Media; Urology; Review [Publication Type]

INTRODUCTION

Recently, Social Media (SoMe) has been one of the most important resources in communication. Unsurprisingly, the medical and scientific community started to utilize the available online platforms in order to facilitate communication, promote scientific knowledge and initiate partnerships with other Institutions.

A 2018 survey of more than 5000 physicians found that 71% of physicians under the age of 40, 50% of those aged 40 to 49 years old, and more than 30% of physicians older than 60 years old regularly use SoMe (1). The American Urological Association survey in 2013 showed a high use of SoMe among its members (74%), of which fellows and residents consisted 86% and attendings 66% (2).

Online platforms such as Twitter, Facebook, and Instagram are available at no cost and provide the opportunity to interact with others around the world using your smartphone. The dynamic use of SoMe became attractive for physicians and scientists, specially allowing a communication pathway that was not available before among the most prominent experts and their peers.

Medical societies, medical meetings and conferences rapidly started to use SoMe to promote their events and engage participants in the discussions using hashtags that facilitated finding the specific content in social media platforms, and also provided metrics for the online engagement of their participants (3, 4).

Another online phenomenon is the spontaneous international workgroups such as #UroSoMe, #SoMe4Surgery, and others. These workgroups rapidly engaged specialists to discuss their clinical practice, to get advice from world experts, promote journal clubs, and also for re-

search collaboration. In the first online event on Twitter of the #UroSoMe group, a reach of more than 2 million users was achieved, in more than 200 different geographic locations (5). Patients are also benefiting from the online discussions, having useful online resources for their education regarding their health issues, surgeries, providers review and other factors that can contribute for their decision--making.

SoMe use also came with controversy about ethics, copyrights and unprofessional use per health care providers and patients, medical associations, and online communities. Recommendations on the appropriate use of SoMe have been published before (6), however, the legislation regarding social media use of scientific content still varies worldwide. The use of pictures, photos, documents, patient's body images or even radiology studies without proper patient consent has been criticized.

In this manuscript we will review potential pitfalls and harms of social media use, focusing in the health care aspect.

Defining characteristics of SoMe

The first challenge concerning SoMe is to define its characteristics and to know all the available platforms, with their own particularities, so that issues can be properly addressed and compared. SoMe can be classified into two large groups (7):

1- Horizontal Social Networks: Mostly common available SoMe, where any user can join and participate, without having a priori common characteristics. Examples: Facebook, Instagram or Twitter.

2- Vertical Social Networks: Users looking to have common interests. These social networks serve one or several specific purposes at a professional level: employment, networking, travel, etc. Examples: LinkedIn, TripAdvisor, Soundcloud, Spotify, Vimeo, etc...

The use of SoMe can vary based on the user purpose. This includes the general user that may be joining SoMe for entertainment only, but several other aspects must be considered knowing that this is also used by corporations, marketing, branding, and others. Below we listed the most common uses for SoMe (8):

- Entertainment: SoMe started with the purpose of entertainment. The users are usually looking for topics related to their personal interest, such as sports, movies, books, travel and others. Content that users find interesting tends to generate likes and shares.
- Information: SoMe can be a great source of information, if the right sources are used. There is no editor on the shared content and inaccurate information can be found. Misinformation may even promote misunderstandings, propagate disbelief in healthcare professionals and lead to non--evidence based movements such as the anti-vaccine campaign.
- Personal contacts: To find and connect with those you already know, like family and friends. SoMe facilitates the contact not only by those that are geographically distant but also for those that cannot see each other often.
- Professional contacts: Professional contacts in your area of work, online networking and meetings. Initial online collaborative efforts via SoMe may eventually bear fruit and lead to formal professional relations.
- Online community: Online communities are created with common interests and to discuss related topics. It can take time to develop an online community.
- Web traffic: Sharing articles, topics, pictures and web links is often used to direct users to other websites. Trusting the source is the key for followers to take the step to research further and click on the shared link.
- Advertising: SoMe became huge for online advertising of brands, services or products. SoMe allowed segmenting users facilitating the achievement of a targeted audience.
- Branding: A new brand in SoMe emerge daily. Multiple publications, linking to other people, responding to followers and

continuous interaction with the audience becomes a brand.

- Recommendation channel: When users like a product or service of a brand, they are likely to recommend it online. When researching for a new product or service, it is very common nowadays for users to research online about product or company reputation before buying. Most online services will follow up purchases with the intent that users share their experience with products. Multiple websites emerged only with the purpose to review and recommend products.

MOST POPULAR SOME PLATFORMS (8)

Facebook

Currently, Facebook is the SoMe with most users in the world, being an easy-to--use platform, allows different posts such as videos, images, or texts. Last year Facebook was involved in lack of security of users data. A wide variety of users of different ages are connected to Facebook. Facebook is the favorite social network for Millennial and Generation X. For Generation Z, the percentage Facebook users diminish in favor of other networks such as Instagram.

Whatsapp

WhatsApp is one of the biggest instant message applications. It is the favorite choice to communicate among Millennials (40%) due to the facility and agility in communication. More than 80% of users connect through a mobile device. Apart from private messages, you can also create groups with several users.

Youtube

The Youtube video platform is the third most commonly used social network. YouTube has a great capacity of interaction with other networks. YouTube allowed the emergence of digital influencers. It is also one of the fastest growing in number of users and is one of the best rated along with Instagram and Spotify. Young users are among those who consume the most audiovisual content. 43% of users between 16 and 23 years old follow at least one digital influencer through YouTube. In Urology, the most common use is sharing of surgical videos highlighting techniques and operative procedures.

Instagram

In fourth position and following closely is Instagram. Younger users consider it the most important and relevant social network. Like Youtube, it is among the younger generations (between 16 and 23 years old) and for the second time in a row it is one of the SoMe that most attracts new users. The platform has been able to integrate the options of photography and video in a simple and attractive way. Brands have already captured this trend and are selling their products or services integrated among the publications of their acquaintances.

Twitter

Twitter is a social network of microblogging, i.e. a network to publish, share, exchange information, through brief comments in text format, with a maximum of 140 characters, called Tweets. These tweets are displayed on each user's main page. Twitter is considered the most important real-time communication nowadays. Users can subscribe to the Tweets of others, and it has the attraction of quickly updating the status from portable devices, such as smartphones. Important news are shared worldwide in real time. It can also be accessed from a PC, a laptop or tablet.

LinkedIn

LinkedIn, unlike the latest SoMe, does not seem to have gained much traction among younger users. The LinkedIn social network seeks professional profile helping to connect professionals of different geographic location. It is also used by headhunters for recruitment.

The medical and scientific community took advantage of SoMe plateforms to colaborate and share knowledge to their peers and to general population. However, some aspects of SoMe use by these comunnites needs to be clarified to protect patients and the online community.

PITFALLS AND CAUTIONS

Scientific aspect of SoMe use

It becomes very common among physicians to share conferences, lectures and slides content on SoMe without certifying if there are any copyrights that must be respected. Some of the most difficult resources to reclaim copyrights is pictures of slides shared during meetings and conferences. Despite being prohibited in most conferences, it is almost impossible to refrain attendants or conference delegates from taking pictures and videos from the audience. Nevertheless, in the urological community, the American Urological Association (AUA) and the European Urological Association (EUA) websites stated that taking pictures or screen captures, or reproducing any materials without informed consent from authors and the society is not allowed. Most scientific journals require authors to sign a copyright release when publishing a manuscript. By doing this, authors cannot share or reproduce the content of the manuscript without journal permission.

We are facing a dilemma regarding the contents that could be reproduced and shared through SoMe. All intellectual property and information shared in SoMe should be followed by proper citations to avoid issues.

National and continental medical associations should provide education to members regarding SoMe in order to avoid and possible legal consequences of inflicting copyrights.

Sharing manuscripts or parts of manuscripts online is also common practice among physicians. However it generally involves a copyright agreement between the corresponding author (on behalf of the rest of the authors) and the journal publisher. Each journal publisher has its own copyright regulations and sharing policies and a list of links has been collated to allow users to quickly access each publisher's sharing policy at <https://www.howcanishareit.com>. Although it does not reveal any specific rules pertaining to SoMe, generally the full article cannot and should not be shared publicly.

Conversly, journal publishers are increasingly recognizing the role of SoMe to help their authors disseminate research findings quickly. This is evidenced by the fact that most urology journals have an official Twitter account through which recently published articles are shared. Some journals, such as European Urology, even have a requirement for authors to submit a 160-character Tweet along with the manuscript for consideration. Although the correlation between Twitter mentions and subsequent citations has not been demonstrated (9, 10), there is a belief that sharing on SoMe may improve an article's overall visibility and encourage more to visit the link embedded in the tweet (11, 12).

We recommend urologists to share a link of their article (either PubMed/MEDLINE or Digital Object Identifier [DOI]), with a screenshot of the title/abstract as an image. We do not recommend sharing the entire publication or it's .PDF files on social media publicly due to potential copyright rules.

Physicians perspective of SoMe use

It must be taken that SoMe is an open environment where everything published will be public domain, which means that other physicians, patients and the general population will have access to that. A major risk associated with the use of SoMe is the posting of unprofessional content that can reflect unfavorable bias or self-promotion.

A survey carried out by the Pew Research Internet Project found that 72% of the patients who responded to it have searched for some information in the social media (13). A similar concern is the exploitation of the patient in online communities that are influenced by marketing. Greene et al. found that a significant proportion (27%) of posts on Facebook about diabetes management in support groups appeared to be promotional, typically in the form of testimonials. Many of these recommendations are very vague and, in most cases, associated with a specific product without any disclosures (14). Another study identified that 69% of Youtube medical

related videos suffer from bias and 73% are low quality videos that can lead patients to take erroneous decisions regarding their treatment. Some physicians take advantage and publish on SoMe health related information in their personal accounts. However, limitations of these informations found online are lack of quality and reliability (15).

A survey of the executive directors of American state medical boards revealed that at least one online professional violation had been reported in 92% (44/48) of responding jurisdictions (16). Inappropriate communication with patients (69%) and misrepresentation of credentials (60%) were the most common violations. As physicians we have to cross a line between our personal and professional opinion. Professionals should not claim treatments that cannot be substantiated or verified. Also, professionals should not advertise their services or results beyond medically verifiable data. Online discussions or posts which could be associated with financial conflicts of interest must be transparent and any conflict of interest should be disclosed.

Researchers analyzed the content of blogs written by health professionals, 11% contained product endorsement of specific healthcare products; none provided conflict of interest disclosures (17). Physician's tweets may also involve product promotions (18). Of note, Federal Trade Commission regulations released in 2009 require material connections between advertisers and endorsers to be disclosed. A blogger who receives cash or payment to review a product is considered an endorser. In addition, both advertisers and endorsers may be liable for false or unsubstantiated claims made in an endorsement in the US (19), but in other countries there is still a lack of regulation.

Patient's perspective of SoMe use

Patients are the epicenter of medicine. Everything should revolve around patients and their wellbeing. Patients hand their lives and health to us with an enormous amount of private information. At the same time, as physicians, it is our duty to educate and share our experience with our peers and this is when it gets complicated. There are multiple factors that should deter increased use of SoMe from the patient's perspective and this includes protection of privacy, "fake news", credibility and source credibility.

In the era of technological mobility, patient's privacy is often compromised. The appeal of medical imagery, especially in rare cases, puts the affected patient in a vulnerable position. The patient could no longer be protected from being exposed to the online crowds. Sharing innapropriate content is more prevalent among junior staffs, as suggested by a recent survey (20). Although patient's privacy is protected by law, regional variations exist thus allowing loopholes to be utilized to 'justify' invasion of privacy. Furthermore, legislation is a tedious process and with the exponential growth of SoMe, the laws governing it will never be at par with the impact of SoMe. Despite the existence of consent documentation and lengthy discussions between physician and patient, the aspect of consent will always be imperfect due to the imbalance between legislation and SoMe outreach.

SoMe has become an indispensible part of the health-seeking-behavior complex. Health information is mostly sought through online and socially networked platforms (21). This is due to its nature of anonymity, ease of access, and abundance of information. Accuracy and relevance of information is often misjudged by the patients and this presents as hazard to them. The feature of SoMe is the remarkable, global importance of social experiences into the online domain (22). In this scenario, the phenomenon of "fake news" has come about. Anyone can upload seemingly good advice online despite it not having strong supportive evidence. The gold standard of medical research is the randomized controlled trial and while evidence-based medicine de-emphasizes anecdotal reports, SoMe tends to emphasize them, relying on individual expericences for collective medical knowledge (23). In addition, the Echo Chamber effect as proposed by Christopher Paul et al. points out how SoMe users often follow like--minded individuals thus allowing polarized opinions to gather momentum (24). This pro-

pagation of inaccurate information represents a serious hazard to the patient and could have fatal consequences.

As with any information, its source is often in question. Health information does not escape this fact. Increased use of SoMe blurs this line of identification. Health information from foreing countries are often quoted online and often times it is quoted as being from a "renowned" physician. Most times the reader has no idea on who this "renowned" physician is and this creates confusion amongst patients. Although there are ways to verify the identity of the physician in question, the widespread availability of this information is not readily available. There are efforts to streamline information and source with the World Health Organization leading a request to the Internet Corporation for Assigned Names and Numbers to establish a suffix for validated information (25) and this could pave the way for the safety of healthcare information online, but it is still a work in progress.

CONCLUSIONS

SoMe revolutionized communication and healthcare professionals are included in this trend. These tools facilitate interaction among physicians, patients, associations, and organizations. Even though SoMe legislation varies broadly worldwide, physicians and scientists should be mindful of their posts, sharing only data with good scientific support with proper citations. Most importantly, patients' privacy must be respected.

ACKNOWLEDGEMENTS

Rodrigo Donalisio Da Silva and Jeffrey J. Leow contributed similarly as first author

On Behalf of #UroSoMe working Group

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Medscape. Medscape Reader Polls - How Do You Use Social Media? Medscape. 2018. Available at. <https://www.medscape.com/viewarticle/891157>
2. Loeb S, Bayne CE, Frey C, Davies BJ, Averch TD, Woo HH, Stork B, et al. Use of social media in urology: data from the American Urological Association (AUA). BJU Int. 2014;113:993-8.
3. Wilkinson SE, Basto MY, Perovic G, Lawrentschuk N, Murphy DG. The social media revolution is changing the conference experience: analytics and trends from eight international meetings. BJU Int. 2015;115:839-46.
4. Matta R, Doiron C, Leveridge MJ. The dramatic increase in social media in urology. J Urol. 2014;192:494-8.
5. Gudaru K, Blanco LT, Castellani D, Santamaria HT, Pelayo-Nieto M, Linden-Castro E, et al. Connecting the Urological Community: The #UroSoMe Experience. Journal of Endoluminal Endourology. 2019;2:e20-e9.
6. Borgmann H, Cooperberg M, Murphy D, Loeb S, N'Dow J, Ribal MJ, et al. Online Professionalism-2018 Update of European Association of Urology (@Uroweb) Recommendations on the Appropriate Use of Social Media. Eur Urol. 2018;74:644-50.
7. Loeb S, Catto J, Kutikov A. Social media offers unprecedented opportunities for vibrant exchange of professional ideas across continents. Eur Urol. 2014;66:118-9.
8. Murphy DG, Basto M. Social media @BJUIjournal--what a start! BJU Int. 2013;111:1007-9.
9. Eysenbach G. Can tweets predict citations? Metrics of social impact based on Twitter and correlation with traditional metrics of scientific impact. J Med Internet Res. 2011;13:e123. Erratum in: doi:10.2196/jmir.2041.
10. Haustein S, Peters I, Sugimoto CR, Thelwall M, Larivière V. Tweeting biomedicine: An analysis of tweets and citations in the biomedical literature. Journal of the Association for Information Science and Technology. 2014;65:656-69.
11. Peoples BK, Midway SR, Sackett D, Lynch A, Cooney PB. Twitter Predicts Citation Rates of Ecological Research. PLoS One. 2016;11:e0166570.
12. Cardona-Grau D, Sorokin I, Leinwand G, Welliver C. Introducing the Twitter Impact Factor: An Objective Measure of Urology's Academic Impact on Twitter. Eur Urol Focus. 2016;2:412-7.
13. Fox S, Duggan M. Health Online 2013. Pew Research Center's Internet & American Life Project. 2013. Available at. <https://www.pewinternet.org/2013/01/15/health-online-2013/>
14. Greene JA, Choudhry NK, Kilabuk E, Shrank WH. Online

social networking by patients with diabetes: a qualitative evaluation of communication with Facebook. J Gen Intern Med. 2011;26:287-92.
15. Rivas JG, Socarrás MR, Blanco LT. Social Media in Urology: opportunities, applications, appropriate use and new horizons. Cent European J Urol. 2016;69:293-8.
16. Greysen SR, Chretien KC, Kind T, Young A, Gross CP. Physician violations of online professionalism and disciplinary actions: a national survey of state medical boards. JAMA. 2012;307:1141-2.
17. Ventola CL. Social media and health care professionals: benefits, risks, and best practices. P T. 2014;39:491-520.
18. Leveridge MJ. The emerging role of social media in urology. Rev Urol. 2014;16:110-7.
19. Chretien KC, Kind T. Social media and clinical care: ethical, professional, and social implications. Circulation. 2013;127:1413-21.
20. Fanti Silva DA, Colleoni R. Patient's Privacy Violation on Social Media in the Surgical Area. Am Surg. 2018;84:1900-5.
21. Kamel Boulos MN, Wheeler S. The emerging Web 2.0 social software: an enabling suite of sociable technologies in health and health care education. Health Info Libr J. 2007;24:2-23.
22. Centola D. Social media and the science of health behavior. Circulation. 2013;127:2135-44.
23. Grindrod K, Forgione A, Tsuyuki RT, Gavura S, Giustini D. Pharmacy 2.0: a scoping review of social media use in pharmacy. Res Social Adm Pharm. 2014;10:256-70.
24. Miller DT. Topics and emotions in Russian Twitter propaganda. 2019. Available at. < https://firstmonday.org/ojs/index.php/fm/article/view/9638>
25. Grajales FJ 3rd, Sheps S, Ho K, Novak-Lauscher H, Eysenbach G. Social media: a review and tutorial of applications in medicine and health care. J Med Internet Res. 2014;16:e13.

Correspondence address:
Marcelo Langer Wroclawski, MD
Hospital Israelita Albert Einstein e
Hospital da Beneficência Brasileira de São Paulo
Rua Iguatemi, 192, 4º andar
São Paulo, SP, 01451-010, Brasil
Telephone: +55 11 3168-2130
E-mail: urologia.marcelo@gmail.com

ARTICLE INFO

Marcelo Wroclawski
https://orcid.org/0000-0001-6835-9085
Int Braz J Urol. 2019; 45: 882-8

Submitted for publication:
July 20, 2019

Accepted after revision:
August 05, 2019

Published as Ahead of Print:
September 15, 2019

REVIEW ARTICLE

Vol. 45 (5): 889-900, September - October, 2019
doi: 10.1590/S1677-5538.IBJU.2019.0080

Brazilian consensus in enuresis–recomendations for clinical practice

José Murillo B. Netto [1], Atila Victal Rondon [2], George Rafael Martins de Lima [3], Miguel Zerati Filho [4], Edison Daniel Schneider-Monteiro [5], Carlos Augusto F. Molina [6], Adriano de Almeida Calado [7], Ubirajara Barroso Jr. [8]

[1] Universidade Federal de Juiz de Fora (UFJF) e Hospital e Maternidade Therezinha de Jesus da Faculdade de Ciências Médicas e da Saúde de Juiz de Fora (HMTJ-SUPREMA), Juiz de Fora, MG, Brasil; [2] Universidade do Estado do Rio de Janeiro (UERJ) e Hospital Federal Cardoso Fontes (HFCF), Rio de Janeiro, RJ, Brasil; [3] Hospital Infantil Albert Sabin, Fortaleza, CE, Brasil; [4] Instituto de Urologia e Nefrologia de São José do Rio Preto (IUN) e Faculdade Regional de Medicina(FAMERP), Hospital de Base, São José do Rio Preto, SP, Brasil; [5] Hospital da Pontifícia Universidade Católica de Campinas (PUC-Campinas), Campinas, SP, Brasil; [6] Hospital das Clinicas da Faculdade de Medicina de Ribeirão Preto da Universidade de São Paulo (HCFMRP-USP), Ribeirão Preto, SP, Brasil; [7] Faculdade de Medicina da Universidade de Pernambuco (UPE), Recife, PE, Brasil ; [8] Universidade Federal da Bahia (UFBA) e Escola Bahiana de Medicina (BAHIANA), Salvador, BA, Brasil

ABSTRACT

Introduction: Enuresis, defined as an intermittent urinary incontinence that occurs during sleep, is a frequent condition, occurring in about 10% of children at 7 years of age. However, it is frequently neglected by the family and by the primary care provider, leaving many of those children without treatment. Despite of many studies in Enuresis and recent advances in scientific and technological knowledge there is still considerable heterogeneity in evaluation methods and therapeutic approaches.

Materials and Methods: The board of Pediatric Urology of the Brazilian Society of Urology joined a group of experts and reviewed all important issues on Enuresis and elaborated a draft of the document. On September 2018 the panel met to review, discuss and write a consensus document.

Results and Discussion: Enuresis is a multifactorial disease that can lead to a diversity of problems for the child and family. Children presenting with Enuresis require careful evaluation and treatment to avoid future psychological and behavioral problems. The panel addressed recommendations on up to date choice of diagnosis evaluation and therapies.

ARTICLE INFO

José Murillo B. Netto
http://orcid.org/0000-0002-9959-6160

Keywords:
Enuresis; Urinary Incontinence; Lower Urinary Tract Symptoms

Int Braz J Urol. 2019; 45: 889-900

Submitted for publication:
February 04, 2019

Accepted after revision:
May 06, 2019

Published as Ahead of Print:
July 15, 2019

INTRODUCTION

Enuresis is defined as an intermittent urinary incontinence that occurs during sleep, having clinical significance after the child completes 5 years of age (1). It is a frequent condition, occurring in about 15 to 20% of 5 years old children and 6.4 to 10.3% of children at 7 years of age (2-4) with a spontaneous re-

solution rate of about 15% per year (5) and will still be presented in approximately 0.5 to 2.3% of adults (6, 7).

Enuresis is a multifactorial condition. Hereditary factors have been described and genetic factors are the most important in the etiology of enuresis (8). Family history of enuresis plays important rule. Studies have shown a risk of a child have enuresis to be 44% if one parent was enuretic and 77% if both had enuresis, and 15% if neither one of the parents suffered from the disorder (9).

Other factors involved on enuresis physiopathology are changes in bladder function (nocturnal bladder overactivity) (10), nocturnal urinary output (nocturnal polyuria due to altered circadian cycle of the antidiuretic hormone) (11-13) and associated with disturbance of awakening (inability of the child to wake up in response to bladder contractions or full bladder sensation) (14, 15).

There is evidence that enuresis is associated with emotional and behavioral changes (16), dysfunctions of the urinary and intestinal tracts (17), and respiratory changes, such as nocturnal apnea (18-20).

About 20 to 30% of enuretic children present with psychological/psychiatric disorders (2 to 4 times more than non-enuretic ones) (21). Enuretic children present more behavioral problems than non-enuretic ones regarding to social and attention problems (22).

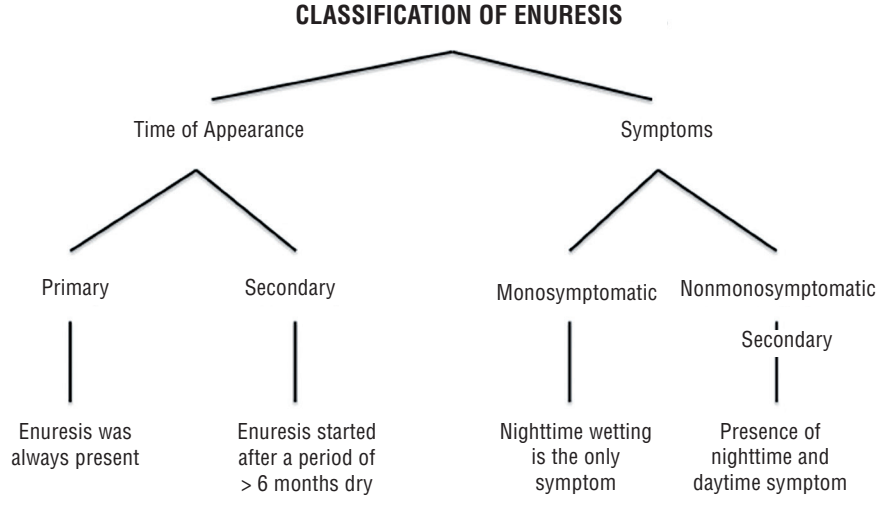
Many enuretic children will present emotional and/or behavioral changes, such as sadness, mood alteration, shame, low self-esteem, feelings of guilt, insecurity, social isolation, low school performance, among others (21). In addition, families are also affected and the consequences of enuresis, for both the child and his family, are often neglected. Recent studies have shown significant loss of quality of life, not only for children, but also for their families (23, 24). Thus, parents, by not well understanding the problem and also for being stressed often become intolerant and punishing the child. Two national studies have shown a high incidence of punishment in enuretic children, being it verbal, physical without contact (chastisement) or with contact (aggression) (25, 26).

When relevant emotional and behavioral changes are identified, especially in secondary enuresis, psychological evaluation and treatment are recommended.

Enuresis can be classified according to the moment of its appearance and to the symptoms presented and both classifications should be added to define the correct type of enuresis (Figure-1).

Evaluating the outcome of a treatment is important for clinical and research purpose. The

Figure 1 - Classification of Enuresis.



standardization document from ICCS considers treatment outcome as "No Response when there is an improvement of less than 50% of the symptoms, "Partial Response" when there is improvement of 50 to 99% of the symptoms and "Complete Response" when 100% of the symptoms are resolved. Relapse is considered when more than one episode occurs per month. Continuous Success is when there is absence of relapse in 6 months and Complete Success when no relapses occur after 2 years (27).

MATERIALS AND METHODS

The board of Pediatric Urology of the Brazilian Society of Urology, noticing the need of a Brazilian guideline on Enuresis, joined a group of experts to review the important issues on Enuresis and elaborated a consensus document. Eight renewed pediatric urologist with known experience in dealing with voiding dysfunction and enuresis were invited to participate in the elaboration a document with the scope of the guiding urologists, pediatricians, nephrologists and all others that deal with enuresis on the most important and up to date aspects of the evaluation and treatment of enuretic children.

All panel members were instructed to perform a literature search on MEDLINE, EMBASE and COCHRANE LIBRARY databases as well as review of the base of practical guidelines database for the last 20 years using the terms "Enuresis", "Nocturnal Enuresis", and "Bedwetting". Papers were selected according to their level of evidence, giving more importance to meta-analysis, systematic reviews, and randomized controlled trials. Criterion of exclusion of bibliography included topics in which neither of those were found or were not of good quality or did not address treatment options. Cohort and series of patients were used to add information. Review papers and guidelines were used as orientation for which topics and aspects would be included.

After the papers were selected, each member of the group were designated one topic to review and write an orientation document based on the recommended literature.

On September 2018, all members joined together during 2 days to review and discuss the previous written documents of each topic and prepare the consensus document. Further discussions, corrections, and revisions were carried out digitally, until all members of the panel have approved this final document. A paragraph containing the panels opinion ("consensus") was added at the end of each section to guide the reader about the information provided and the most common practice on that subject.

CLINICAL EVALUATION AND DIAGNOSIS

A careful and meticulous clinical history is the best tool to understand and diagnose the correct type of enuresis and propose the most appropriate treatment. For this, it is necessary to differentiate between primary or secondary, and mono or non-monosymptomatic enuresis.

Anamnesis should include the child's age, if any dry period had occurred, presence of voiding symptoms throughout the day (incontinence, increased voiding frequency, urinary urgency and low volume voiding), bowel habits, number of enuresis episodes per night and per week, information on child's sleep pattern, sleep apnea, and difficulties in awakening, presence of any behavioral problem, such as attention deficit and hyperactive disorder (ADHD), anxiety, stress, abuse, bullying (28) and punishment (25). Past and familial histories are also important.

A careful history of bowel habits should be included to investigate constipation. The use of Rome IV criteria and Bristol Stool Scale is recommended to help making the proper diagnosis. In cases when the child presents signs of increase urine output diabetes mellitus should be investigated (glycosuria and glycaemia) and excluded.

Physical exam is helpful in identifying associated comorbidities. Genitalia should be careful examined and also abdomen, where signs of constipation (impacted feces in the left colon) can be found. Examination of the back is important to exclude any cutaneous sign of occult spinal cord malformation (29). As a complement to the clinical history, the child should be asked to complete both a voiding and a night diary. The voiding diary increases the reliability of the information gi-

ven by the family and makes the parents aware of their child voiding habits. It should be performed for two to three days, not necessarily consecutive, and include all void and drink episodes. The voiding diary collects information on frequency, time of void, volume at each micturition, episodes of urgency and/or incontinence, liquid intake. It is considered normal 4 to 7 void per day, and an average of voided volume between 65 and 150% of the expected bladder capacity for the age, calculated by the formula ((Age+1) x 30) (27). A carefully orientation, explaining any doubts, on how to fulfill the voiding diary is important to avoid problems in its outcome.

A dry night diary of 14 consecutive days should also be obtained with the purpose of recording the frequency that enuresis occurs. To obtain the night-voided volume, the child is asked to sleep wearing a diaper. The sum of the diaper's weight (Kg), the voided volume of the first micturition, and the voided volume of any episode of nocturia, if present will give the nocturnal diuresis. Night polyuria is considered to be a nocturnal diuresis volume >130% estimated bladder capacity or > the volume expressed by the formula ((age+9) x 20) (27).

No other test is necessary in the evaluation of monosymptomatic enuresis. Children with polyuria and polydipsia should be investigated for diabetes insipidus.

Evaluation of non-monosymptomatic enuresis will be discussed at the end of the document.

Consensus: The panel believes that a careful and meticulous clinical history considering all aspects discussed above, associated with a voiding diary are the most important tools in the evaluation of an enuretic child. It is important to address all behavioral problems the child may have. The addition of any other diagnostic test is rarely necessary in children with monosymptomatic enuresis.

TREATMENT (Figure-2)

Urotherapy

Urotherapy or behavioral therapy consists in a series of orientations indicated as initial treatment in all patients with enuresis it should be maintained throughout the treatment even when other therapeutic modalities are chosen (27, 30). The aims of urotherapy are to inform and demystify enuresis and give orientation on habits modifications to improve symptoms.

Urotherapy orientation includes (27, 31): 1) information and demystification. Explanation about the normal function of the urinary tract and how that particular child deviates from the normal pattern; 2) regular voiding habits (voiding every 3 to 4 hours); 3) avoid retention maneuvers; 4) appropriate voiding position on the toilet to promote relaxation of the pelvic floor (appropriate-sized seat, footrest, forward bending of the torso); 5) regular bowel habits (standard time for evacuation, preferably after meals); 6) increase fluid intake during the day, specially in the mornings and early afternoon; 7) change dietary habits (eliminate caffeine, avoid citric fruits and juices, reduce sodium intake at nigh); 8) avoid any liquids at least 2 hours before bedtime; 9) void prior to go to bed; 10) no use of diapers; 11) dry nights calendar; and 12) support and encouragement through regular follow-up with the child and caregiver, providing positive reinforcement (award system for dry nights).

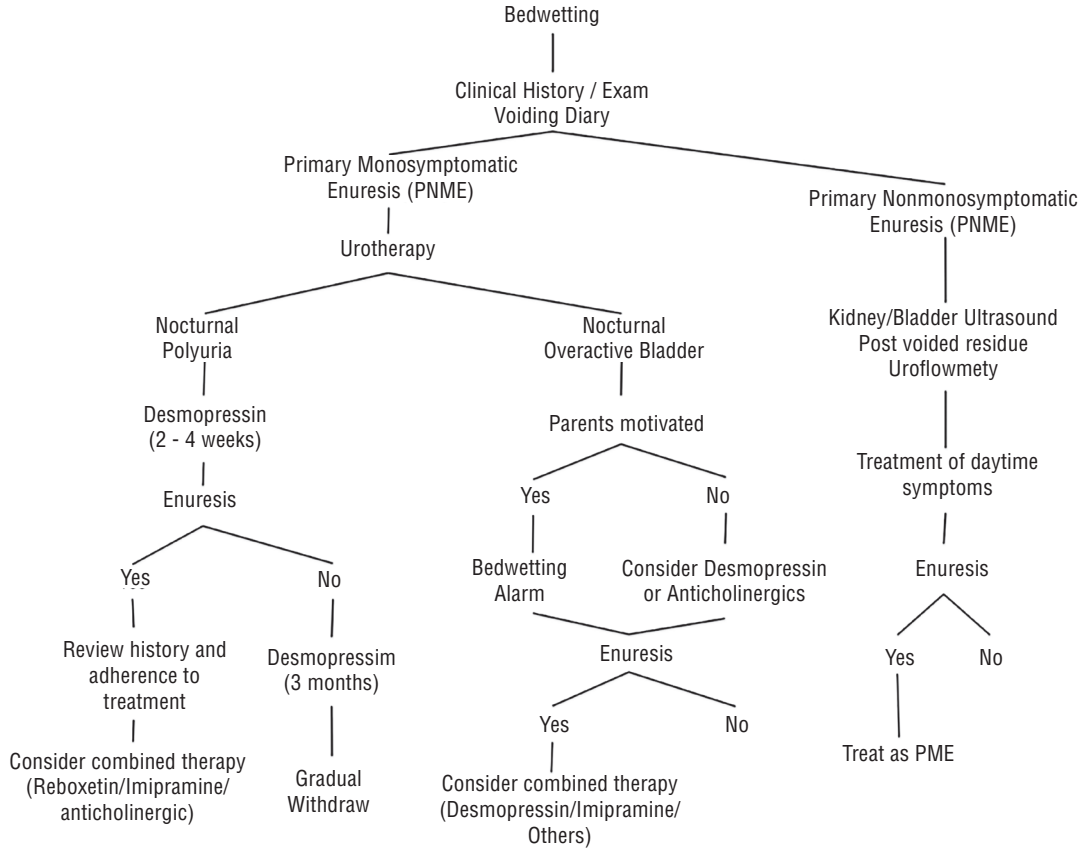
Behavioral interventions were superior when compared to no intervention, but had worse results when used alone to treat associated with alarm or drug therapy (32, 33). The resolution rate of enuresis with urotherapy alone is about 20% (34).

Consensus: The panel agrees that urotherapy orientations should be offered to any child with enuresis as first line treatment, regardless if it is mono or non-monosymptomatic. The health professional should provide clear information and reinforce all orientation given in every clinical consult. The addition of other therapeutic modalities will increase success of urotherapy.

Enuresis Alarm

The Enuresis Alarm is an electronic device that uses a humidity sensor placed at underwear and connected to a sound and/or vibrating circuit that will be activated when the child wets the bed. The purpose of the alarm is to wake the child up during the enuretic episode and make the user (or a responsible person) go to the toilet to com-

Figure 2 - Treatment Algorithm.



plete micturition. It is considered as behavioral or conditioning treatment. The age recommended to start treatment with alarm is six to seven years when the child is mature enough to accept and understand the treatment (35).

The alarm is considered the most effective long-term treatment for enuresis (Level of evidence and recommendation 1A). Results suggest a success rate of 62 to 75% after treatment, with a relapse rate of 15 to 30% during long-term follow-up (36-38).

The greatest problem with enuresis alarm is that a high number of patients discontinue treatment (30% to 50%) due to loss of motivation or other factors, such as waking up other members of the family during the night and the duration of the treatment that may take about six to eight weeks (38, 39). Due to this, a supportive approach to the family is necessary to reduce these numbers (35).

Results with alarm therapy improves if urine volume is greater than or equal to 65% of the expected capacity for age, in children who have more number of wet nights (40), and when parents and child are motivated (34). Factors related to failure of poor results are punitive parental reactions to the bedwetting (41), child behavioral problems, higher socioeconomic status (41), and if treatment is started out of the winter (41).

It is recommended that the child should keep using the alarm after 14 consecutive dry nights before stopping treatment.

Alarm should not be indicated when punishment is identified and should be used with caution in cases of night sweats in hot climate areas, due to activation of the alarm by humidity caused by sweat, and when the child sleeps with other children and a risk of bullying exists.

Compared to desmopressin, studies suggest that enuresis alarm is better in the long run, but some show that results may equivalent if the

highest alarm dropout rate is considered (35, 39).

The addition of desmopressin to alarm treatment improves dry nights rate initially, but in the long term there is no benefit with this addition. Therefore, there is no recommendation that these methods be used together except in cases that fail when either one is used alone (42).

Treatment with alarm is better than oxybutynin alone and the addition of oxybutynin to the alarm does not improve the success rate in the initial treatment. This association is only indicated in refractory cases (43). Alarm was shown to be better than imipramine in improving the number of dry nights (44).

Regarding the two types of alarm, the body-worn alarm and the bed pad alarm, the alarm fixed on the body is preferred by the children (44). There is no evidence on which type of alarm is better than the other. Further studies need to be performed.

Consensus: The panel highly recommends the use of alarm in children with primary monosymptomatic enuresis that presents with a high number of wet nights and parents are motivated. A careful evaluation of the child and family, discarding behavioral problems and punishment is important for treatment success. Alarm treatment should be followed closely due to a high number of children that discontinue treatment. When no or poor response is found, other drugs such as desmopressin may be added to the treatment.

Desmopressin

Desmopressin acetate is a synthetic analogue of the natural hormone, arginine vasopressin (antidiuretic hormone). It acts increasing the reabsorption of water through the renal tubules, leading to an increase in urinary osmolality and decreased diuresis (45, 46).

Desmopressin is a first-line treatment for enuresis with grade 1A of evidence. Its best results can be obtained in children with nocturnal polyuria (diuresis >130% estimated bladder capacity or > (age+9) x 20mL, adding the volume of the first void in the morning) (27) and normal bladder reservoir function (maximum voided volume greater than 70% of estimated bladder capacity for age) (47), and children with a greater age, and limited number of wet nights a week (31, 46).

Its administration is orally and should be taken one hour prior to bedtime and about 2 hours after dinner. It is recommended to start with a low dose of 0.1 to 0.2mg and adjust, when necessary, up to a maximum dose of 0.6mg/day, regardless of age or weight (48). One hour before taking the medication, children should stop fluid intake in order to avoid risk of hyponatremia and water intoxication, as well as obtain an optimal urine concentrating capacity (49).

Initial treatment should be maintained for 2 to 4 weeks in order to obtain maximum effect and if there is improvement in the number of dry night it should be continued for at least 3 more months. If the child is dry, withdrawn of the medication may be done gradually, which seems to reduce relapse (48, 49). In cases when symptoms worsen after beginning medication withdraw, dose should be increased again and treatment maintained for three more months.

The overall success rate of desmopressin is up to 65% and the relapse rate, especially if a gradual withdraw is not done, goes up to 80% (50). However, the outcome is lower in the long term follow-up.

Consensus: The panel believes that desmopressin should be used as first line treatment for all children with nocturnal polyuria and those that the use of enuresis alarm is not suitable. Dose should be increased gradually until dryness is achieved and treatment maintained for at least 3 months. There is evidence that withdrawn should be done gradually. Parents and child should be aware that, although rare, increase fluid intake and desmopressin may lead to side effects (hyponatremia and water intoxication).

Other Drugs

Anticholinergics

The use of anticholinergics in enuresis is limited (grade 1B evidence) (51, 52). Children with increased voiding frequency and low bladder volume (<65% of estimated bladder capacity) and with nocturnal bladder overactivity are the most suitable candidates as anticholinergics act inhi-

biting bladder contractions. The most commonly used anticholinergic in our setting is oxybutynin, although others such as solifenacine, and tolterodine can be used.

The results with anticholinergics in enuresis, although better than placebo, are poor when used as monotherapy and they should only be indicated if other treatments have failed (31). In these cases, anticholinergics can be helpful in up to 40% of the patients, especially in combination with desmopressin (53).

The major problem with the use of anticholinergics is, in addition to bad results, that a significant amount of patients present side effects, such as constipation, increased post-voided residual, dry mouth, among others. Therefore, when using anticholinergic drugs, constipation should first be assessed (53).

Consensus: In the panel's opinion, anticholinergics should be used only in cases where no success with desmopressin and alarm was achieved and in selected patients with overactive bladder. Evaluation of constipation and post-voided residual should be done prior to prescription.

Tricyclic Antidepressants

The leading tricyclic antidepressant prescribed for enuresis is imipramine. Randomized trials have shown that imipramine is slightly better than placebo, but due to safety concerns (cardiotoxicity) and side effects, it is considered to be a third line treatment for enuresis (Grade 1C evidence) (54-56), and should only be used in cases of failure of the first therapeutic options or when patients cannot afford them.

The mechanism of action of tricyclic antidepressants in enuresis is still controversial. It is known that they decrease the amount of time spent in REM sleep, stimulate vasopressin secretion, and relax the detrusor muscle.

Imipramine has shown to be more effective particularly for short-term outcomes, with fewer wet nights per week when compared to placebo (54, 57). Imipramine promotes a reduction of 1 wet night per week, with about 20% of children being dry for at least 14 consecutive nights. However, no sustained results are seen after ceasing treatment, and the majority of patients relapse after discontinuation of treatment (54).

Combination therapy including imipramine and anticholinergics showed improved results when compared to imipramine alone or placebo in the short and long term, with fewer relapses than imipramine monotherapy (54, 58). The combination of tricyclic antidepressants and desmopressin did not show any improvement in treatment results (54, 59).

It is advised to take the medicine 1h before bedtime, in the initial dosage of 10-25mg, and it can be increased in 25mg after 1 week of treatment. The maximum dose in children aged 6-12 years is 50mg/day and in those over 12 years of age up to 75mg/day. Every 3 months, treatment should be discontinued for at least 2 weeks to decrease the risk of drug tolerance and observation of therapeutic efficacy.

Imipramine presents limitations due to its cardiotoxicity with potential risk of cardiac conduction disorders and myocardial depression in overdose. Other possible side effects are possible behavioral changes, irritability and drowsiness, dizziness, headache, sweating, lethargy, sleep disturbance or restlessness, apathy, depression, among others.

Consensus: It is the panel's opinion that imipramine and other tricyclic antidepressants should be reserved to those that failed other treatment. It should be used with caution and the family should be aware of possible side effects and the medication should be kept away from child's reach. Due to its low cost compared to other treatment modalities, it can be an option in patients with low income. Combination with anticholinergics should be in mind to improve results.

Alternative Treatments

So-called alternative, or rather unconventional, treatments such as acupuncture, hypnosis, homeopathy, herbalism, chiropractic, faradization, among others, have been tested but there is no scientific evidence to support their use.

The results with neuromodulation (transcutaneous electroneurostimulation) are controversial, and there may be a reduction in dry nights but no complete response in primary monosymptomatic enuresis. Further studies are need to validate its use in enuresis (60).

Consensus: The panelists agree that alternative treatment should not be used, except for electroneurostimulation, that could be tried in cases in which other therapies have failed.

THERAPY RESISTANT ENURESIS

Some patients will not respond to alarm nor desmopressin and those are considered the therapy resistant ones, and, for them, other therapeutic modalities should be tried.

Assessment of these children includes a careful clinical history and physical exam trying to identify any missed information in their previous evaluation and treatment (49).

Possible factors related to poor therapeutic response should be careful investigated prior to considering the child resistant to treatment (61). Some questions should be addressed, such as: 1) is enuresis really monosymptomatic or daytime symptoms are present?; 2) does the patient present nocturnal polyuria?; 3) is the child reducing/stopping fluid intake at least 2 hours prior to bedtime; 4) is he/she following dietary orientation (low caffeine and citric intake)?; 5) is desmopressin being taking as prescribed (1 hour before bedtime and 2 hours after dinner)?; 6) is desmopressin dose adequate or it can be raised?; 7) is the alarm being used correctly?; 8) is there any behavioral disorder, such as attention deficit and hyperactivity disorders or others?

Children not responding to enuresis alarm correctly should be reinforced and reoriented on how the alarm works and how to be used and, those not motivated, other therapeutic options, such as desmopressin or other drugs should be started.

In desmopressin resistant patients, possible causes are excess urine solute due to increased sodium intake or altered sodium circadian rhythm or influence of vasoactive hormones and prostaglandins, such as renin, aldosterone and atrial natriuretic peptide. After defining the patient as resistant to desmopressin, we can divide them among those with absence of response despite decreased diuresis and those with nocturnal bladder overactivity as a possible etiology (61).

Therapeutic options for desmopressin resistant children includes indomethacin, a cyclooxygenase inhibitor, although significantly reduces nocturnal sodium, urea and osmotic excretion, no significant decrease in the number of wet nights was observed neither as mono therapy nor as combined to desmopressin (62, 63).

Furosemide has also been tried as a second line treatment for those resistant to desmopressin. It should be taken in the morning to increase diuresis and excretion of sodium and solutes during the day and, when associated to desmopressin, it has shown decrease of frequency of enuresis (64).

Reboxetine, an antidepressant with noradrenergic action, significantly reduced enuresis episodes in therapy resistant patients when compared to placebo, although must of the responses were partial (65).

Combination therapy is an option in those patients. The combined use of desmopressin and an anticholinergic agent is well tolerated and results in a significant improvement in enuresis episodes and the results suggest a better response if higher anticholinergic dose is used (up to 10mg) (66, 67). The effect of combined therapy of desmopressin and alarm is still controversial and immediate positive effect has been presented (68, 69), although some have lacked to show long term effect (42).

Consensus: It is the panel's opinion that therapy resistant enuresis should always be addressed by an experienced professional in the field, since those cases requires expertise due to its complexity and poor results with the current available treatment options. A careful clinical history and physical exam are keys to achieve success in those cases.

NON-MONOSYMPTOMATIC ENURESIS

Non-monosymptomatic nocturnal enuresis (NME) is characterized by the presence of diurnal symptoms associated with enuresis and is present in about 1/3 of all enuretic children (70). It has peculiarities that differ it from the monosymptomatic enuresis, as increased risk of urinary tract infection and greater association with constipation and emotional/behavioral disorders.

Those children with NME need proper evaluation and treatment. Greater attention should be

taken on diurnal voiding symptoms (urgency, frequency, incontinence, straining to void) and constipation. Besides the voiding diary, evaluation of the post-void urinary residue by ultrasound and uroflowmetry are required to make the correct diagnosis and propose the ideal treatment. Assessment of bowel function with Rome IV criteria and Bristol Stool Scale should be careful evaluated as those children frequently present constipation.

Initial treatment should address daytime symptoms first focusing on treating the lower urinary tract dysfunction, constipation, and any behavioral disorder if present (1).

Urotherapy is the initial therapy and should be performed for at least one month. In the persistence of symptoms, more specific treatments are indicated and added to urotherapy. Therapeutic options include anticholinergics, alpha--blockers, electro neurostimulation, biofeedback, and botulin toxin.

Children who do not improve diurnal and nocturnal symptoms should continue with other therapeutic modalities that remain focused on the treatment of daytime symptoms such as increased dose of medication or combination of medications and of medication with other therapies (electro neurostimulation or biofeedback). Constipation, when present, should be assessed in the beginning of treatment. Improvement of bowel habits is a key point for improvement of voiding function.

If enuresis is still persistent after improvement of daytime symptoms; its treatment follows those described for monosymptomatic enuresis.

Consensus: The panel believes that children presenting with non-monosymptomatic enuresis should be evaluated as those presenting lower urinary tract dysfunction, which includes voiding diary, uroflowmetry and ultrasound with evaluation of post-voided residual. Assessment of constipation and behavioral disorders should not be forgotten during clinical investigation and should be treated if present. Daytime symptoms are the focus of the initial therapy and enuresis is treated after improvement of daytime lower urinary tract symptoms.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Franco I, von Gontard A, De Gennaro M; International Childrens's Continence Society. Evaluation and treatment of nonmonosymptomatic nocturnal enuresis: a standardization document from the International Children's Continence Society. J Pediatr Urol. 2013;9:234-43.
2. Fergusson DM, Horwood LJ, Shannon FT. Factors related to the age of attainment of nocturnal bladder control: an 8-year longitudinal study. Pediatrics. 1986;78:884-90.
3. Hellström AL, Hanson E, Hansson S, Hjälmås K, Jodal U. Micturition habits and incontinence in 7-year-old Swedish school entrants. Eur J Pediatr. 1990;149:434-7.
4. Järvelin MR, Vikeväinen-Tervonen L, Moilanen I, Huttunen NP. Enuresis in seven-year-old children. Acta Paediatr Scand. 1988;77:148-53.
5. Feehan M, McGee R, Stanton W, Silva PA. A 6 year follow-up of childhood enuresis: prevalence in adolescence and consequences for mental health. J Paediatr Child Health. 1990;26:75-9.
6. Hirasing RA, van Leerdam FJ, Bolk-Bennink L, Janknegt RA. Enuresis nocturna in adults. Scand J Urol Nephrol. 1997;31:533-6.
7. Yeung CK, Sihoe JD, Sit FK, Bower W, Sreedhar B, Lau J. Characteristics of primary nocturnal enuresis in adults: an epidemiological study. BJU Int. 2004;93:341-5.
8. von Gontard A, Schaumburg H, Hollmann E, Eiberg H, Rittig S. The genetics of enuresis: a review. J Urol. 2001;166:2438-43.
9. Bakwin, H.: The Genetics of Enuresis. In: Bladder Control and Enuresis. Edited by I. M. Kolvin, R. C.; Meadow, S. R. London: William Heinemann Medical Books, 1973.
10. Yeung CK, Sit FK, To LK, Chiu HN, Sihoe JD, Lee E,et al. Reduction in nocturnal functional bladder capacity is a common factor in the pathogenesis of refractory nocturnal enuresis. BJU Int. 2002;90:302-7.
11. Rittig S, Schaumburg HL, Siggaard C, Schmidt F, Djurhuus JC. The circadian defect in plasma vasopressin and urine output is related to desmopressin response and enuresis status in children with nocturnal enuresis. J Urol. 2008;179:2389-95.

12. Aikawa T, Kasahara T, Uchiyama M. The arginine-vasopressin secretion profile of children with primary nocturnal enuresis. Eur Urol. 1998;33(Suppl 3):41-4.
13. Dossche L, Walle JV, Van Herzeele C. The pathophysiology of monosymptomatic nocturnal enuresis with special emphasis on the circadian rhythm of renal physiology. Eur J Pediatr. 2016;175:747-54.
14. Yeung CK, Diao M, Sreedhar B. Cortical arousal in children with severe enuresis. N Engl J Med. 2008;358:2414-5.
15. Hunsballe JM. Increased delta component in computerized sleep electroencephalographic analysis suggests abnormally deep sleep in primary monosymptomatic nocturnal enuresis. Scand J Urol Nephrol. 2000;34:294-302.
16. Von Gontard A, Hollmann E. Comorbidity of functional urinary incontinence and encopresis: somatic and behavioral associations. J Urol. 2004;171(6 Pt 2):2644-7.
17. Hoffmann A, Sampaio C, Nascimento AA, Veiga ML, Barroso U. Predictors of outcome in children and adolescents with overactive bladder treated with parasacral transcutaneous electrical nerve stimulation. J Pediatr Urol. 2018;14:54.e1-54.e6.
18. Dahan P, de Bessa J Jr, de Oliveira DM, Gomes CC, Cardoso JC, Macedo IT, de Almeida Belo M, de Figueiredo AA, Netto JM. Association between Asthma and Primary Nocturnal Enuresis in Children. J Urol. 2016;195(4 Pt 2):1221-6.
19. Sousa AS, Veiga ML, Braga AA, Carvalho MC, Barroso U Jr. Enuresis and overactive bladder in children: what is the relationship between these two conditions? Int Braz J Urol. 2016;42:798-802.
20. Sampaio C, Sousa AS, Fraga LG, Veiga ML, Bastos Netto JM, Barroso U Jr. Constipation and Lower Urinary Tract Dysfunction in Children and Adolescents: A Population-Based Study. Front Pediatr. 2016;4:101.
21. von Gontard A, Baeyens D, Van Hoecke E, Warzak WJ, Bachmann C. Psychological and psychiatric issues in urinary and fecal incontinence. J Urol. 2011;185:1432-6.
22. Akyüz M, Koca O, Karaman B, Özcan ZY, Öztürk Mİ, Kutluhan MA, et al. Evaluation of behavioral problems in patients with monosymptomaticnocturnal enuresis: a prospective controlled trial. Turk J Med Sci. 2016;46:807-11.
23. Van Herzeele C, Dhondt K, Roels SP, Raes A, Groen LA, Hoebeke P, et al. Neuropsychological functioning related to specific characteristics of nocturnal enuresis. J Pediatr Urol. 2015;11:208.e1-6.
24. Kilicoglu AG, Mutlu C, Bahali MK, Adaletli H, Gunes H, Duman HM, et al. Impact of enuresis nocturna on health-related quality of life in children and their mothers. J Pediatr Urol. 2014;10:1261-6.
25. Sá CA, Gusmão Paiva AC, de Menezes MC, de Oliveira LF, Gomes CA, de Figueiredo AA, et al. Increased Risk of Physical Punishment among Enuretic Children with Family History of Enuresis. J Urol. 2016;195(4 Pt 2):1227-30.
26. Sapi MC, Vasconcelos JS, Silva FG, Damião R, Silva EA. Assessment of domestic violence against children and adolescents with enuresis. J Pediatr (Rio J). 2009;85:433-7.
27. Austin PF, Bauer SB, Bower W, Chase J, Franco I, Hoebeke P, et al. The standardization of terminology of lower urinary tract function in children and adolescents: Update report from the standardization committee of the International Children's Continence Society. Neurourol Urodyn. 2016;35:471-81.
28. Akan S, Ürkmez A, Yildirim C, Sahin A, Yüksel ÖH, Verit A. Late-onset secondary nocturnal enuresis in adolescents associated with post-traumatic stress disorder developed after a traffic accident. Arch Ital Urol Androl. 2015;87:250-1.
29. Haid B, Tekgül S. Primary and Secondary Enuresis: Pathophysiology, Diagnosis, and Treatment. Eur Urol Focus. 2017;3(2-3):198-206.
30. Maternik M, Krzeminska K, Zurowska A. The management of childhood urinary incontinence. Pediatr Nephrol. 2015;30:41-50.
31. Neveus T, Eggert P, Evans J, Macedo A, Rittig S, Tekgül S, et al. Evaluation of and treatment for monosymptomatic enuresis: a standardization document from the International Children's Continence Society. J Urol. 2010;183:441-7.
32. Glazener CM, Evans JH, Peto RE. Complex behavioural and educational interventions for nocturnal enuresis in children. Cochrane Database Syst Rev. 2004;(1):CD004668.
33. Hjalmas K, Arnold T, Bower W, Caione P, Chiozza LM, von Gontard A, et al. Nocturnal enuresis: an international evidence based management strategy. J Urol. 2004;171(6 Pt 2):2545-61.
34. Devlin JB, O'Cathain C. Predicting treatment outcome in nocturnal enuresis. Arch Dis Child. 1990;65:1158-61.
35. Perrin N, Sayer L, While A. The efficacy of alarm therapy versus desmopressin therapy in the treatment of primary mono-symptomatic nocturnal enuresis: a systematic review. Prim Health Care Res Dev. 2015;16:21-31.
36. Houts AC, Berman JS, Abramson H. Effectiveness of psychological and pharmacological treatments for nocturnal enuresis. J Consult Clin Psychol. 1994;62:737-45.
37. Butler RJ, Robinson JC. Alarm treatment for childhood nocturnal enuresis: an investigation of within-treatment variables. Scand J Urol Nephrol. 2002;36:268-72.
38. Butler RJ, Gasson SL. Enuresis alarm treatment. Scand J Urol Nephrol. 2005;39:349-57.

39. Evans J, Malmsten B, Maddocks A, Popli HS, Lottmann H; UK study group. Randomized comparison of long-term desmopressin and alarm treatment for bedwetting. J Pediatr Urol. 2011;7:21-9.
40. Jensen IN, Kristensen G. Alarm treatment: analyses of response and relapse. Scand J Urol Nephrol Suppl. 1999;202:73-5.
41. Moffatt ME, Cheang M. Predicting treatment outcome with conditioning alarms. Scand J Urol Nephrol Suppl. 1995;173:119-22.
42. Ozden C, Ozdal OL, Aktas BK, Ozelci A, Altinova S, Memis A. The efficacy of the addition of short-term desmopressin to alarm therapy in the treatment of primary nocturnal enuresis. Int Urol Nephrol. 2008;40:583-6. Erratum in: Int Urol Nephrol. 2008;40:587.
43. Yucel S, Kol A, Guntekin E, Baykara M. Anticholinergics do not improve cure rate of alarm treatment of monosymptomatic nocturnal enuresis. Urology. 2011;77:721-4.
44. Glazener CM, Evans JH, Peto RE. Alarm interventions for nocturnal enuresis in children. Cochrane Database Syst Rev. 2005;(2):CD002911.
45. Alloussi SH, Mürtz G, Lang C, Madersbacher H, Strugala G, Seibold J, et al. Desmopressin treatment regimens in monosymptomatic and nonmonosymptomatic enuresis: A review from a clinical perspective. J Pediatr Urol. 2011;7:10-20.
46. Tauris LH, Andersen RF, Kamperis K, Hagstroem S, Rittig S. Reduced anti-diuretic response to desmopressin during wet nights in patients with monosymptomatic nocturnal enuresis. J Pediatr Urol. 2012;8:285-90.
47. Rushton HG, Belman AB, Zaontz MR, Skoog SJ, Sihelnik S. The influence of small functional bladder capacity and other predictors on the response to desmopressin in the management of monosymptomatic nocturnal enuresis. J Urol. 1996;156(2 Pt 2):651-5.
48. Sinha R, Raut S. Management of nocturnal enuresis - myths and facts. World J Nephrol. 2016;5:328-38.
49. Vande Walle J, Rittig S, Bauer S, Eggert P, Marschall-Kehrel D, Tekgul S; et al. European Society for Paediatric Urology; European Society for Paediatric Nephrology; International Children's Continence Society. Practical consensus guidelines for the management of enuresis. Eur J Pediatr. 2012;171:971-83. Erratum in: Eur J Pediatr. 2013;172:285. Eur J Pediatr. 2012;171:1005.
50. Keatin JC Jr. Functional nocturnal enuresis. J Manipulative Physiol Ther. 1995;18:44-6.
51. Koşar A, Arikan N, Dinçel C. Effectiveness of oxybutynin hydrochloride in the treatment of enuresis nocturna--a clinical and urodynamic study. Scand J Urol Nephrol. 1999;33:115-8.
52. Austin PF, Ferguson G, Yan Y, Campigotto MJ, Royer ME, Coplen DE. Combination therapy with desmopressin and an anticholinergic medication for nonresponders to desmopressin for monosymptomatic nocturnal enuresis: a randomized, double-blind, placebo-controlled trial. Pediatrics. 2008;122:1027-32.
53. Nevéus T, Läckgren G, Tuvemo T, Olsson U, Stenberg A. Desmopressin resistant enuresis: pathogenetic and therapeutic considerations. J Urol. 1999;162:2136-40.
54. Caldwell PH, Sureshkumar P, Wong WC. Tricyclic and related drugs for nocturnal enuresis in children. Cochrane Database Syst Rev. 2016;(1):CD002117.
55. Glazener CM, Evans JH, Peto RE. Tricyclic and related drugs for nocturnal enuresis in children. Cochrane Database Syst Rev. 2003;(3):CD002117.
56. Gepertz S, Nevéus T. Imipramine for therapy resistant enuresis: a retrospective evaluation. J Urol. 2004;171(6 Pt 2):2607-10.
57. Nevéus T, Tullus K. Tolterodine and imipramine in refractory enuresis; a placebo-controlled crossover study. Pediatr Nephrol. 2008;23:263-7.
58. Tahmaz L, Kibar Y, Yildirim I, Ceylan S, Dayanç M. Combination therapy of imipramine with oxybutynin in children with enuresis nocturna. Urol Int. 2000;65:135-9.
59. Burke JR, Mizusawa Y, Chan A, Webb KL. A comparison of amitriptyline, vasopressin and amitriptyline with vasopressin in nocturnal enuresis. Pediatr Nephrol. 1995;9:438-40.
60. de Oliveira LF, de Oliveira DM, da Silva de Paula LI, de Figueiredo AA, de Bessa J Jr, de Sá CA, et al. Transcutaneous parasacral electrical neural stimulation in children with primary monosymptomatic enuresis: a prospective randomized clinical trial. J Urol. 2013;190:1359-63.
61. Kamperis K, Van Herzeele C, Rittig S, Vande Walle J. Optimizing response to desmopressin in patients with monosymptomatic nocturnal enuresis. Pediatr Nephrol. 2017;32:217-226.
62. Kamperis K, Rittig S, Bower WF, Djurhuus JC. Effect of indomethacin on desmopressin resistant nocturnal polyuria and nocturnal enuresis. J Urol. 2012;188:1915-22.
63. Kamperis K, Hagstroem S, Faerch M, Mahler B, Rittig S, Djurhuus JC. Combination treatment of nocturnal enuresis with desmopressin and indomethacin. Pediatr Nephrol. 2017;32:627-633.

64. De Guchtenaere A, Vande Walle C, Van Sintjan P, Donckerwolcke R, Raes A, Dehoorne J, Van Laecke E, Hoebeke P, Vande Walle J. Desmopressin resistant nocturnal polyuria may benefit from furosemide therapy administered in the morning. J Urol. 2007;178:2635-9.
65. Lundmark E, Stenberg A, Hägglöf B, Nevéus T. Reboxetine in therapy-resistant enuresis: A randomized placebo-controlled study. J Pediatr Urol. 2016;12:397.e1-397.e5.
66. Berkenwald A, Pires J, Ellsworth P. Evaluating use of higher dose oxybutynin in combination with desmopressin for refractory nocturnal enuresis. J Pediatr Urol. 2016;12:220.e1-6.
67. Chua ME, Silangcruz JM, Chang SJ, Yang SS. Immediate 1-month efficacy o desmopressin and anticholinergic combination therapy versus desmopressin monotherapy in the treatment of pediatric enuresis: A meta-analysis. J Pediat Urol. 2016;12:156.e1-9.
68. Vogt M, Lehnert T, Till H, Rolle U. Evaluation of different modes of combined therapy in children with monosymptomatic nocturnal enuresis. BJU Int. 2010;105:1456-9.
69. Leebeek-Groenewegen A, Blom J, Sukhai R, Van Der Heijden B. Efficacy of desmopressin combined with alarm therapy for monosymptomatic nocturnal enuresis. J Urol. 2001;166:2456-8.
70. Butler RJ, Golding J, Northstone K; ALSPAC Study Team. Nocturnal enuresis at 7.5 years old: prevalence and analysis of clinical signs. BJU Int. 2005;96:404-10.

Correspondence address:
José Murillo B. Netto, MD
Universidade Federal de Juiz de Fora (UFJF) and
Hospital e Maternidade Therezinha de Jesus da
Faculdade de Ciências Médicas e
da Saúde de Juiz de Fora (HMTJ-SUPREMA)
Av. Rio Branco, 2985/605
Juiz de Fora, MG, 36010-012, Brasil
E-mail: jose.murillo@ufjf.edu.br

REVIEW ARTICLE

Vol. 45 (5): 901-909, September - October, 2019
doi: 10.1590/S1677-5538.IBJU.2019.0061

Association between calcitonin receptor gene polymorphisms and calcium stone urolithiasis: A meta-analysis

Jiaxuan Qin [1], Zonglong Cai [2], Jinchun Xing [1], Bo Duan [1], Peide Bai [1]

[1] Department of Urology Surgery, the First Affiliated Hospital of Xiamen University; Center of Diagnosis and Treatment of Urinary System Diseases, the First Affiliated Hospital of Xiamen University; the Key Laboratory of Urinary Tract Tumors and Calculi of Xiamen City, the First Affiliated Hospital of Xiamen University. Xiamen, Fujian, China; [2] The First Clinical Medical School of Fujian Medical University. Xiamen, Fujian, China

ABSTRACT

Purpose: It has been reported that calcitonin receptor (CALCR) gene polymorphisms might be associated with calcium stone urolithiasis. Owing to mixed and inconclusive results, we conducted a meta-analysis to summarize and clarify this association.
Materials and Methods: A systematic search of studies on the association between CALCR gene polymorphisms and calcium stone urolithiasis susceptibility was conducted in databases.
Results: Odds ratios and 95% confidence intervals were used to pool the effect size. Five articles were included in our meta-analysis.
Conclusions: CALCR rs1801197 might be associated with increased risk of calcium stone urolithiasis. There is insufficient data to fully confirm the association between CALCR rs1042138 and calcium stone urolithiasis susceptibility. Well-designed studies with larger sample size and more subgroups are required to validate the risk identified in the current meta-analysis.

ARTICLE INFO

Jinchun Xing
http://orcid.org/0000-0002-1651-5223

Keywords:
Receptors, Calcitonin; Urolithiasis; Meta-Analysis [Publication Type]

Int Braz J Urol. 2019; 45: 901-9

Submitted for publication:
January 29, 2019

Accepted after revision:
May 06, 2019

Published as Ahead of Print:
July 15, 2019

INTRODUCTION

Urolithiasis is a relatively common health problem which is likely associated with the effects of multiple genes in combination with lifestyles and environmental factors (1, 2). Majority of urolithiasis is calcium stone. The calcitonin receptor (CALCR) is a 7-pass transmembrane G-protein--coupled receptor which reacts in response to the calcium metabolism-related hormone calcitonin (3). By binding calcitonin receptor on the osteoclasts in the bone and the renal tubular cells, calcitonin causes inhibition of bone resorption and lowers serum calcium concentration (4). Therefore, calcitonin receptor gene polymorphisms might cause calcium metabolic disorders. Some calcitonin receptor gene polymorphisms, like SNP rs1801197 (1377C>T) and rs1042138 (3'UTR+18C>T), have

shown association with bone mineral density (5, 6). The SNP rs1801197 alters the encoded amino acid from proline to leucine (7). Researchers have also investigated the association of those SNPs with urolithiasis.

Association between calcitonin receptor gene polymorphisms and calcium stone urolithiasis susceptibility has been studied in several populations. Sample sizes in these studies are relatively small. Therefore, we decided to perform a meta-analysis to estimate it.

MATERIALS AND METHODS

Identification of eligible studies

A systematic search in Pubmed by Medline, Embase, Cochrane Library, clinicaltrials.gov, CNKI (China National Knowledge Infrastructure) databases were carried out by two independent investigators. The following terms were used: "calcitonin Receptor OR CTR OR CALCR" AND "stone OR calculus OR calculi OR lithiasis OR Nephrolithiasis OR urolithiasis" AND "polymorphisms OR polymorphism", without any limitation applied. The last search update was performed on August 3, 2017. References of related studies and reviews were also manually searched for additional studies.

Inclusion and exclusion criteria

Studies selected in this meta-analysis should have met the following inclusion criteria: (1) evaluation of the association between calcitonin receptor gene polymorphisms and calcium stone urolithiasis susceptibility; (2) case-control study; (3) studies focusing on tissues of human beings; (4) detailed genotype data could be acquired to calculate the odds ratios (ORs) and 95% confidence intervals (95% CIs). Exclusion criteria: (1) duplication of previous publications (when there were multiple publications from the same population, only the largest study was included); (2) comment, review and editorial; (3) study without detailed genotype data; (4) GWAS; (5) studies focusing on cell lines. Dissertation thesis were included in the analysis.

Study selection was achieved by two investigators independently, according to the inclusion and exclusion criteria by screening the title, abstract and full-text. Any dispute was solved by discussion.

Data extraction

Two investigators extracted data of the eligible studies independently. In the case of a conflict, an agreement was reached by discussion. If the dissent still existed, the third investigator would be involved to adjudicate the disagreements. Try to contact the author by email for detailed genotype data.

The following contents were collected: first author's surname, year of publication, chemical composition of urinary calculi, the characteristics of cases and controls, source of control groups, country of origin, the detective sample, ethnicity, genotyping method, Hardy-Weinberg equilibrium, number of cases and controls for each genotype.

Methodological quality assessment

The qualities of included studies were evaluated independently by two investigators according to Newcastle-Ottawa Scale (NOS) (8) and the most important factor was "age, gender and country". Quality scores range from 0 to 9, and higher scores meant better quality of the study. Disagreement was resolved through discussion.

Statistics analysis

Our meta-analysis was conducted according to the PRISMA checklists (9). Hardy-Weinberg equilibrium (HWE) was evaluated for each study by Chi-square test in control groups, and $P < 0.05$ was considered as a significant departure from HWE. OR and 95% CIs were calculated to evaluate the strength of the association between calcitonin receptor gene polymorphisms and calcium stone urolithiasis susceptibility. Pooled ORs were obtained from combination of single studies by allelic comparison (T vs. C), dominant model (CT+TT vs. CC), recessive model (TT vs. CC+CT), homozygote comparison (TT vs. CC) and heterozygote comparison (CT vs. CC), respectively. The statistical significant level was determined by Z-test with P value less than 0.05.

Heterogeneity was evaluated by Q-test and I^2 index (10). When Q-test's P-value was less than 0.10 and/or I^2 index was more than 50%, the random-

-effects model (DerSimonian and Laird method) was used; otherwise, the fixed-effects model (Mantel and Haenszel method) was conducted (11).

Sensitivity analyses were performed towards each genetic model to evaluate effect of each study on combined ORs by sequentially excluding each study in total and in any subgroup including more than two studies. Potential publication bias was checked by Begg's funnel (12) plots and Egger's test (13). An asymmetric plot, the P value of Begg's test (P_B) less than 0.05, and the P value of Egger's test (P_E) less than 0.05 was considered a significant publication bias. All statistical analyses were performed with Stata 12.0 software (StataCorp, College Station, Texas, USA). A two-tailed P <0.05 was considered significant except for specified conditions, where a certain P value was declared.

RESULTS

Characteristics of studies

A total of 70 articles were acquired from databases (Pubmed by Medline=6, Embase=9, Cochrane=0, clinicaltrials.gov=0, CNKI=55, other sources (from manually search) =0). The selection process is shown in Figure-1. Three full--text articles were excluded (1 duplicate study (14), 1 GWAS (15), 1 not about urolithiasis (16)). Finally, 5 articles (17-21) were included in our meta-analysis. The characteristics of each study are shown in Table-1 and Table-2. Different genotyping methods were utilized including PCR--RFLP, PCR-SSCP and Sequencing. Blood samples were used for genotyping in all studies. The control group of study NO 1.4, 1.5 and 1.5.1 had shown significant departure from HWE.

Overall analyses and Subgroup analyses

Summary results of each genetic model are listed in Table-3. In pediatric urolithiasis subgroup and overall, significantly increased risk of calcium stone urolithiasis was found in CALCR rs1801197 in all genetic models. In adult urolithiasis and its male subgroup, significantly increased risk of calcium stone urolithiasis was found in CALCR rs1801197 in heterozygote comparison (CT vs. CC) and dominant model (CT+TT vs. CC). In adult urolithiasis, significantly increased risk

Figure 1 - Flow Chart of study selection.

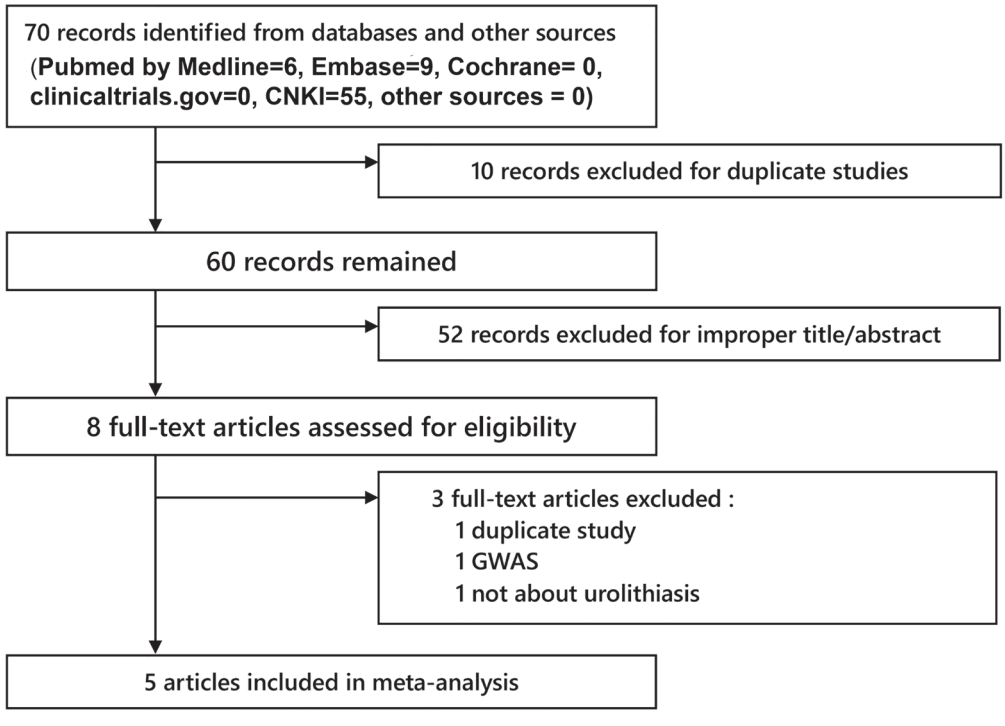


Table 1 - Characteristics of studies included in the meta-analysis.

NO.	Study ID	Year	Country or Area	Ethnicity	Control Type	Genotyping Method	Case			Control			P for HWE*	Quality
	CALCR rs1801197						TT	CT	CC	TT	CT	CC		
1.1	**Bid HK (17)**	2005	Northern India	Indian	PB*	PCR-RFLP	10	24	16	4	26	30	0.604	9
1.2	**Song GL (18)**	2014	China	Uyghur	PB	PCR-RFLP	4	23	62	0	21	100	0.296	9
1.3	**Chen WC (19)**	2001	Taiwan	Asian	PB	PCR-RFLP	2	25	75	0	6	99	0.763	7
1.3.1	Male						2	18	56	0	0	60	NA*	
1.3.2	Female						0	7	19	0	6	39	0.632	
1.4	**Shakhssalim N (20)**	2014	Iran	Caucasian	PB	PCR-SSCP	17	47	37	17	38	51	0.039*	8
1.5	**Mitra P (21)**	2017	West India	Indian	PB	Sequencing	24	78	50	6	69	69	0.026	9
1.5.1	Male						16	59	37	2	47	56	0.026	
1.5.2	Female						8	19	13	3	22	14	0.159	
	CALCR rs1042138						TT	CT	CC	TT	CT	CC		
2.1	**Shakhssalim N (20)**	2014	Iran	Caucasian	PB	PCR-SSCP	1	27	73	0	0	101	NA	8
2.2	**Mitra P (21)**	2017	West India	Indian	PB	Sequencing	6	51	95	2	49	93	0.110	9
2.2.1	Male						6	40	66	2	34	69	0.344	
2.2.2	Female						0	11	29	0	15	24	0.137	

*** HWE:** Hardy–Weinberg equilibrium; **PB:** population-based; **NA:** not available.
* Results with statistical significant difference were marked as bold.

Table 2 - Characteristics of cases and controls.

Study ID	Case	Control
Bid HK (17)	50 **pediatric patients** (age range 2–14 years) with renal stones from the Northern Indian states of Uttar Pradesh and Bihar. Patients who had history of bowel disease, renal tubular acidosis and urinary tract anomalies were excluded. Stone composition was verified using Xray crystallography. **38 patients had sufficient specimens available for stone composition analysis: 64% were whewellite, 28% whewellite and weddellite, and 8% whewellite and uricite.**	60 healthy children (age range 4–16 years) who had no history of stone disease were drawn from the general population taking care to match age, socioeconomic status, dietary habits, religion and gender.
Song GL (18)	89 **pediatric patients** (age range 0.5–7 years) with upper urinary tract calculi from southern Xinjiang of China. Patients who had rickets, thyroid dysfunction, parathyroid dysfunction, symptomatic urinary tract infection, ureteropelvic junction obstruction, renal insufficiency, renal tubular acidosis, tumor, osteoporosis, taking vitamin D or calcium supplements were. All stone composition was **calcium stone**, such as calcium oxalate, calcium phosphate, calcium carbonate, etc.	121 healthy children (same age range) who had no history of stone disease were drawn from the general population of southern Xinjiang taking care to match age, gender, socioeconomic status, dietary habits and religion.
Chen WC (19)	102 **adult patients** (age range 23–76 years) with **recurrent calcium oxalate stones** who had been treated in the department of urology were included. Patients who showed symptoms of urinary tract infections during the period of stone treatment were excluded. Stone composition was verified by infrared spectroscopy revealing calcium oxalate monohydrate, dihydrate, or a combination of the two.	105 healthy volunteers (age range 40–87 years) with no familial history of stone disease, or renal calcification (following renal ultrasonography tests, as well as routine tests made from urinary microscopic hematuria).
Shakhssalim N (20)	105 **adult men** (age range 30–55 years) with a history of **recurrent calcium urinary stones** who had at least two recurrent episodes during the past 5 years. Patients with histories of known metabolic, gastrointestinal, hepatic, renal or endocrinological diseases, with any anatomic abnormality or obstruction in the urinary tract, or taking any drugs which may affect urine composition were excluded.	101 adult men (age range 30–55 years) were selected from volunteers who had been referred to the Ophthalmology Clinic of the Labbafinejad hospital, or unrelated healthy friends of the patients who did not express any personal or family history of urolithiasis. Ultrasonographic examination was performed and men with any evidence of urolithiasis were excluded. Other exclusion criteria were similar to those for the case group.
Mitra P (21)	152 **adult patients** (age range 18–75 years) with **calcium containing renal stone(s)** in Kolkata, West Bengal, India. Patients with histories of known metabolic, gastrointestinal, renal, endocrinological disorders or patients taking any drugs like steroids, diuretics were excluded. The composition of stone was analyzed by chemical tests. Only patients with calcium containing kidney stones were included.	144 age and sex matched healthy individuals from the same geographical region and socioeconomic status who was negative in family history for kidney stone. Ultrasonographic examination was performed to confirm no evidence of renal stone.

of calcium stone urolithiasis was also found in CALCR rs1801197 in allelic comparison (T vs. C). No statistically significant changes of calcium stone urolithiasis risk was found in other analyses.

Sensitivity analyses

Sensitivity analyses were performed in any comparison and any subgroup including more than two studies. In adult urolithiasis and its male subgroup, when study NO 1.4 or 1.5 was excluded, statistically different results were obtained in all genetic models of CALCR rs1801197. Overall, when study NO 1.1 or 1.5 was excluded, statistically different results were obtained in recessive model (TT vs. CC+CT) of CALCR rs1801197 (Table-3).

Less than three studies were included in PU, Female of AU, ARU, Male of ARU subgroup of rs1801197, and AU, Male of AU subgroup of rs1042138, so that sensitivity analyses could not be performed.

Other results showed stability in sensitivity analyses (Table-3).

Publication bias

Begg's funnel plot and Egger's test were used to assess the publication bias. Symmetry of funnel plot, P value of Begg's test (P_B) and P value of Egger's test (P_E) were evaluated overall (including studies NO 1.1, 1.2, 1.3, 1.4 and 1.5). No significant publication bias was found in Egger's test in all genetic models of CALCR rs1801197. However, in allelic comparison (T vs. C), heterozygote comparison (CT vs. CC) and dominant model (CT+TT vs. CC) of CALCR rs1801197, study NO 1.3 extended beyond the diagonal line which represents pseudo-95% CI limits about the effect estimate in funnel plot, meanwhile, the u value=1.96 (u=z in Begg's test) in those three genetic models. In Begg's test, 1.96 is a critical value.

DISCUSSION

Overall, we found CALCR rs1801197 was associated with increased risk of calcium stone urolithiasis in homozygote comparison (TT vs. CC), and the results showed stability in sensitivity analyses and no publication bias (Figure-2).

Figure 2 - Forest plot with a fixed effect model for the association between CALCR rs1801197 and calcium stone urolithiasis in homozygote comparison (TT vs. CC). For each study, the estimate of OR and its 95% CI is plotted with a box and a horizontal line. Rhombus: pooled OR and its 95% CI.

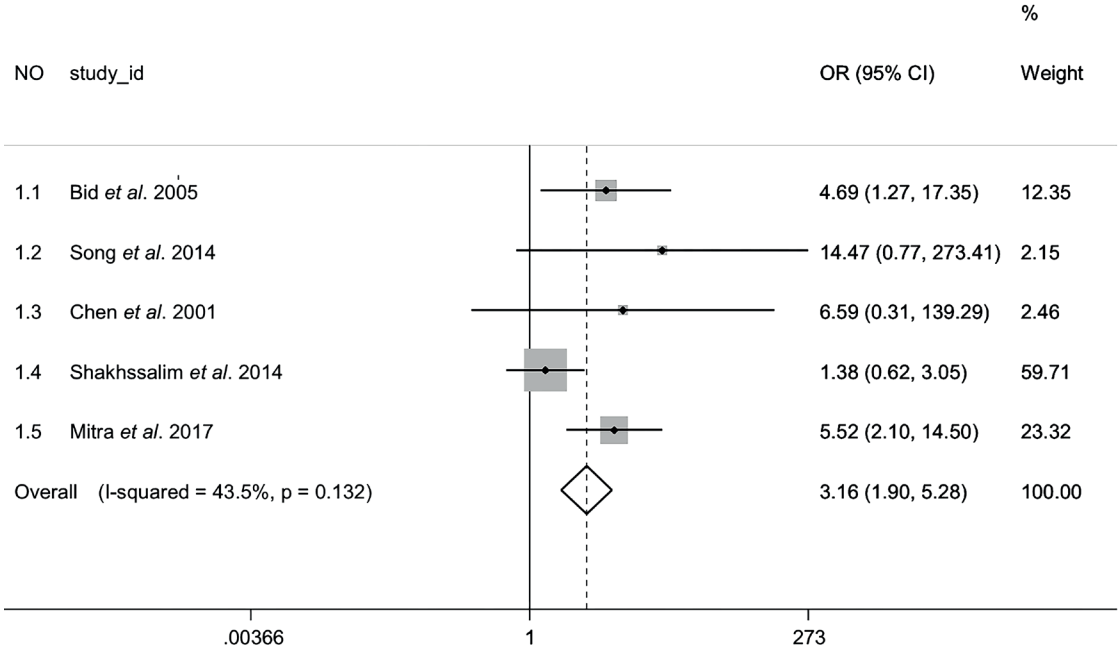


Table 3 - Summary of pooled ORs in the meta-analysis.

	Number	T vs C		TT vs CC		CT vs CC		CT+TT vs CC		TT vs CC+CT	
CALCR rs1801197	(cases/ controls)	OR*(95%CI*)	I2(%)	OR(95%CI)	I2(%)	OR(95%CI)	I2(%)	OR(95%CI)	I2(%)	OR(95%CI)	I2(%)
Overall (1.1*,1.2,1.3,1.4,1.5)	494/536	**1.987(1.401-2.819)***	**56.9**	**3.163(1.895-5.279)**	43.5	**1.910(1.433-2.544)**	30.7	**2.117(1.608-2.787)**	32.2	**2.805(1.195-6.587)***	**51.0**
PU* (1.1, 1.2)	139/181	**2.094(1.394-3.145)**	0.0	**6.140(1.895-19.89)**	0.0	**1.752(1.042-2.947)**	0.0	**2.095(1.270-3.456)**	0.0	**4.631(1.527-14.04)**	0.0
AU* (1.3, 1.4, 1.5)	355/355	**2.052(1.141-3.690)**	**76.9**	2.955(0.937-9.315)	**61.3**	**2.188(1.161-4.126)**	**64.9**	**2.329(1.255-4.323)**	**66.1**	2.305(0.708-7.500)	**66.2**
Male of AU (1.3.1, 1.4, 1.5.1)	289/271	2.036(0.963-4.305)	**76.3**	3.862(0.724-20.61)	**70.0**	**2.234(1.011-4.935)**	**61.2**	**2.437(1.063-5.586)**	**67.1**	2.855(0.557-14.64)	**70.1**
Female of AU (1.3.2, 1.5.2)	66/84	1.541(0.881-2.694)	0.0	NA*	NA	1.335(0.627-2.840)	29.1	1.509(0.722-3.154)	0.0	NA	NA
ARU* (1.3, 1.4)	203/211	2.557(0.605-10.81)	**88.4**	1.584(0.746-3.366)	0.0	2.891(0.918-9.101)	**76.5**	2.920(0.808-10.55)	**82.2**	1.201(0.595-2.421)	1.0
Male of ARU (1.3.1, 1.4)	177/166	5.796(0.141-239.1)	**85.7**	1.552(0.728-3.307)	0.0	6.349(0.218-184.6)	**82.0**	6.576(0.184-234.4)	**84.1**	1.173(0.580-2.371)	0.0
CALCR rs1042138											
AU (2.1, 2.2)	253/245	7.502(0.067-843.2)	**91.1**	3.150(0.738-13.43)	0.0	7.477(0.049-1144)	**92.0**	7.871(0.054-1144)	**91.8**	2.940(0.694-12.46)	0.0
Male of AU (2.1, 2.2.1)	213/206	8.130(0.084-787.7)	**90.4**	3.321(0.773-14.26)	0.0	8.191(0.064-1041)	**91.3**	8.663(0.073-1029)	**91.1**	2.938(0.690-12.51)	0.0

***OR:** Odds ratio; CI: confidence interval; PU: Pediatric Urolithiasis; AU: Adult Urolithiasis; ARU: adult recurrent urolithiasis; NA: not available.

*NO of studies included in the meta-analysis.

*Results with statistical significant difference were marked as bold. Unstable results in sensitivity analyses were marked as italic. Less than three studies were included in PU, Female of AU, ARU, Male of ARU subgroup of rs1801197, and AU, Male of AU subgroup of rs1042138, so that sensitivity analyses could not be performed.

Significantly increased risk was also found in other four genetic models, however, the result in recessive model (TT vs. CC+CT) lacked stability, and we got a critical value of u in Begg's test in allelic comparison (T vs. C), heterozygote comparison (CT vs. CC) and dominant model (CT+TT vs. CC). Study NO 1.3 might play a negative role in the publication bias analyses.

In AU and its male subgroup of rs1801197, significantly increased risk was found in heterozygote comparison (CT vs. CC) and dominant model (CT+TT vs. CC). In AU, significantly increased risk was also found in allelic comparison (T vs. C) of rs1801197. However, those results lacked stability and publication bias analyses could not be performed.

In PU, Female of AU, ARU, Male of ARU subgroup of rs1801197, and AU, Male of AU subgroup of rs1042138, sensitivity analyses and publication bias analyses could not be performed.

Meanwhile, the limitations of this meta-analysis need to be addressed. To date, the number of available studies which could be included in this meta-analysis were small. Data for subgroup analyses were scanty. Sensitivity analyses and publication bias analyses could not be performed in some groups or subgroups. Studies NO 1.4, 1.5 and 1.5.1 had shown significant departure from HWE. Related studies published in other languages or unpublished were possibly missed. With those limitations, the study provided some insights on the potential association between CALCR gene polymorphims and calcium stone urolithiasis.

In conclusion, our results suggested that: CALCR rs1801197 might be associated with increased risk of calcium stone urolithiasis. There is insufficient data to fully confirm the association between CALCR rs1801197 and calcium stone urolithiasis susceptibility in pediatric urolithiasis, adult urolithiasis, adult recurrent urolithiasis subgroup and gender subgroup, and the results should be interpreted with caution. There is insufficient data to fully confirm the association between CALCR rs1042138 and calcium stone urolithiasis susceptibility, and the results should be interpreted with caution. Well-designed studies with larger sample size and more subgroups are required to validate the risk identified in the current meta-analysis.

Funding

This study was funded by:

1) The Science Fund founded by the First Affiliated Hospital of Xiamen University for Young Scholars (Project Number: XYY2016011; Recipient: Jiaxuan Qin).
2) Natural Science Foundation of Fujian (Project Number: 2017D0010; Recipient: Peide Bai).
3) Young and Middle-aged Backbone Talents Training Project of Fujian (Project Number: 2017-ZQN-81; Recipient: Peide Bai).

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Danpure CJ. Genetic disorders and urolithiasis. Urol Clin North Am. 2000;27:287-99, viii.
2. Devuyst O, Pirson Y. Genetics of hypercalciuric stone forming diseases. Kidney Int. 2007;72:1065-72.
3. Lin HY, Harris TL, Flannery MS, Aruffo A, Kaji EH, Gorn A, et al. Expression cloning of an adenylate cyclase-coupled calcitonin receptor. Science. 1991;254:1022-4.
4. Copp DH, Cameron EC, Cheney BA, Davidson AG, Henze KG. Evidence for calcitonin--a new hormone from the parathyroid that lowers blood calcium. Endocrinology. 1962;70:638-49.
5. Zhang H, Tao X, Wu J. Association of calcitonin receptor gene polymorphism with bone mineral density in postmenopausal Chinese women: a meta-analysis. Arch Gynecol Obstet. 2015;291:165-72.
6. Lee HJ, Kim SY, Kim GS, Hwang JY, Kim YJ, Jeong B, et al. Fracture, bone mineral density, and the effects of calcitonin receptor gene in postmenopausal Koreans. Osteoporos Int. 2010;21:1351-60.
7. Nakamura M, Zhang ZQ, Shan L, Hisa T, Sasaki M, Tsukino R, et al. Allelic variants of human calcitonin receptor in the Japanese population. Hum Genet. 1997;99:38-41.
8. [Last accessed on June 21, 2017]. GA Wells, B Shea, D O'Connell, J Peterson, V Welch, M Losos, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. Available from: <http://www.ohri.ca/programs/clinical_epidemiology/nosgen.pdf>

9. Moher D, Liberati A, Tetzlaff J, Altman DG; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. J Clin Epidemiol. 2009;62:1006-12.
10. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med. 2002;21:1539-58.
11. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials. 1986;7:177-88.
12. Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. Biometrics. 1994;50:1088-101.
13. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ. 1997;315:629-34.
14. Aji K, Song GL, Yasen A, Azad B, Tursun H. [Association of vitamin D receptor gene polymorphisms with urolithiasis in Uyghur children from southern Xinjiang, China]. Zhongguo Dang Dai Er Ke Za Zhi. 2012;14:956-9.
15. Franceschini N, Reiner A, Chi T, Stoller M, Kahn A, Carty C, Edwards T, Jackson R. Novel loci for kidney stone using diverse ancestry populations: The women's health initiative study. J Urol. 2012;187:e834-e5.
16. Yang Y, Wang SG, Ye ZQ, Yang WM. Single nucleotide polymorphism of calcitonin receptor gene and idiopathic hypercalciuria. Chinese Journal of Urology 2006;27: 695-8.
17. Bid HK, Chaudhary H, Mittal RD. Association of vitamin-D and calcitonin receptor gene polymorphism in paediatric nephrolithiasis. Pediatr Nephrol. 2005;20:773-6.
18. Song GL, Guo XL, Sun H, Anniwaer Yashen, Wang YJ. Correlation between calcitonin receptor gene polymorphism and upper urinary tract stone in Uyghur children in Xinjiang. Journal of Xinjiang Medical University 2014;37:1172-5.
19. Chen WC, Wu HC, Lu HF, Chen HY, Tsai FJ. Calcitonin receptor gene polymorphism: a possible genetic marker for patients with calcium oxalate stones. Eur Urol. 2001;39:716-9.
20. Shakhssalim N, Basiri A, Houshmand M, Pakmanesh H, Golestan B, Azadvari M, et al. Genetic polymorphisms in calcitonin receptor gene and risk for recurrent kidney calcium stone disease. Urol Int. 2014;92:356-62.
21. Mitra P, Guha M, Ghosh S, Mukherjee S, Bankura B, Pal DK, et al. Association of calcitonin receptor gene (CALCR) polymorphism with kidney stone disease in the population of West Bengal, India. Gene. 2017;622:23-28.

Correspondence address:
Jinchun Xing, MD, PhD
Department of Urology Surgery, the First Affiliated Hospital of Xiamen University
Center of Diagnosis and Treatment of Urinary System Diseases, the First Affiliated Hospital of Xiamen University; the Key Laboratory of Urinary Tract Tumors and Calculi of Xiamen City, the First Affiliated Hospital of Xiamen University
Xiamen, Fujian, China, 361003.
E-mail: jinchun_xing@163.com

ORIGINAL ARTICLE

Vol. 45 (5): 910-915, September - October, 2019
doi: 10.1590/S1677-5538.IBJU.2018.0604

Non-functional paraganglioma of urinary bladder managed by transurethral resection

Baochao Zhang [1], Zhenrui Fu [1], Liwei Liu [1], Baomin Qiao [1], Chunyu Liu [1]

[1] *Department of Urology, Tianjin Institute of Urology, The Second Hospital of Tianjin Medical University, Tianjin, China*

ABSTRACT

Purpose: As a rare bladder tumor, paraganglioma of the urinary bladder (PUB) is frequently misdiagnosed as bladder cancer, particularly for the non-functional type. To date, transurethral resection remains a controversial treatment for non-functional PUB. This study aimed to identify the clinical features, pathological characteristics, prognosis, and safe/effective treatment of non-functional PUB using transurethral resection of the bladder tumor (TURBT).

Materials and Methods: The clinical records, radiological data, pathological characteristics and follow-up times were retrospectively reviewed in 10 patients with clinically and pathologically proven non-functional PUB in our hospital from January 2008 to November 2016. All patients underwent TURBT treatment.

Results: The incidence of non-functional PUB in patients with bladder cancer was 0.17%. The mean age at diagnosis was 44.5 ± 13.6 years (range, 29-70 years), and the patient population had a female: male ratio of 3: 2. No patients had excess catecholamine (CA) whilst four patients had painless hematuria. All neoplasms were completely resected via TURBT. The majority of samples were positive for immunohistochemical markers including chromogranin A (CgA) and Synaptophysin (Syn), but were negative for cytokeratins (CKs). Only a single recurrence was observed from the mean follow-up period of 36.4 ± 24.8 months.

Conclusion: Complete TURBT is a safe and efficient treatment that serves both diagnostic and therapeutic purposes. Histopathological and immunohistochemistry examinations are mandatory for diagnostic confirmation. Long-term follow-up is recommended for patients with non-functional PUB.

ARTICLE INFO

Baomin Qiao
https://orcid.org/0000-0003-1509-9720

Keywords:
Paraganglioma; Urinary Bladder; Transurethral Resection of Prostate

Int Braz J Urol. 2019; 45: 910-5

Submitted for publication:
August 28, 2018

Accepted after revision:
January 30, 2019

Published as Ahead of Print:
March 22, 2019

INTRODUCTION

Paraganglioma of the urinary bladder (PUB) is a rare type of bladder tumor that accounts for approximately 0.06% of bladder tumors, 1% of pheochromocytomas, and 10% of paragangliomas (extra-adrenal pheochromocytoma) (1). PUB is a neuroendocrine neoplasm, which arises from chromaffin cells located in the muscle layer of the bladder wall (2). The majority of PUB is solitary, arising on the dome or the trigone of bladder (3). Based on the content and activity of catechola-

mine (CA) that arise from the tumor, extra-adrenal paraganglioma can be classified as functional (chromaffin) or non-functional (non-chromaffin). The former clinically manifests as hematuria or catecholamine-related symptoms including micturition syncope, hypertension, headache, palpitations and transient hypertension after urination. The latter shows no obvious symptoms, but compression occurs when the tumor becomes large. In 1953, Zimmerman et al. (4) reported the first case of PUB. As the clinical presentation of PUB did not occur during the early disease stages, clinicians were unaware of the tumor, frequently leading to misdiagnosis or missed diagnosis, particularly for the non-functional type.

In this study, we report the clinical features, pathological characteristics, and prognosis of patients diagnosed as non-functional PUB treated by transurethral resection of the bladder tumor (TURBT) in our hospital. This enhances our knowledge and understanding of non-functional PUB and the safety and effectiveness of TURBT.

MATERIALS AND METHODS

From January 2008 to November 2016, 10 patients were diagnosed as non-functional PUB according to postoperative pathologic reports at our hospital. These accounted for approximately 0.17% of all bladder tumors reported during the same term. We retrospectively reviewed the clinical records, operative notes, pathologic reports, and follow-up records of the patients. To evaluate both the position and clinical stage of the tumors, all patients received preoperative abdominal ultrasound, urine cytology and computed tomography (CT) examinations. Cystoscopy was performed in a single case. All neoplasms were completely resected by TURBT. During tumor resection, blood pressure, heart rate, and microcirculation status modestly changed. All surgical specimens were diagnosed by at least two urological pathologists. The pathological tumor stage was estimated according to the Cancer tumor-node-metastasis (TNM) staging system on bladder cancer. Long-term follow-up was performed to evaluate the therapeutic outcome of non-functional PUB. The mean follow-up period was 36.4 ± 24.8 months (range: 8-95 months).

RESULTS

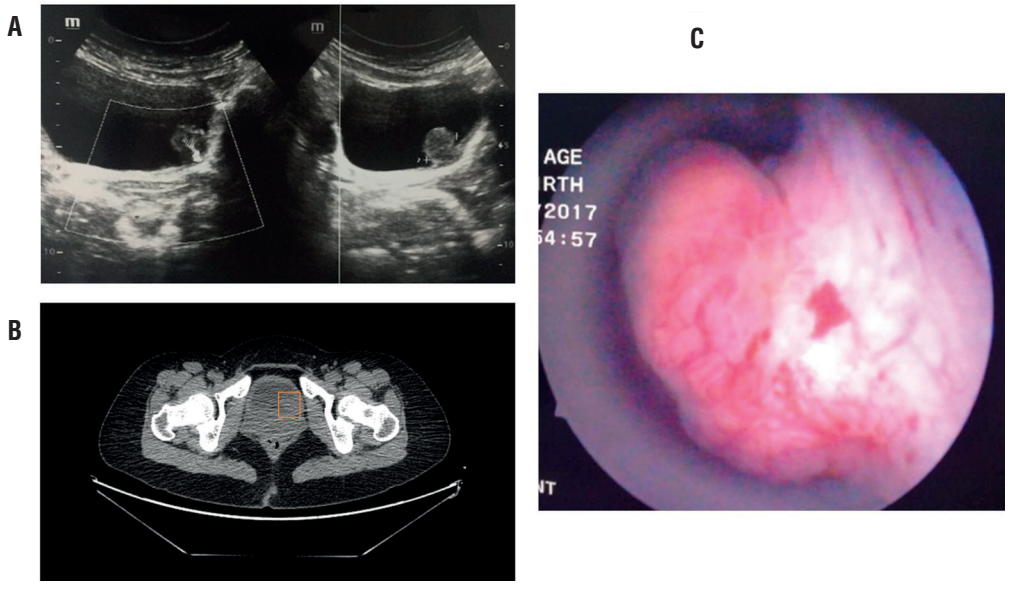
Clinical Features

In this study, 10 cases of non-functional PUB were identified, accounting for 0.17% (10/5680) of patients with bladder cancer. In all patients, tumors were incidentally detected on imaging studies. The mean age of the non-functional PUB patients were 44.5 ± 13.6 years (range 29-70 years), the mean body mass index (BMI) was 23.5 ± 3.6, 4 patients were male and 6 were female, and the mean blood pressure of patients was ≤ 140/90mmHg. All patients had no symptoms of excess CA and four patients had painless hematuria. Tumor sizes ranged from 1.5cm x 1.3cm to 3.5cm x 2.1cm.

Surgical treatment and Pathological findings

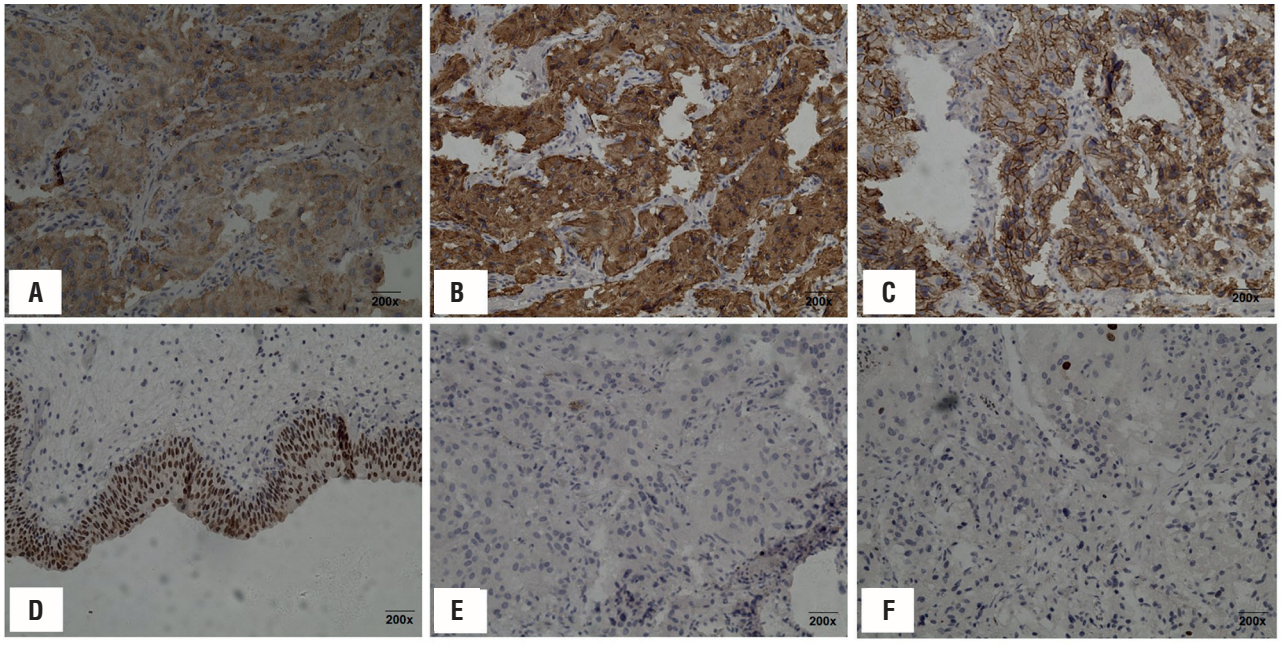
All patients underwent ultrasonography, urine cytology and CT examinations after hospitalization. Most PUBs were localized, solitary, spherical, broad basal tumors and extended towards the bladder cavity according to imaging films (Figures 1A and B). During cystoscopy (Figure-1C), neoplasms were covered by smooth vesical mucosa, with an abundant blood supply and calcification observed in two cases. TURBT was performed in all patients and all surgical margins were negative. The mean surgery time was 39.6 ± 6.7 minutes. All patients had a high tolerance for the operational procedures with no significant postoperative complications. From pathological macroscopic findings, the non-functional PUB specimens were daffodil or dark yellow. Microscopically, tumor tissues were comprised of a dense array that was divided into numerous small nests. Tumor cells were polygonal and fusiform with stippled chromatin and inconspicuous nucleoli. Immunohistochemistry revealed that the majority of tumor cells were positive for chromogranin A (CgA) and Synaptophysin (Syn), but negative for cytokeratin markers (CKs), including CK7 and CK20. Three tumors were positive for GATA3 and two were positive for CD56. The Ki-67 positive percentage of PUB cells was 1% to 30% (Figure-2A-F). According to the pathological reports, 7 cases were at stage T1, and 3 cases were at T2.

Figure 1 - Imaging and cystoscopy.



A) Ultrasound examination showing a hypoechoic mass with abundant blood flow inside in the urinary bladder. B) CT scan showing a polypoid mass (box) in the left wall of the urinary bladder. C) Cystoscopy showing solitary and broad basal neoplasms covered by smooth vesical mucosa in the left anterior wall of the urinary bladder.

Figure 2 - Immunohistochemistry.



A) CgA; B) Syn; C) CD56; D) GATA3; E) CK7; F) Ki67; expressed in PUB cells (IHC 200x).

Follow-up

All patients received no further treatment and were followed up with physical examinations, laboratory tests, and abdominal ultrasound or pelvic cavity CT and cystoscopy examination every 3-6 months, and then annually. The mean postoperative follow-up period was 36.4 ± 24.8 months (range, 8-95 months). Only one case of T2 relapsed on the 37th month and TURBT was again performed in this patient. A single patient died of myocardial infarction 8 months post-operation.

DISCUSSION

Paraganglioma, also named extra-adrenal pheochromocytoma, is a rare tumor that is derived from chromaffin tissues of the sympathetic and parasympathetic nervous system (5). Paragangliomas are mostly located in the abdomen and pelvic cavity, whilst paraganglioma in the bladder is extremely rare, constituting ≤ 1% of all urinary bladder tumors, 6% of all paragangliomas (6). In additon, only 10% of PUB cases are malignant (1). To-date, the diagnosis of malignant paraganglioma relies on the invasiveness of the neoplasm; namely, the primary lesion metastasizes to non-chromaffin tissues or organs, including the lymph nodes, liver, spleen, and bone (7). Honma et al. (8) illustrated that paraganglia can occur in any position of the bladder wall, mostly in the anterior and posterior walls of bladder and is rarely seen in the bladder trigone.

CA is a neurogenic substance consisting of catechol and amino groups, including norepinephrine, adrenaline and dopamine, which are mainly secreted by chromaffin cells. The level of CA is often found to significantly increase in pheochromocytoma and functional paraganglioma due to the symptoms of paroxysmal hypertension. According to catecholamine secretion, PUB can be classified into functional and non-functional types. The majority of PUBs are functional groups characterized by excess catecholamines and symptoms, including paroxysmal hypertension, palpitation, micturition syncope, and headaches (5). In some cases, these features are absent. As such, patients are often misdiagnosed as urothelial cancer during pre-operative evaluations (9, 10). According to previous studies, 61.6% of PUB, confirmed by postoperative pathologic diagnosis, were misdiagnosed as bladder tumors or intramucosal bladder tumors, and only 28.9% were diagnosed prior to surgery (11).

Image analysis and biochemical examinations are crucial for non-functional PUB, as these neoplasms are often found in routine imaging examinations. However, the performance of the tumors are non-specific, making them difficult to distinguish from other bladder tumors. As similar with other types of bladder tumor, conventional imaging studies for bladder paraganglioma often reveal a mural or extramural tumor with wide basilar areas and calcification. Some features that provide clues to bladder paraganglioma include its small intramural lesions that are accentuated in contrast-enhanced MRI and hyperintense on T2 weighted images (12). Metaiodobenzylguanidine (MIGB) is highly specific for functional pheochromocytoma and is often used to distinguish functional and non-functional types. However, this technique is less sensitive than MRI for the detection of paragangliomas (12). The assessment of urinary vanillyl mandelic acid in 24-hour urinary sample contributes to the preoperative diagnosis of functional PUB (13). However, in the absence of the characteristic symptoms of excess CA, these tests are not appreciated by urologists prior to operation. According to previous studies, fluctuating blood pressure and tachyarrhythmia occur in non-functional PUB cases during the TURBT procedure (13). In this study, the blood pressure remained stable at the time of admission and operation. As such, we did not perform a diagnosis of PUB, contrast-enhanced MRI, MIGB examinations, or an investigation of CA metabolites in all patients.

It is difficult to correctly diagnose non-functional PUB pre-operation. Definitive diagnosis is based on histopathology and immunohistochemistry of the excised tumor. From histopathology, paraganglioma cells display characteristic zellballen or nesting patterns with delicate fibrovascular stroma and abundant eosinophilic or amphophilic cytoplasm divided by

delicate vascular stroma (10). Similar to the immunophenotypes of other paraganglioma, positive neuroendocrine markers combined with negative epithelial and mesenchymal markers are of significance to its diagnosis. Neuroendocrine markers including CgA, Syn, CD56 and NSE were strongly expressed in the cytoplasm of tumor cells, whilst supporting cells were positively stained for S-100 (1, 14). A metastatic lesion confirmed by pathology is critical to malignant diagnosis. The differential diagnoses of PUB includes urothelial carcinoma, metastatic renal cell carcinoma, prostatic carcinoma, malignant melanoma, and granular cell tumors (10). Histological appearance and immune profiles help to distinguish PUB from other differential diagnoses.

At present, there are no uniform standard treatment options for PUB. The most effective therapy for local PUB remains complete resection. Various surgical options are available including transurethral resection, partial cystectomy and radical cystectomy. Specialists have indicated that partial cystectomy is the mainstream treatment for the disease (15). However, endourethral surgeries, including electro-excision and laser resection have been reported for the treatment of non-functional PUB and lead to the same therapeutic effect as partial cystectomy (16). Katiyar et al. (13) reported 2 non-functional PUB patients who underwent TURBT in which no recurrence after follow-up for 6 and 10 months occurred, indicating that TURBT is an optional and effective treatment for non-functional PUB. In contrast, it has been suggested that resection rarely excises all the tumor residue as the neoplasms often invade the submucosa and muscularis of the urinary bladder, leading to potential tumor recurrence (17). In this study, we successfully treated all patients with PUB with TURBT, and most displayed no recurrence or metastasis after long-term follow-up. Therefore, the complete resection of the tumors through the transurethral approach is a curative option for patients with non-functional PUB, and may represent the mainstream future treatment.

In general, PUB is a rare tumor with uncertain biological behavior, but most PUBs have a good prognosis and slow development. However, paraganglioma has a tendency for recurrence and metastasis, and no standard reference of the duration of follow-up has been reported. Although Beilan et al. (15) indicated that a long-term follow-up is not necessary in benign and local PUB, it has been reported that the rate of local recurrence ranges from 5 to 15%, and that metastasis can occur 20 to 40 years post-surgery (7, 13, 18). As such, Katiyar et al. (13) argued that even non-functional cases should be followed up for an extended time period. In this study, all patients had regular follow-up and only a single recurrence occurred. Despite this, we recommend that long-term periodical CA/metabolite testing, and image-based examinations are performed since the prognosis of PUB remains poorly established.

The limitation of this study includes the lack of case-control studies owing to the unique nature of PUB. However, we are confident that the study holds significance for urologists and enhances our understanding of the diagnosis and treatment of non-functional PUB.

CONCLUSIONS

Despite its controversial nature, complete transurethral resection of bladder tumor is a safe and curative approach that serves both diagnostic and therapeutic purposes, and avoids the need for partial or radical cystectomy. Histopathological examination and immunohistochemistry are mandatory for a definitive diagnosis, and confirmation of a metastatic lesion through pathology provides the only definite evidence of malignancy in paraganglioma. Regular follow-up through CA and metabolite testing, combined with image-based examinations are recommended to fully eliminate recurrence in these patients.

COMPLIANCE WITH ETHICAL STANDARDS

The research was approved by the Research Ethics Committee of the Second Hospital of Tianjin Medical University. Informed consents were obtained from the participants. The leader of the Second Hospital of Tianjin Medical University and the ethics committees made an agreement on this research and approved this consent procedure.

ACKNOWLEDGEMENTS

To the second hospital of Tianjin Medical University, the Department of Urology for their assistance in the writing of our report.

Baochao Zhang and Zhenrui Fu contributed equally to this work and should be considered co-first authors.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Zhai H, Ma X, Nie W, Li H, Peng C, Li X, et al. Paraganglioma of the Urinary Bladder: A Series of 22 Cases in a Single Center. Clin Genitourin Cancer. 2017;15:e765-e771.
2. Siatelis A, Konstantinidis C, Volanis D, Leontara V, Thoma-Tsagli E, Delakas D. Pheochromocytoma of the urinary bladder: report of 2 cases and review of literature. Minerva Urol Nefrol. 2008;60:137-40.
3. Lazareth H, Cohen D, Vasiliu V, Tinel C, Martinez F, Grünfeld JP, et al. Paraganglioma of the bladder in a kidney transplant recipient: A case report. Mol Clin Oncol. 2017;6:553-5.
4. Zimmerman IJ, Biron RE, Macmahon HE. Pheochromocytoma of the urinary bladder. N Engl J Med. 1953;249:25-6.
5. Deng JH, Li HZ, Zhang YS, Liu GH. Functional paragangliomas of the urinary bladder: a report of 9 cases. Chin J Cancer. 2010;29:729-34.
6. Pastor-Guzmán JM, López-García S, Giménez-Bachs JM, Ruíz-Mondejar R, Cañamares-Pabolaza L, Atiénzar-Tobarra M, et al. Paraganglioma of the bladder: controversy regarding treatment. Urol Int. 2004;73:270-5.
7. Huang KH, Chung SD, Chen SC, Chueh SC, Pu YS, Lai MK, et al. Clinical and pathological data of 10 malignant pheochromocytomas: long-term follow up in a single institute. Int J Urol. 2007;14:181-5.
8. Honma K. Paraganglia of the urinary bladder. An autopsy study. Zentralbl Pathol. 1994;139:465-9.
9. Lai Y, Chen D, Yu Z, Ni L, Yang S. Non-functioning paraganglioma of the urinary bladder: A case report and review of the literature. Oncol Lett. 2014;7:891-3.
10. Zhou M, Epstein JI, Young RH. Paraganglioma of the urinary bladder: a lesion that may be misdiagnosed as urothelial carcinoma in transurethral resection specimens. Am J Surg Pathol. 2004;28:94-100.
11. Iwamoto G, Kawahara T, Tanabe M, Ninomiya S, Takamoto D, Mochizuki T, et al. Paraganglioma in the bladder: a case report. J Med Case Rep. 2017;11:306.
12. Adraktas D, Caserta M, Tchelepi H. Paraganglioma of the urinary bladder. Ultrasound Q. 2014;30:233-5.
13. Katiyar R, Dwivedi S, Trivedi S, Patne SC, Dwivedi US. Non-Functional Paraganglioma of the Urinary Bladder Treated by Transurethral Resection: Report of Two Cases. J Clin Diagn Res. 2016;10:XD01-XD03.
14. Kovacs K, Bell D, Gardiner GW, Honey RJ, Goguen J, Rotondo F. Malignant paraganglioma of the urinary bladder: Immunohistochemical study of prognostic indicators. Endocr Pathol. 2005;16:363-9.
15. Beilan JA, Lawton A, Hajdenberg J, Rosser CJ. Pheochromocytoma of the urinary bladder: a systematic review of the contemporary literature. BMC Urol. 2013;13:22.
16. Yang Y, Wei ZT, Lu JS, Zu Q, Wang H, Zhang X. Transurethral partial cystectomy with 2 μm thulium continuous wave laser in the treatment of bladder pheochromocytoma. J Endourol. 2012;26:686-90.
17. Pahwa HS, Kumar A, Srivastava R, Misra S, Goel MM. Urinary bladder paraganglioma-a case series with proposed treating algorithm based on our experience and review of literature. Indian J Surg Oncol. 2013;4:294-7.
18. Hanji AM, Rohan VS, Patel JJ, Tankshali RA. Pheochromocytoma of the urinary bladder: a rare cause of severe hypertension. Saudi J Kidney Dis Transpl. 2012;23:813-6.

Correspondence address:
Baomin Qiao, MD
Department of Urology
Tianjin Institute of Urology
The Second Hospital of Tianjin Medical University
Pingjiang Road 23, Hexi District, Tianjin, 300211, China
Telephone: + 86 022 8832-9692
E-mail: qiaobaomin2@126.com

ORIGINAL ARTICLE

Vol. 45 (5): 916-924, September - October, 2019
doi: 10.1590/S1677-5538.IBJU.2018.0535

Biological roles of filamin a in prostate cancer cells

Xue-Chao Li [1], Chuan-Xi Huang [2], Shi-Kui Wu [1], Lan Yu [3], Guang-Jian Zhou [3], Li-Jun Chen [1]

[1] Department of Urology, the Fifth Medical Center, Chinese PLA General Hospital, Beijing, China; [2] College of Life Science, Hebei University, Hebei, China; [3] Laboratory of Medical Molecular Biology, Beijing Institute of Biotechnology, Beijing, China

ABSTRACT

Objective: This study aims to investigate the association of filamin A with the function and morphology of prostate cancer (PCa) cells, and explore the role of filamin A in the development of PCa, in order to analyze its significance in the evolvement of PCa.
Materials and Methods: A stably transfected cell line, in which filamin A expression was suppressed by RNA interference, was first established. Then, the effects of the suppression of filamin A gene expression on the biological characteristics of human PCa LNCaP cells were observed through cell morphology, in vitro cell growth curve, soft agar cloning assay, and scratch test.
Results: A cell line model with a low expression of filamin A was successfully constructed on the basis of LNCaP cells. The morphology of cells transfected with plasmid pSilencer-filamin A was the following: Cells were loosely arranged, had less connection with each other, had fewer tentacles, and presented a fibrous look. The growth rate of LNCap cells was faster than cells transfected with plasmid pSilencer-filamin A ($P < 0.05$). The clones of LNCap cells in the soft agar cloning assay was significantly fewer than that of cells stably transfected with plasmid pSilencer-filamin A ($P < 0.05$). Cells stably transfected with plasmid pSilencer-filamin A presented with a stronger healing and migration ability compared to LNCap cells (healing rate was 32.2% and 12.1%, respectively; $P < 0.05$).
Conclusion: The expression of the filamin A gene inhibited the malignant development of LNCap cells. Therefore, the filamin A gene may be a tumor suppressor gene.

ARTICLE INFO

Li-Jun Chen
https://orcid.org/0000-0002-3012-4018

Keywords:
Prostatic Neoplasms; Filamins; RNAi Therapeutics

Int Braz J Urol. 2019; 45: 916-24

Submitted for publication:
September 20, 2018

Accepted after revision:
April 14, 2019

Published as Ahead of Print:
May 22, 2019

INTRODUCTION

The incidence of prostate cancer (PCa) is increasing globally. In Europe and the United States, PCa has the highest incidence among malignant tumors in men, accounting for 25% of malignant tumors in men; and its mortality rate ranks second in men with malignant tumors (1, 2). Approximately 40.000 American men die from PCa annually (3). Most of the newly diagnosed PCa patients have low-risk or benign tumors (4). However, there are still approximately 20-30% of patients with localized PCa who have high-risk tumors (5).

PCa is caused by the transformation and canceration of prostatic epithelial cells. This is a multi-step, multi-stage process (6, 7). Androgen receptors (ARs) play a key role in the deve-

lopment and growth of the prostate gland (8). It also plays an important role in the growth, survival, apoptosis, metastasis and differentiation of PCa cells (9). Filamin A is a 280kDa cytoskeletal protein, which consists of two large fragments of 170kDa and 110kDa, respectively; and the latter can be divided into two parts: 90kDa and 20kDa (10). The 90kDa part can bind with ARs and affect the chromosomal translocation of the nucleus (11, 12). Savoy et al. confirmed that filamin A could regulate AR Nrdp1 in PCa, and affect the growth and survival of PCa (13). Bismar et al. used 12 molecules screened by proteomics combined with gene chip analysis technology as the combination of candidate molecular markers for PCa progression, which were ordered according to the significance of difference in clinical specimens; and the result revealed that filamin A ranked first (14). The Filamin A gene has also been proven to be associated with PCa metastasis. Sun et al. found that filamin A could inhibit the metastasis and invasion of PCa by regulating the expression of MMP-9 (15). Mooso et al. also verified that the level of filamin A in the nucleus and cytoplasm was correlated to the metastatic ability of PCa (16). Narain et al. considered that Filamin-B instead of filamin A could be used as a biomarker for PCa (17). The degree of damage to PCa is positively correlated with its disease progression. How to effectively delay the conversion of hormone-sensitive PCa to castration-resistant prostate cancer (CRPC) is more important in the overall treatment of PCa (18). The Filamin A gene has been shown to be involved in the development and progression of PCa, and further studies of the biological function of Filamin A may provide a new perspective for the overall treatment of prostate cancer.

In order to reveal the realistic biological function of the filamin A gene, in the present study, a stably transfected cell line, in which the expression of filamin A was suppressed by RNA interference, was first established, and the effect caused by filamin A expression levels on cell characteristics was observed, thereby providing experimental data for further research on the function of the filamin A gene.

Experimental materials

Cell lines and plasmids

Human PCa cell line LNCaP and plasmid pSilencer-filamin A were previously preserved in our Department.

Major reagents

The RPMI1640 and trypsin were purchased from GIBCO®. The quality fetal bovine serum was purchased from Hyclone® (USA). The calf serum was purchased from Sijiqing Bioengineering Co., Ltd.® (Hangzhou, China). HEPES was purchased from Amersham Life Science®. The cell transfection reagent Lipofectamine 2000 was purchased from Invitrogen® (USA). Dimethyl sulfoxide (DMSO) was purchased from Sigma® (USA). The western blot color kit was purchased from Pierce®. The nitrocellulose membrane was purchased from Bio-Rad®. The filamin A antibody was purchased from Chemicon®. Other reagents were analytical pure products made in China. The eukaryotic transfection reagent Lipofectamine 2000 was purchased from Vigorous Biotechnology®.

Major instruments

The Trans-Blot SD semi-dry electric transfer apparatus and Steri-cycle carbon dioxide incubator were purchased from Thermo Electron Corporation®. The XDS-1B microscope was purchased from Chongqing Optical Instrument Co. Ltd®. The IX70 fluorescent inverted microscope was purchased from Olympus®. The 3K18 refrigerated centrifuge was purchased from Sigma® (USA). The PCR machine was purchased from Biometra®. The-EL311SX ELISA kit was purchased from BioTek® (US). The clean bench was purchased from Beijing Semiconductor Equipment First Factory®.

Experimental methods

Cell cultivation

(1) The preserved cells were taken out from the liquid nitrogen or -80°C refrigerator. The frozen storage tube that contained the cells were rapidly placed in a water bath at 37-42°C, and shaken slightly to promote it to melt.

(2) Cells were gently suspended with RPMI1640 containing 8% fetal bovine serum, and transferred to the centrifuge tube.

(3) Cells were centrifuged at 1.000rpm, and the supernatant was discarded.

(4) Cells were added with 5mL of RPMI1640 containing 8% Hyclone fetal bovine serum, transferred in a cell culture flask, and cultured in an incubator at 37°C with 5% CO2 and saturated humidity.

Transfection of eukaryotic cells

(1) Cells were first inoculated on six--well plates, and cultured until approximately 75% fusion.

(2) Plasmid DNA was diluted with serum--free RPMI1640 medium, and the Lipofectamine2000 was diluted with the same medium. The above two were mixed and placed at room temperature for 20 minutes.

(3) The cells were transferred to a serum--free medium. After 20 minutes, the mixture was added to the cell medium.

(4) These were converted into normal medium after 4-6 hours.

(5) Cells were cultured in an incubator at 37°C with 5% CO2 and saturated humidity.

(6) Cells were screened by hygromycin until the formation of cell clones could be visually observed. Then, cells were extracted by capillary and subjected to amplification culture.

Cell morphology

(1) Cells were inoculated in culture dishes at a lower density, and placed in an incubator for culture at 37°C with 5% CO2 and saturated humidity.

(2) The morphological changes in cells were observed, photographed and recorded.

Western blot

(1) These cells were obtained in the normal state, and the protein was extracted.

(2) After the protein was processed, the absorption value of the optical density was read in the microplate reader at a wavelength of 562nm, and the protein concentration was calculated based on the formula: y=1.1308x+0.0802.

(3) The protein was loaded and processed. Then, coloration, tabletting and development were performed using the chemiluminescence method.

Tetrazolium salt colorimetry assay

(1) Cells were inoculated in 96-well plates at a density of 2.000 cells/well, and eight duplicated wells were set.

(2) Cells were allowed to stand overnight to adhere to the wall, added with 20μl of tetrazolium salt MTT (5mg/mL dissolved in phosphate buffered solution [PBS]), and cultured in an incubator at 37°C with 5% CO2 and saturated humidity for four hours.

(3) The supernatant was discarded and 150μl of DMSO was added to dissolve it.

(4) The OD490 value was read on the microplate reader at a 490nm wavelength, which was defined at the OD490 value of day one.

(5) Then, the OD490 value was read daily, the ratio of the value of day n to the value of day one was defined at the relative value of day n. Finally, the growth curve was drawn based on the relative value and analyzed.

Agar colony forming experiment

(1) Preparation of the bottom gel: One volume of 5% agar was mixed with nine volumes of preheated medium, and were poured into six-well plates.

(2) Cells were inoculated on six-well plates according to gradients of 2.000, 4.000 and 6.000 cells/well, and mixed with a certain proportion of agar. Each cell density occupied two wells.

(3) Cells were placed in an incubator and cultured at 37°C with 5% CO2 and saturated humidity for three weeks.

(4) Clones with diameters of >75μM or >50 cells were counted.

Cell scratch test

(1) Photographing and measurement of the scratch width under a microscope.

(2) Cells were placed in an incubator at 37°C with 5% CO2 and saturated humidity for culture. Then, continuous observation was performed, cells were photographed with a camera, and the width of the scratch was measured after healing.

(3) The scratch repair rate was calculated according to the formula: scratch repair rate= (initial scratch width-scratch width after healing) /initial scratch width ×100%.

Data analysis

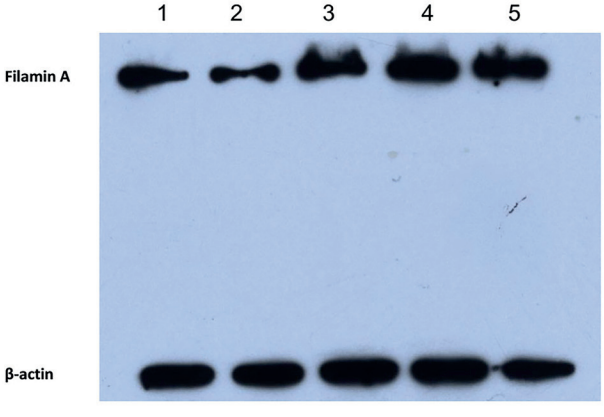
All statistics analysis of the experimental data were processed and analyzed using SAS software. Data obtained from MTT assay and soft agar colony formation experiments were analyzed using one-way analysis of variance. Data obtained from in vitro cell migration experiments were analyzed using the t-test in the SAS software.

Experimental results

The establishment of the PCa cell line where the expression of filamin A was inhibited

The PCa LNCaP cell line was transfected with recombinant plasmids of pSilencer-filamin A and pSilencer-negative, which were screened and identified by hygromycin and Western blot. Then, the PCa cell line, in which the expression of filamin A was effectively inhibited, was obtained (Figure-1).

Figure 1 - The expression of Filamin A in LNCaP cell line that was transfected with different plasmids by Western-blot. Stably transfected pSilencer-Filamin A recombinant plasmid LNCaP cell line (1, 2). Stably transfected pSilencer-negative recombinant plasmid LNCaP cell line (3, 4). Normal cell line (5). The result of 2 is significant.



Change in cytomorphology

The cells in logarithmic growth phase in each group were observed under light microscopy. These results revealed the following LNCap cell phenotypic characteristics: cells were closely arranged, had rich contact with each other, and were thick and had increased adherent antennae (Figure-2A). The morphology of cells transfected with plasmid pSilencer-filamin A: These cells were loosely arranged with less connection with each other and fewer tentacles, presenting a fibrous look (Figure-2B).

In vitro growth curve of cells

In order to investigate the effect of the expression of filamin A gene on the proliferation of LNCap cells, the in vitro growth of LNCap cells and cells transfected with plasmid pSilencer-filamin A were observed by tetrazolium salt colorimetry assay. In order to eliminate the error caused by the cell count, the count of cells on day zero was set as 100%, and the relative number of cells in each group on each day (%) was calculated respectively. That is, the relative cell number (%) = the OD value on day n/ the OD value on day 0×100 (n=1, 2, 3). With time as the abscissa and the relative cell number (%) as the ordinate, the cell growth curve was drawn (Figure-3). The results of this test revealed that the growth rate of LNCap cells was significantly faster than of cells transfected with plasmid pSilencer-filamin A, and difference was statistically significant ($P < 0.05$). The growth rate of LNCap cells on day five and seven was 1.5 times faster than that of cells transfected with plasmid pSilencer-filamin A. Under the same culture condition, the number of proliferated cells transfected with plasmid pSilencer-filamin A was less than that of LNCap cells. This appears to be in contradiction with the phenomenon in our clinical practice, in which the higher the malignant degree is, the larger the tumor tissue in the PCa patient becomes.

Soft agar cloning assay

The anchorage-independent growth ability of cells on soft agar is an important indicator to measure the malignant degree of cells under the condition of in vitro culture. Therefore, the ability of these two groups of cells to grow on soft agar was measured. Under a low magnification microscope, the number of clones with more than 50 cells was counted, and the effect of different inoculation densities on cloning efficiency was compared. These results reveal that no matter how many cells were inoculated, the clones of LNCap cells in the soft agar cloning assay was significantly fewer than that of cells

Figure 2 - Changes in cell morphology. Morphological characteristics of Normal LNCaP cell line (A). Morphological characteristics of LNCap cell line that transfected pSilencer-Filamin A (B), compared to LNCaP , the cells are loosely arranged, with less contact with each other, fibrous, and with fewer tentacles.

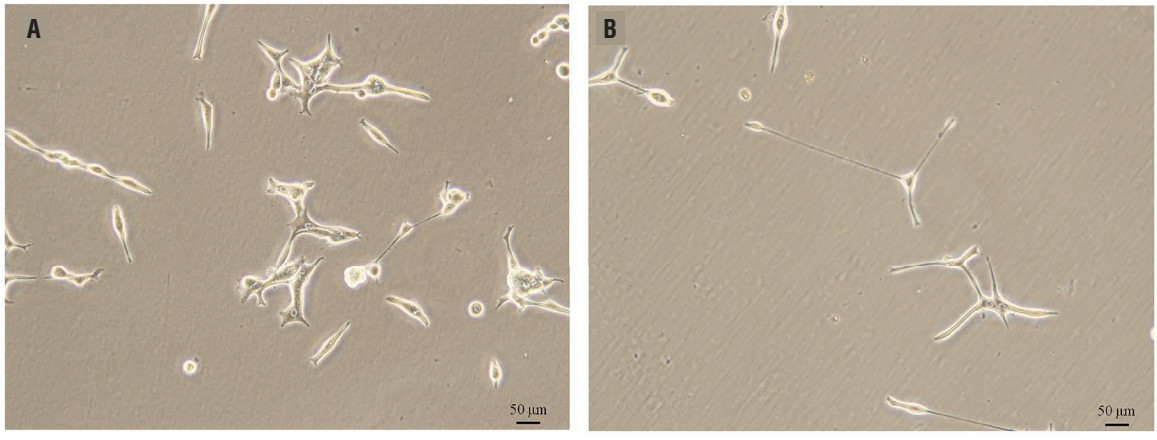
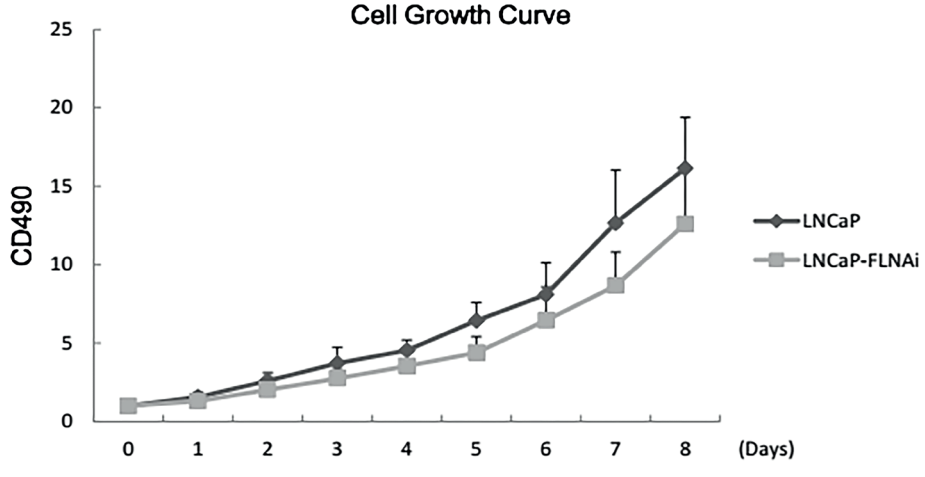


Figure 3 - Observe the growth status in vitro by MTT assay. The experimental results show that the growth of LNCaP cells (Blue) is faster than cells transfected with pSilencer-Filamin (Pink, p <0.05), fifth and seventh days, the growth rate of LNCaP cells is 1.5 times of cells transfected with plasmid pSilencer-Filamin A. Filamin A gene expression inhibits cell division and growth process.

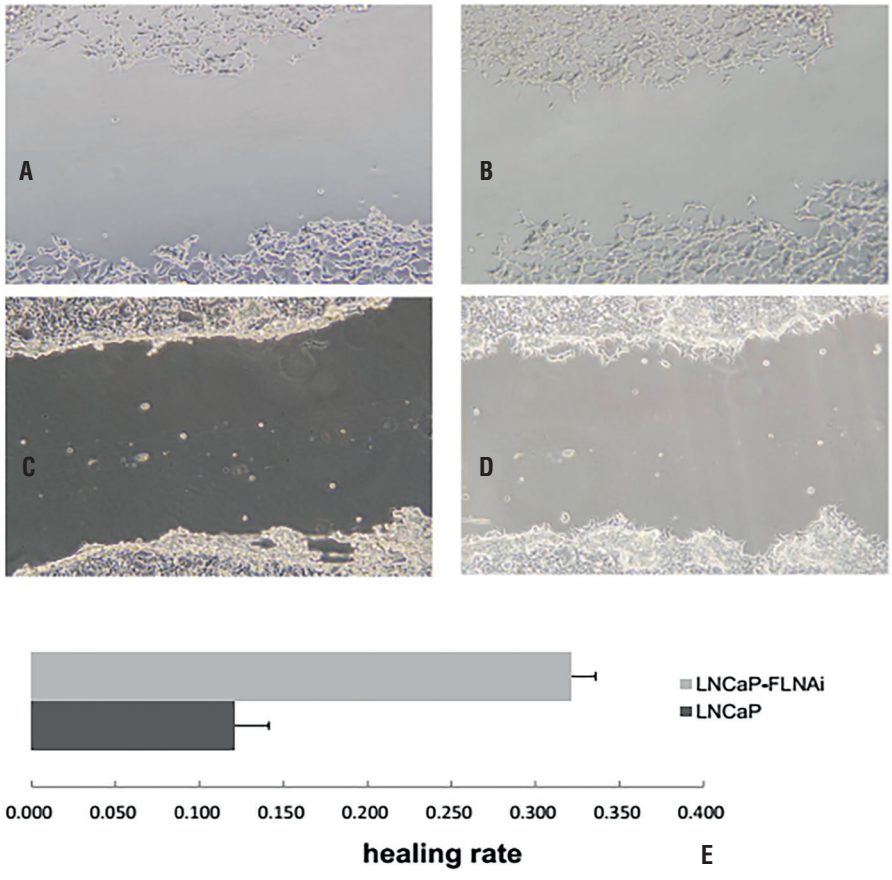


stably transfected with plasmid pSilencer-filamin A, and the difference was statistically significant (P <0.05). When these two groups of cells were inoculated at densities of 2.000, 4.000 and 6.000 cells/well, the clones of the cells stably transfected with plasmid pSilencer-filamin A in the soft agar cloning assay increased by 3.1, 2.7 and 3.8 times, compared with LNCap cells, respectively. This suggests that the inhibition of the filamin A gene can significantly promote the cloning of LNCap cells in vitro.

Scratch test

The scratch test is a common method for measuring cell migration ability. The scratch healing ability of cells in these two groups under the same conditions was determined by observation, photography and measurement using a fluorescence inverted microscope. These results revealed that cells stably transfected with plasmid pSilencer-filamin A presented with stronger healing and migration ability than LNCap cells (healing rates were 32.2% and 12.1%, respectively; Figure-4),

Figure 4 - Wound Healing of Cells transfected plasmid pSilencer-Filamin A. LNCaP cell lines transfected plasmid pSilencer-Filamin A with 0 hours (A). LNCaP cell lines transfected plasmid pSilencer-Filamin A with 48 hours (B). Wound Healing of LNCaP cell lines with 0 hours(C). Wound Healing of LNCaP cell lines with 48 hours (D). Stable transfected plasmid pSilencer-Filamin A cells had higher migration ability than LNCap cells (E, healing rates were 32.2% and 12.1%; p <0.05)



and the difference was statistically significant (P <0.05). This suggests that the inhibition of the filamin A gene can significantly strengthen the migration ability of LNCap cells in vitro. This indicates that the malignant degree of LNCap cells transfected with plasmid pSilencer-filamin A was elevated.

DISCUSSION

In the present study, the PCa LNCaP cell was chosen as the study subject. The LNCaP cell is an androgen-dependent (AD) PCa cell, and is equivalent to the early stage of clinical PCa. A study reported that on the basis of LNCaP cells, a sub cell line, androgen-independent (AI) PCa cell line C4-2, has been developed. On this basis, the occurrence and development of clinical PCa were constructed by simulation: ADPCa→AIPCa (19, 20). At present, the LNCaP cell line has become an effective model for studying PCa, the process of PCa progressing into AI, and the occurrence of metastasis (3).

After the LNCap cell line was transfected with plasmid pSilencer-filamin A, the expression of the filamin A gene was inhibited, which is consistent with previous literature (21-23). Compared to LNCaP cells, the morphology of these was characterized as follows: cells were loosely arranged, had less connection with each other, had fewer tentacles, and presented a fibrous look. These coincide with the basic characteristics of cancer cells, that is, the adhesion of cancer cells decreases, and cancer cells lose contact inhibition on

growth. This trend increases with the increase in the malignant degree of cancer cells. In these experimental results, it was manifested as that with the increase in the malignant degree of PCa cells, the degree that cancer cells lose contact inhibition on growth increased.

Cell proliferation ability is an important index to determine cell viability. In the present study, the in vitro growth curves of LNCap cells and cells transfected with plasmid pSilencer-filamin A were determined by MTT colorimetry assay. The results revealed that under the same culture condition, the number of proliferated cells transfected with plasmid pSilencer-filamin A was less than that of LNCap cells. This seems to be inconsistent with the perception that the degree of malignancy of the tumor is positively correlated with the growth rate of the tumor cells and the ability to spread. In fact, the reason for the slower growth rate of the plasmid pSilencer-filamin A transfected LNCap cells was significantly associated with decreased expression of filamin A. Studies have shown that filamin A is one of the substrates for CDK1 binding (24). CDK1 regulates the cell cycle and determines whether the cell cycle enters the cell division phase from the intercellular phase (25, 26). A significant decrease in filamin A expression affects the cell cycle, resulting in pSilencer-filamin A transfected LNCap cells. At the same time, filamin A is an important actin cross-linking protein that is involved in actin rearrangement. Before cell division, the morphology of the cells is deformed, the cytoskeleton rearranges, and the microfilaments composed of actin are involved. Filamin A may affect the cell cycle by affecting the rearrangement of the cytoskeleton (27, 28). It is well-known that some limitations exist in the simulation of in vivo growth of tumor cells in cell experiments in vitro, since the growth environment of tumor cells in vivo is very different from the experimental environment of in vitro cultivation. The soft agar cloning assay can reflect the population dependence and proliferation ability of cells, and is more similar to the internal environment, compared with cells simply cultured in the culture dish with a culture medium. The cloning efficiency of cells was positively correlated with the malignant degree of cells, which is a common basis for detecting tumor cells. These results reveal that no matter how many cells were inoculated, the clones of cells stably transfected with plasmid pSilencer-filamin A in the soft agar cloning assay were significantly more than that of LNCap cells, and the anchorage-independent growth ability was enhanced; that is, the malignant degree increased.

When tumor cells separate from the mother tumor cells, cross the vessel wall and invade surrounding normal tissues, it requires cells to have certain movement ability (29, 30). Highly metastatic tumor cells usually have strong motility. In the present study, the scratch test was used to compare the movement ability of LNCap cells transfected with pSilencer-filamin A and untransfected LNCap cells. The scratch healing ability of cells in these two groups under the same condition was determined. These results reveal that cells stably transfected with plasmid pSilencer-filamin A presented with stronger healing and migration abilities than LNCap cells. This suggests that the malignant degree of LNCap cells transfected with plasmid pSilencer-filaminA increased.

The degree of harm of PCa is positively correlated with the progression of the disease. Once entering the CRPC stage, the condition of patients often deteriorates rapidly within a short time. Finding effective therapeutic targets before the emergence of CRPC is bound to play a key role in preventing the progression of disease. The inhibition of the expression of the filamin A gene increases the malignant degree of LNCap cells. From this, we can speculate that the filamin A gene may be a tumor suppressor gene, and play an important role in the occurrence and development of tumors, especially in PCa. Furthermore, it may become a new molecular marker for the occurrence and development of PCa. In hormone sensitive stage PCa, if adding filamin A gene expression, or reduce inhibition of filamin A gene expression will reduce hormone sensitivity prostate proliferation, transfer ability, and slow down the transformation process of PCa from hormone sensitive phase to CRPC phase, will bring benefit to the overall treatment in patients with PCa. In summary, this study provides an experimental and theoretical basis for pre-clinical studies on gene

diagnosis and gene therapy of PCa. These are the primary explorations on the function of the filamin A gene in PCa cells. In future studies, we will further investigate the expression of the filamin A gene at the PCa tissue level.

ABBREVIATIONS

PCa = prostate cancer
Ars = Androgen receptors
DMSO = Dimethyl sulfoxide
PBS = phosphate buffered solution
AD = androgen-dependent
AI = androgen-independent

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Principles of Laboratory Animal Care' (NIH Publication Vol 25, No. 28 revised 1996; http://grants.nih.gov/grants/guide/notice-files/not96-208.html) were followed, as well as specific national laws (e.g. the current version of the German Law on the Protection of Animals) where applicable.

FUNDING

This work was supported by the National Key Research and Development Program of China 2017YFC0906602.

Author's Contribution LXC carried out the study, LXC and HCX drafted the manuscript. HCX, WSK, YL and ZGJ participated in the design of the study. CLJ conceived of the study, and participated in its design and helped to draft the manuscript. All authors read and approved the final manuscript.

ACKNOWLEDGEMENTS

Xue-Chao Li, Chuan-Xi Huang, contributed similarly as first author

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin. 2018;687-30.
2. Groeben C, Wirth MP. Prostate cancer: Basics on clinical appearance, diagnostics and treatment. Med Monatsschr Pharm. 2017;40:192-201.
3. Zhau HE, Li CL, Chung LW. Establishment of human prostate carcinoma skeletal metastasis models. Cancer. 2000;88(12 Suppl):2995-3001.
4. Schröder FH, Hugosson J, Roobol MJ, Tammela TL, Ciatto S, Nelen V, et al. Screening and prostate-cancer mortality in a randomized European study. N Engl J Med. 2009;360:1320-8.
5. Weiner AB, Matulewicz RS, Schaeffer EM, Liauw SL, Feinglass JM, Eggener SE. Contemporary management of men with high-risk localized prostate cancer in the United States. Prostate Cancer Prostatic Dis. 2017;20:283-8. Erratum in: Prostate Cancer Prostatic Dis. 2017;20:442.
6. Feldman BJ, Feldman D. The development of androgen-independent prostate cancer. Nat Rev Cancer. 2001;1:34-45.
7. Chung LW, Baseman A, Assikis V, Zhau HE. Molecular insights into prostate cancer progression: the missing link of tumor microenvironment. J Urol. 2005;173:10-20.
8. Castoria G, Auricchio F, Migliaccio A. Extranuclear partners of androgen receptor: at the crossroads of proliferation, migration, and neuritogenesis. FASEB J. 2017;31:1289-300.
9. Loy CJ, Sim KS, Yong EL. Filamin-A fragment localizes to the nucleus to regulate androgen receptor and coactivator functions. Proc Natl Acad Sci U S A. 2003;100:4562-7.
10. Yamazaki M, Furuike S, Ito T. Mechanical response of single filamin A (ABP-280) molecules and its role in the actin cytoskeleton. J Muscle Res Cell Motil. 2002;23:525-34.
11. Wang Y, Kreisberg JI, Bedolla RG, Mikhailova M, deVere White RW, Ghosh PM. A 90 kDa fragment of filamin A promotes Casodex-induced growth inhibition in Casodex-resistant androgen receptor positive C4-2 prostate cancer cells. Oncogene. 2007;26:6061-70.
12. Ozanne DM, Brady ME, Cook S, Gaughan L, Neal DE, Robson CN. Androgen receptor nuclear translocation is facilitated by the f-actin cross-linking protein filamin. Mol Endocrinol. 2000;14:1618-26.
13. Savoy RM, Chen L, Siddiqui S, Melgoza FU, Durbin-Johnson B, Drake C, et al. Transcription of Nrdp1 by the androgen receptor is regulated by nuclear filamin A in prostate cancer. Endocr Relat Cancer. 2015;22:369-86.

14. Bismar TA, Demichelis F, Riva A, Kim R, Varambally S, He L, et al. Defining Aggressive Prostate Cancer Using a 12-Gene Model. Neoplasia. 2006:8;59-68.
15. Sun GG, Lu YF, Zhang J, Hu WN. Filamin A regulates MMP-9 expression and suppresses prostate cancer cell migration and invasion. Tumour Biol. 2014;35:3819-26.
16. Mooso BA, Vinall RL, Tepper CG, Savoy RM, Cheung JP, Singh S, et al. Enhancing the effectiveness of androgen deprivation in prostate cancer by inducing Filamin A nuclear localization. Endocr Relat Cancer. 2012;19:759-77.
17. Narain NR, Diers AR, Lee A, Lao S, Chan JY, Schofield S, et al. Identification of Filamin-A and -B as potential biomarkers for prostate cancer. Future Sci OA. 2016;3:FSO161.
18. DE Nunzio C, Presicce F, Giacinti S, Bassanelli M, Tubaro A. Castration-resistance prostate cancer: what is in the pipeline? Minerva Urol Nefrol. 2018;70:22-41.
19. Thalmann GN, Sikes RA, Wu TT, Degeorges A, Chang SM, Ozen M, et al. LNCaP progression model of human prostate cancer: androgen-independence and osseous metastasis. Prostate. 2000;44:91-103;44.
20. Wu HC, Hsieh JT, Gleave ME, Brown NM, Pathak S, Chung LW. Derivation of androgen-independent human LNCaP prostatic cancer cell sublines: role of bone stromal cells. Int J Cancer. 1994;57:406-12.
21. Huang C, Miller RT, Freter CE. Signaling regulation and role of filamin A cleavage in Ca2+-stimulated migration of androgen receptor-deficient prostate cancer cells. Oncotarget. 2017;8:3840-53.
22. Salimi R, Bandaru S, Devarakonda S, Gökalp S, Ala C, Alvandian A, et al. Blocking the Cleavage of Filamin A by Calpain Inhibitor Decreases Tumor Cell Growth. Anticancer Res. 2018;38:2079-85.
23. Panigrahi GK, Praharaj PP, Kittaka H, Mridha AR, Black OM, Singh R, et al. Exosome proteomic analyses identify inflammatory phenotype and novel biomarkers in African American prostate cancer patients. Cancer Med. 2019;8:1110-23.
24. Szeto SG, Williams EC, Rudner AD, Lee JM. Phosphorylation of filamin A by Cdk1 regulates filamin A localization and daughter cell separation. Exp Cell Res. 2015;330:248-66.
25. Jackman M, Lindon C, Nigg EA, Pines J. Active cyclin B1-Cdk1 first appears on centrosomes in prophase. Nat Cell Biol. 2003;5:143-8.
26. Vagnarelli P. Mitotic chromosome condensation in vertebrates. Exp Cell Res. 2012;318:1435-41.
27. Uotila LM, Guenther C, Savinko T, Lehti TA, Fagerholm SC. Filamin A Regulates Neutrophil Adhesion, Production of Reactive Oxygen Species, and Neutrophil Extracellular Trap Release. J Immunol. 2017;199:3644-53.
28. Chiang TS, Wu HF, Lee FS. ADP-ribosylation factor-like 4C binding to filamin-A modulates filopodium formation and cell migration. Mol Biol Cell. 2017;28:3013-28.
29. Reymond N, d'Água BB, Ridley AJ. Crossing the endothelial barrier during metastasis. Nat Rev Cancer. 2013;13:858-70.
30. Zeeshan R, Mutahir Z. Cancer metastasis - tricks of the trade. Bosn J Basic Med Sci. 2017;17:172-82.

Correspondence address:
Li-Jun Chen, MD
Department of Urology
The Fifth Medical Center, Chinese PLA General Hospital
No.8 Dongda Street, Fengtai District,
Beijing, 100071, China
Fax: + 86 10 6694-7321
E-mail:lijunchen_1962@21cn.com

ORIGINAL ARTICLE

Vol. 45 (5): 925-931, September - October, 2019
doi: 10.1590/S1677-5538.IBJU.2018.0737

Outcomes of endovascular treatment of renal arterial stenosis in transplanted kidneys

Alexandre Sallum Bull [1], Affonso Celso Piovesan [1], Giovanni Scala Marchini [1], Kleiton Gabriel Ribeiro Yamaçake [1], Ioannis Michel Antonopoulos [1], Renato Falci [1], Hideki Kanashiro [1], Gustavo Ebaid [1], Francisco César Carnevale [2], Gustavo Messi [1], William Carlos Nahas [1]

[1] Divisão de Urologia, Unidade de Transplante Renal, Faculdade de Medicina da Universidade de São Paulo - USP, São Paulo, SP, Brasil; [2] Unidade de Radiologia Intervencionista da Faculdade de Medicina da Universidade de São Paulo - USP, São Paulo, SP, Brasil

ABSTRACT

Objective: To evaluate the effectiveness and outcomes of endovascular treatment of TRAS with PTA.
Materials and Methods: We searched our prospectively collected database looking at cases of TRAS between January 2005-December 2011. CCT was the gold-standart for diagnosis of TRAS. Parameters analysed comprised technical aspects, arterial blood pressure variation, and renal function. A minimum follow-up of 24 months was considered.
Results: Of the 2221 renal transplants performed in the selected period, 22 (0.9%) patients were identified with TRAS. Fourteen (63.6%) were male and mean age was 377±14.8years (12-69). Kidney graft was from deceased donnors in 20 (80%) cases. On doppler evaluation, mean blood flow speed after transplantation, at TRAS diagnosis and after TAP was 210.6±99.5, 417±122.7 and 182.5±81.6mL/sec, respectively (p<0.001). For SBP and DBP, there was a significant difference between between pre-intervention and all post-treatment time points (p<0.001). After 1 month of the procedure, there was stabilization of the Cr level with a significant difference between mean Cr levels along time (p<0.001). After a mean follow-up of 16±4.2 (3-24) months, overall success rate was 100%.
Conclusions: Endovascular treatment with PTA/stenting is a safe and effective option for managing TRAS, ensuring the functionality of the graft and normalization of blood pressure and renal function.

ARTICLE INFO

Giovanni Scala Marchini
https://orcid.org/0000-0003-4334-9803

Keywords:
Transplantation; Kidney; Arteries

Int Braz J Urol. 2019; 45: 925-31

Submitted for publication:
January 06, 2019

Accepted after revision:
April 14, 2019

Published as Ahead of Print:
May 22, 2019

INTRODUCTION

Renal transplantation remains the gold-standart treatment for patients with chronic renal failure, not only for a medical stand-point but also for an economic and social perspective. Indications include: end-stage renal disease (ESRD), dyalitic patients and in a preenptive matter.

The most common vascular complication in transplanted kidneys is transplant renal artery stenosis (TRAS), with a reported incidence of 1% in selected cohorts. However, literature incidence

of TRAS is heterogeneous and usually higher, affecting almost 25% of patients according to some series (1-5). Therefore, appropriate diagnosis and treatment is crutial for organ preservation. TRAS usually occurs within the first three years after transplantation and its main cause is artheriosclerosis (6, 7).

Percutaneous transluminal angioplasty (PTA) is considered the less invasive approach for initial management of TRAS with proved efficacy (8). The purpose of our investigation was to evaluate the effectiveness of endovascular treatment of TRAS with PTA in regards to clinical and renal functional parameters, also comparing the results according to the site and etiology of TRAS.

MATERIALS AND METHODS

Case Selection

After Institutional Ethical Approval, a retrospective search on our prospectively collected database was performed searching for cases of TRAS between January 2005 and December 2011. TRAS was suspected if patient had refractory hypertension and transplant graft dysfunction, or when imaging exams were strongly suggestive of the disease. Initial evaluation was performed with doppler ultrassound and confirmed with contrasted computed tomography (CCT) with arterial phase reconstruction. Only cases confirmed by CCT were considered to have definitive diagnosis of TRAS. Of the 2221 renal transplants performed at our Institution in the selected period, 22 (0.9%) patients were identified with TRAS and all cases were treated with PTA.

Surgical Technique

Initial angiography was performed to evaluate stenosis under ipsilateral common femoral artery approach with a 6-French guiding catheter using a nonionic isosmolar contrast medium. After a intravenous bolus of 5000UI of heparin a 0.014 inches guidewire was used to cross the stenosis and was positioned into a distal renal branch. A 6 x 15mm metallic balloon-expandable Palmaz Blue® stent (Cordis, Johnson & Johnson) was positioned and primary stent placement was performed under roadmap technique. Arteriography showed ideal stent positioning without residual stenosis or complications. An Exoseal® closure device (Cordis, Johnson & Johnson) was used for hemostasis.

Analysed Parameters

Analysed parameters comprised technical aspects, arterial blood pressure variation-systolic and dyastolic; need for antihypertensive drugs; and renal function-serum creatinine (Cr) variation. Blood pressure measurements considered the month before PTA, 2 days, 1 week, 1 month, 6 months, 1 and 2 years after the intervention. Cr values were obtained one week before intervention, 1 day, 3 days, 1 week, 1 month, 6 months, 1 and 2 years after PTA. Blood flow speed at doppler ultrassound evaluation was also obtained after transplantation, at TRAS diagnosis, and after angioplasty.

A subgroup analysis was performed to seek for differences regarding the above-mentioned parameters between groups divided by specific cause of TRAS and donnor characteristics. A minimum follow-up of 24 months was considered for all cases. Final success of PTA was considered if the procedure underwent uneventfull and if there was improvement of renal function paralell to blood pressure normalization.

Statistical analysis

Data was analyzed with SPSS V.20 (Chicago, IL). Results were expressed in mean±standard variation and range, or absolute number and frequencies. The Chi-Square or Fisher Exact Test were used to compare categorical variales. To compare measurements for each parameter between individuals the Levene test was used. One-Way Analysis of Variance (ANOVA) with repeated measures was used to compare continuous variables among different time periods for each patient. ANOVA with fixed factor was used to compare continuous variables among groups. Post-Hoc test was performed with Tukey test. Statistical siginificance was set at $p < 0.05$ and considered a 95% Confidence Interval.

RESULTS

Demographic Data

In the six-year period analyzed, 22 consecutive patients presented with TRAS after kidney transplantation-TRAS incidence of 0.9%. Of those, 14 (63.6%) were male and 8 (46.4%) female. Mean age was 37.7±14.8 (12-69) years.

Kidney graft was from deceased donnors in 20 (80%) cases and from living donors in 5 (20%). All presented with refractory hypertension and/or increased serum creatinine levels. The mean interval time from transplantation to diagnosis was 145 day±111 (4 to 232) days.

In 6 (27.3%) patients the site of renal artery stenosis was at the anastomosis. In 3 (13.6%) cases it was due to kinking and the remaining 13 (59.1%) cases it was distal to the anastomosis.

Imaging Evaluation

On doppler evaluation, mean blood flow speed after transplantation, at TRAS diagnosis, and after TAP was 210.6±99.5, 417±122.7 and 182.5±81.6mL/sec, respectively (p <0.001). There was a significant difference between mean blood flow speed at time of TRAS diagnosis compared to after transplantation and after angioplasty, these two moments being similar. For each time point, there was no mean blood flow speed difference among patients (p=0.381), showing a homogeneous normalization of the speed after angioplasty. Figure-1 illustrates a case of TRAS due to kinking (Figure-1A) which was successfully treated with stenting (Figure-1B).

Blood Pressure Analysis

Mean diastolic blood pressure (DBP) showed a mean decrease of 23.4-33.0mmHg from pre-treatment to after intervention. Mean systolic blood pressure (SBP) also presented an average decrease of 11.8 to 17.3mmHg in the same period. After PTA, both showed a relative stabilization (Figure-2).

At each time point, there was no statistical significant difference among patients for SBP and DBP (p=0.11). For SBP and DBP, there was a significant difference between mean pressure value along the time period analysed (p <0.001) due to a significant difference between pre-intervention and all post-treatment time points (p <0.001). After the procedure, mean pressure value was similar for all patients at all time points (p non-significant).

Finally, when considering the amount of use of antihypertensive drugs, there was no statistically significant reduction in the number of medications used to control the disease.

Figure 1 - Three-dimensional reconstruction of arteriography showing TRAS due to kinking (A). Arteriography evidenciating successful outcome after stent placement at the kinking site (B).

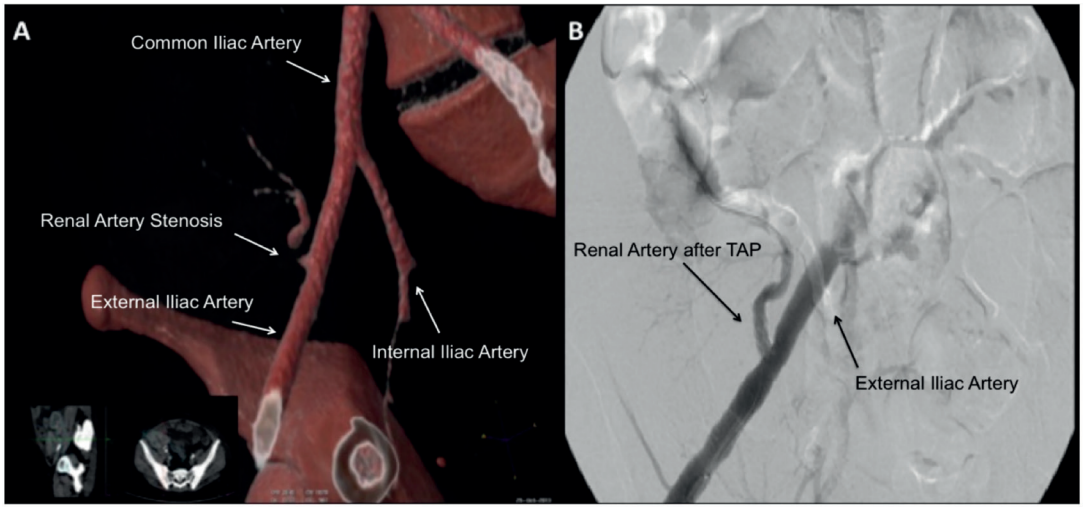
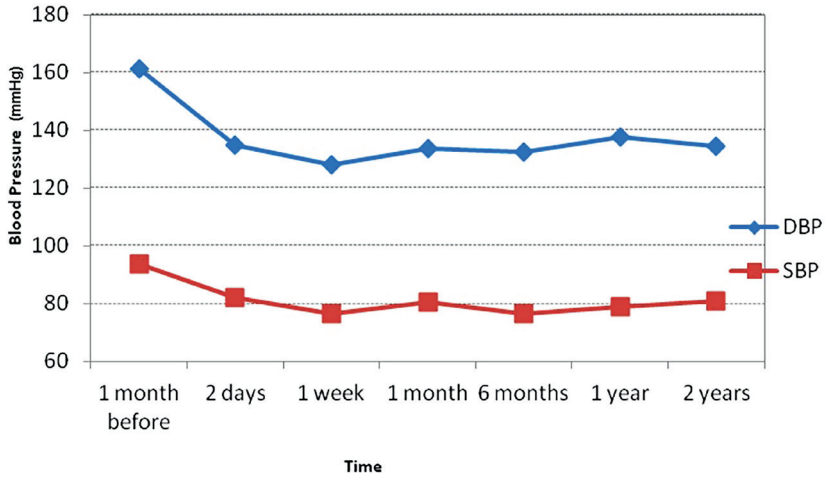


Figure 2 - Systolic and diastolic blood pressure curves before and after TRAS treatment with TAP.



Renal Function Evaluation

Mean serum Cr level pre-intervention was 4.07±2.82mg/dL, falling to 2.1±1.94 after 2 years of TAP. At each time point, there was no significant mean Cr level between patients.

The mean drop of creatinine values from pre-intervention to post-treatment days 1, 3, 7 and 30 was 0.72, 1.16, 1.38 and 2.28mg/dL, respectively. After 1 month of the procedure, there was stabilization of the Cr level (Figure-3). Overall, there was a significant difference between mean Cr levels along time ($p < 0.001$). Although there is a pronounced decrease in mean Cr level from pre to post-intervention, it turned statisticaly significant after the first month of TAP ($p < 0.01$).

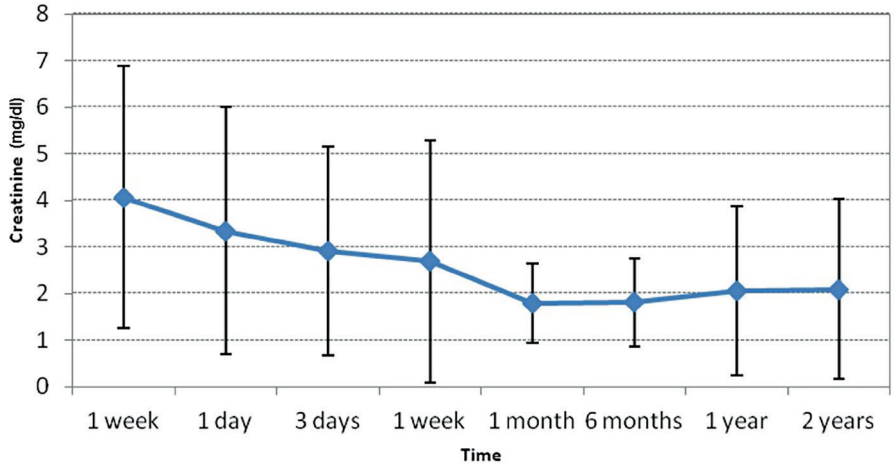
Outcome Analysis

After a mean follow-up of 16±4.2 (3-24) months, overall success rate was 100%. No complications neither restenosis occured. Six patients died due to unrelated causes: 3 due to pneumonia, 1 due to vascular cerebral accident, 1 gastric tumor and 1 acute myocardial infarction.

Stenotic Site Analysis

Age was found to be significantly different between groups ($p=0.018$). The kinking cohort (30.5±20.4 years old) was significantly younger than the anastomotic (50.3±10.2 years old) and post-anastomotic groups (56.2±6.2 years old)($p=0.002$).

Figure 3 - Curve progress of creatinine levels after PTA/stenting.



There was no difference for SBP between groups (p=0.34). However, DBP was different among cohorts (p=0.036). DBP was significantly lower in the kinking group compared to the anastomosis (p=0.029) but not to the post-anastomotic group (p=0.11).

The cohorts were also similar in terms of Cr decrease (p=0.47), blood flow speed at doppler ultrasound (p=0.63), and prevalence of hypertension (p=0.11). Improvement of renal function showed a tendency to be better when due to kinking compared to other sites of stenosis. However this data was not statistically significant, probably due to the small sample size (Figure-4).

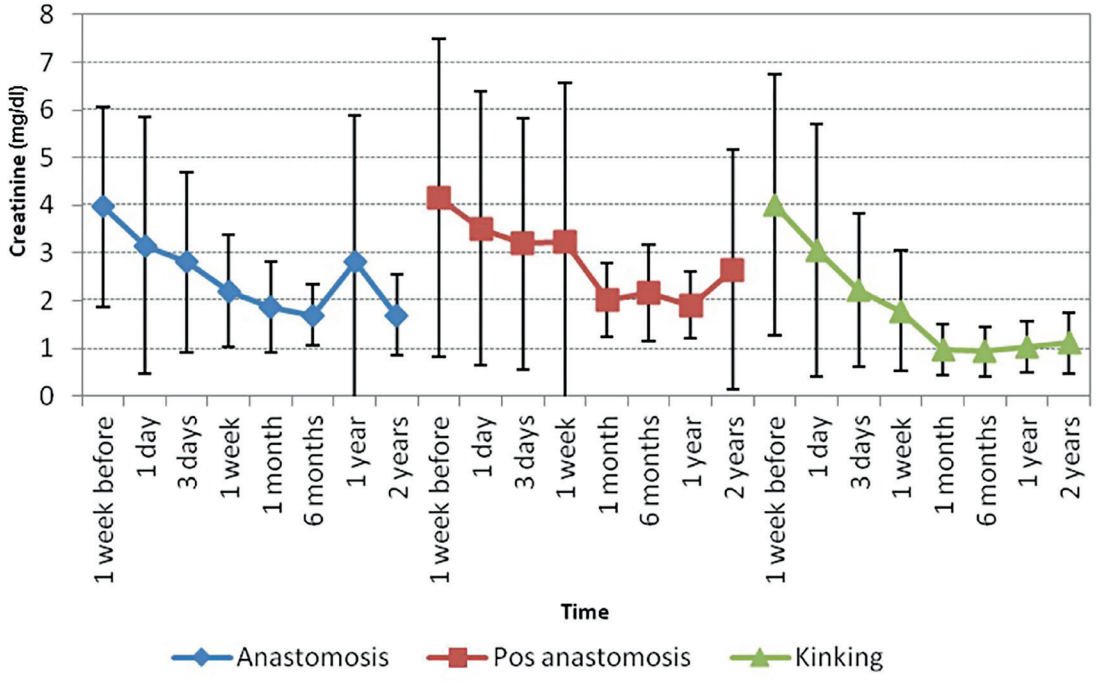
Classical vs expanded donation criteria

Comparison regarding the outcomes of renal function, blood pressure behaviour and mean blood flow speed between patients with classical versus expanded donation criteria (>60 years-old, or donors between 50-59 years with 2 of 3 of the following criteria: hypertension; creatinine greater than 1.5mg/dL or creatinine clearance between 50-70mL/s before removal of the organ; and cause of death stroke), was performed. There was no statistically significant difference (p >0.05).

DISCUSSION

TRAS remains the most frequent vascular complication in patients undergoing renal transplantation. It is responsible for 1 to 5% of cases with refractory hypertension following transplantation. The incidence of TRAS is extremely variable; it was reported an incidence of 2.3% in a large series of 2.013 kidney-transplanted patients (9); it was reported an increase in TRAS diagnosis, with the incidence rising from 2.4% to 12.4% since the routine introduction of diagnostic doppler ultrasonography (10); these results are similar to those of Mammen et al. (11), who reported a rise from 1.7% to 7.9%. The same group also reported that patients with TRAS treated with PTA/stenting had a 5-year survival rate of 76.3%, higher than kidney transplant patients who did not develop TRAS (72.9%), emphasising the effectiveness of the treatment. Although we performed doppler

Figure 4 - Comparison of creatinine progress regarding the site and cause of anastomosis. Data with mean+-Standard Deviation.



ultrassound routinelly after the renal transplant, our incidence of 0.9% is lower than the previously reported, possibly because we use a strict definition of imaging and clinical criteria instead of solely doppler characterization.

Early diagnosis and immediate therapeutic work-up plays an important role in the graft function. The angioplasty of renal artery stenosis has been an excellent therapeutic method in the management of these cases, since it presents few complications with significantly improvement in renal function and blood pressure control. This retrospective evaluation highlights the safety of endovascular management of TRAS and we could succesfully treat all cases without any complication. Furthermore, TAP's efficiency was symbolized by renal function and graft preservation with significant reduction of mean blood pressure to normal parameters. After a two-year follow-up, no cases of restenosis occurred and these patients are being followed closely for an extended period.

TRAS is a serious complication of renal transplantation. There is a higher incidence of TRAS in the first 6 months of transplantation and in elderly transplanted patients. Early appearing stenosis are mainly due to traumatic intimal injury during recovering of the organ or vessel manipulation, kinking of the artery when it is longer than the vein, or technical problems with the vascular suture. Stenosis occurring later, sometimes in terms of years, reflect allograft renal artery hyperplasia or renal and/or iliac atherosclerotic evolution. Late and diffuse stenosis might also reflect endothelial damage related to immune response. In our series, the median time to presentation was 145 days and is consistent with the literature. We found cases of kinking to present in younger patients and with lower dyastolic blood pressure than in patients with anastomotic or post-anastomotic stenosis.

We realize the importance of both blood pressure and renal function normalization to improve endurance of the transplanted graft and survival of recipients. We could successfully normalize both parameters in all patients identified with TRAS who were treated with TAP within a 2-year follow-up, which is longer than some recent series (12-14). Our results are consistent with the literature (12-15) and corroborate the effectiveness of endovascular treatment of TRAS with PTA/stenting. Despite a reduction in blood pressure values, a reduction in the number of antihypertensive drugs was not observed. This is similar to the findings of a recent publication by Braga et al. (14) We attribute this fact to be a result of a closer and more strict outpatient follow-up with close monitoring of the blood pressure to assure normal levels at all times and a more precise pressure control.

This investigation has potential draw--backs that cannot be overlooked. It is a retrospective analysis and therefore suscetible to selection and measurement bias. However, our database is collected in a prospective manner and missing data is extremelly rare. Also, the concise group of urologists and nephrologists following those patients together minimize such biases. Another limitation is the small number of patients analyzed. The number of individuals with suspected TRAS is much larger, nevertheless we have a strict criteria to define TRAS which explain the low amount of patients identified with the disease at our Institution. In addition, most differences found in the investigation achieved significance even in this relatively short series. A larger prospective multi-institutional study could provide a better insight into the subgroup analysis.

Endovascular treatment with PTA/stenting is a safe and effective option for managing TRAS as it preserves vascular permeability in short and medium term, ensuring the functionality of the graft and normalization of blood pressure and renal function. In addition, patients with TRAS due to kinking showed a tendency of better results in terms of renal function improvement.

ABBREVIATIONS

CT = contrasted computed tomography

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Bruno S, Remuzzi G, Ruggenenti P. Transplant renal artery stenosis. J Am Soc Nephrol. 2004;15:134-41.
2. Fervenza FC, Lafayette RA, Alfrey EJ, Petersen J. Renal artery stenosis in kidney transplants. Am J Kidney Dis. 1998;31:142-8.
3. Roberts JP, Ascher NL, Fryd DS, Hunter DW, Dunn DL, Payne WD, et al. Transplant renal artery stenosis. Transplantation. 1989;48:580-3.
4. Wheatley K, Ives N, Gray R, Kalra PA, Moss JG, Baigent C, et al. Revascularization versus medical therapy for renal-artery stenosis. N Engl J Med. 2009;361:1953-62.
5. Henning BF, Kuchlbauer S, Böger CA, Obed A, Farkas S, Zülke C, et al. Percutaneous transluminal angioplasty as first-line treatment of transplant renal artery stenosis. Clin Nephrol. 2009;71:543-9. Erratum in: Clin Nephrol. 2009;72:82.
6. Patel NH, Jindal RM, Wilkin T, Rose S, Johnson MS, Shah H, et al. Renal arterial stenosis in renal allografts: retrospective study of predisposing factors and outcome after percutaneous transluminal angioplasty. Radiology. 2001;219:663-7.
7. Krishnamoorthy S, Gopalakrishnan G, Kekre NS, Chacko N, Keshava S, John G. Detection and treatment of transplant renal artery stenosis. Indian J Urol. 2009;25:56-61.
8. Ghazanfar A, Tavakoli A, Augustine T, Pararajasingam R, Riad H, Chalmers N. Management of transplant renal artery stenosis and its impact on long-term allograft survival: a single-centre experience. Nephrol Dial Transplant. 2011;26:336-43.
9. Hurst FP, Abbott KC, Neff RT, Elster EA, Falta EM, Lentine KL, et al. Incidence, predictors and outcomes of transplant renal artery stenosis after kidney transplantation: analysis of USRDS. Am J Nephrol. 2009;30:459-67.
10. Voiculescu A, Schmitz M, Hollenbeck M, Braasch S, Luther B, Sandmann W, et al. Management of arterial stenosis affecting kidney graft perfusion: a single-centre study in 53 patients. Am J Transplant. 2005;5:1731-8.
11. Mammen NI, Chacko N, Ganesh G, Jacob CK, Shastry JC, Pandey AP. Aspects of hypertension in renal allograft recipients. A study of 1000 live renal transplants. Br J Urol. 1993;71:256-8.
12. Benoit G, Moukarzel M, Hiesse C, Verdelli G, Charpentier B, Fries D. Transplant renal artery stenosis: experience and comparative results between surgery and angioplasty. Transpl Int. 1990;3:137-40.
13. Clements R, Evans C, Salaman JR. Percutaneous transluminal angioplasty of renal transplant artery stenosis. Clin Radiol. 1987;38:235-7.
14. Braga AF, Catto RC, Dalio MB, Tenório EJ, Ribeiro MS, Piccinato CE, et al. Endovascular Approach to Transplant Renal Artery Stenosis. Ann Transplant. 2015;20:698-706.
15. Li CM, Shang T, Tian L, Zhang HK. Short-Term Outcomes Using a Drug-Coated Balloon for Transplant Renal Artery Stenosis. Ann Transplant. 2018;23:75-80.

Correspondence address:
Giovanni Scala Marchini, MD
Departamento de Urologia, Unidade de Transplante Renal, Universidade de São Paulo - USP
Av. Dr. Enéas de Carvalho Aguiar, 255 / 7º andar - Sala 710F
Cerqueira César, São Paulo, SP, 05403-000, Brasil
Telephone: +55 11 2661-8080
E-mail: marchinism@gmail.com

ORIGINAL ARTICLE

Vol. 45 (5): 932-940, September - October, 2019
doi: 10.1590/S1677-5538.IBJU.2018.0776

Preoperative proteinuria is associated with increased rates of acute kidney injury after partial nephrectomy

Önder Kara [1, 2], Matthew J. Maurice [1], Pascal Mouracade [1], Ercan Malkoc [1], Julien Dagenais [1], Mustafa Çapraz [3], Jaya S. Chavali [1], Merve Yazici Kara [4], Jihad H. Kaouk [1]

[1] Department of Urology, Glickman Urological and Kidney Institute, Cleveland Clinic, Cleveland, OH, USA; [2] Kocaeli University, Medical School, Kocaeli, Turkey; [3] Amasya University, Medical School, Amasya, Turkey; [4] Kocaeli Derince Training and Research Hospital, Kocaeli, Turkey

ABSTRACT

Purpose: We investigated the association between preoperative proteinuria and early postoperative renal function after robotic partial nephrectomy (RPN).

Patients and Methods: We retrospectively reviewed 1121 consecutive RPN cases at a single academic center from 2006 to 2016. Patients without pre-existing CKD (eGFR≥60 mL/min/1.73m^2) who had a urinalysis within 1-month prior to RPN were included. The cohort was categorized by the presence or absence of preoperative proteinuria (trace or greater (≥1+) urine dipstick), and groups were compared in terms of clinical and functional outcomes. The incidence of acute kidney injury (AKI) was assessed using RIFLE criteria. Univariate and multivariable models were used to identify factors associated with postoperative AKI.

Results: Of 947 patients, 97 (10.5%) had preoperative proteinuria. Characteristics associated with preoperative proteinuria included non-white race (p<0.01), preoperative diabetes (p<0.01) and hypertension (HTN) (p<0.01), higher ASA (p<0.01), higher BMI (p<0.01), and higher Charlson score (p<0.01). The incidence of AKI was higher in patients with preoperative proteinuria (10.3% vs. 4.6%, p=0.01). The median eGFR preservation measured within one month after surgery was lower (83.6% vs. 91%, p=0.04) in those with proteinuria; however, there were no significant differences by 3 months after surgery or last follow-up visit. Independent predictors of AKI were high BMI (p<0.01), longer ischemia time (p<0.01), and preoperative proteinuria (p=0.04).

Conclusion: Preoperative proteinuria by urine dipstick is an independent predictor of postoperative AKI after RPN. This test may be used to identify patients, especially those without overt CKD, who are at increased risk for developing AKI after RPN.

ARTICLE INFO

Önder Kara
https://orcid.org/0000-0003-1197-2932

Keywords:
Kidney Neoplasms; Proteinuria; Acute Kidney Injury

Int Braz J Urol. 2019; 45: 932-40

Submitted for publication:
November 22, 2018

Accepted after revision:
January 13, 2019

Published as Ahead of Print:
March 20, 2019

INTRODUCTION

Partial nephrectomy (PN) is the gold standard treatment for T1a, and when technically feasible T1b renal masses, due to improved renal functional preservation (1). Given the benefits offered by the robotic platform, there has been an upward trend in the utilization of robotic partial nephrectomy (RPN) for the treatment of renal masses, and relatedly robotic adoption has increased the overall utilization of nephron-sparing surgery (2, 3). PN reduces the amount of renal parenchymal volume loss, more so when performed robotically; however; it does not eli-

minate nephron loss entirely (4-6). Furthermore, the remaining kidney may experience ischemic damage as a result of the temporary vascular clamping required during PN (5). Acute kidney injury (AKI) is associated with increased hospital length of stay and in hospital mortality (7), and following AKI patients have an increased risk of residual structural and functional disease (8). Therefore, preoperative prediction of AKI, especially for patients with presumed normal renal function (Estimated Glomerular Filtration Rate (eGFR)>60) is difficult, and important in patients' counseling.

The glomerular filtration rate (GFR) has been used for a long time as the primary indicator in diagnosing and staging CKD (9). However, Kidney Disease Improving Global Outcomes (KDIGO) recently included etiology, eGFR, and proteinuria as vital components for CKD identification, as each of them has a prognostic value on survival and renal function stability in the population (10-12). The association between CKD severity and AKI risk after PN was quantified based on one component of KDIGO classification (as measured by levels of estimated GFR) in previously published series (13-15). Our primary objective was to assess proteinuria as a marker of early renal dysfunction in patients undergoing RPN.

PATIENTS AND METHODS

Using our institutional review board-approved database, we abstracted data on 1121 RPN cases performed at our center from 2006 to 2016. Patients without pre-existing CKD (eGFR≥60 mL/min/1.73m^2) who had a urinalysis within 1-month prior to RPN were included in the study (n=947). The cohort was categorized by the presence or absence of preoperative proteinuria, and groups were compared in terms of clinical and functional outcomes. The incidence of AKI was assessed using RIFLE criteria. Univariate and multivariable models were used to identify factors associated with postoperative AKI.

Definition of proteinuria

Urine dipstick analysis was used to detect proteinuria. Proteinuria was defined as presence (trace or greater (≥1+) urine dipstick), and absence (negative urine dipstick).

Definition of AKI

The diagnosis of AKI was based on RIFLE criteria (16). Grade 1 (risk) is characterized by a 1.5-2.0-fold increase in serum creatinine or urine output (UO)<0.5mL/kg/h for 6 hours; grade 2 (injury) is characterized by a 2.0-3.0-fold increase in serum creatinine or UO 0.5mL/kg/h for 12 hours; grade 3 (failure) is characterized by any increase >3.0-fold in serum creatinine, temporary need for dialysis, UO <0.3mL/kg/h for 12 hours, or anuria for 12 hours. There were no cases of renal loss or end-stage renal failure in this cohort of patients.

Study variables

Demographic and tumor characteristics included patient age; race (white and non-white); gender; body mass index (BMI); Charlson Comorbidity Index (CCI); American Society of Anesthesiology (ASA) score; history of preoperative diabetes mellitus (DM), hypertension (HTN), and/or smoking; preoperative estimated glomerular filtration rate (eGFR); solitary kidney status; tumor size; R.E.N.A.L. score; and tumor pathology (benign or malignant). Intraoperative variables included operative time, ischemia time, estimated blood loss (EBL), and intraoperative transfusion. Postoperative variables included 30-day postoperative complications, length of hospital stay, and 30-day readmissions. Complications were graded using the Clavien-Dindo classification system (17) and were characterized as minor (Clavien 1-2) and major (Clavien 3-5). Tumor complexity was assessed based on the R.E.N.A.L nephrometry classification system (18). Functional outcomes were assessed using eGFR, which was calculated using the modification of diet in renal disease (MDRD) formula (19). eGFR preservation was defined as follow-up postoperative eGFR divided by baseline eGFR x 100. CKD upstaging was defined as any increase in CKD stage (20) from the time of preoperative assessment to the time of latest postoperative follow-up.

Surgical technique

We used our standard RPN technique as described previously (21). The transperitoneal

approach was used in all cases. Intraoperative ultrasound was used routinely for intraoperative tumor identification and surgical planning. Intracorporeal renal parenchymal cooling was used selectively when ischemia times were expected to be greater than 25 minutes.

Study outcomes

The primary outcome was postoperative AKI. AKI was assessed using RIFLE criteria. Univariate and multivariable models were used to identify factors associated with postoperative AKI. Secondary outcomes included operative time, EBL, ischemia time, perioperative transfusion, length of hospital stay, 30-day readmission, overall and major complications.

Statistical analysis

Continuous variables, presented as mean ± standard deviation (SD) if normally distributed or as median (interquartile range (IQR)) if non-normally distributed, were compared using the t-test or Mann-Whitney U test, respectively. Categorical variables were compared using the chi-squared test. Multivariable analysis was conducted using logistic regression to identify independent predictors of postoperative AKI. Significance was set at $p<0.05$. Analyses were performed using SPSS v24 software (IBM SPSS Statistics, Armonk, NY: IBM Corp).

RESULTS

In the final cohort, 947 patients were included. Preoperative proteinuria was observed in 97 (10.5%) patients on urine dipstick. Of these, 18 (18.5%) had trace (<30 mg/dL), 78 (80.4%) had 30 to 299 mg/dL, and 1 (1.1%) had >300 mg/dL urinary protein preoperatively. Characteristics associated with preoperative proteinuria included non-white race ($p<0.01$), pre-existing DM ($p<0.01$), pre-existing HTN ($p<0.01$), higher BMI ($p<0.01$), higher ASA ($p<0.01$), and higher Charlson score ($p<0.01$). Tumor characteristics, including mass size ($p=0.08$), R.E.N.A.L score ($p=0.13$), and malignant disease ($p=0.06$), were not associated with preoperative proteinuria (Table-1).

Postoperative AKI was more prevalent in patients with preoperative proteinuria (10.3% vs. 4.6%, $p=0.01$). The median eGFR preservation measured within one month after surgery was lower (83.6 (73.3-89.8) % vs. 91 (79-101) %, $p=0.04$) in those with proteinuria; however, there were no significant differences by 3 months after surgery (88 (77.3-98.4) % vs. 89 (78.2-97.5) %, $p=0.9$) or last follow-up visit (85.1 (72.9-96.2) % vs. 86.9 (76.1–98.2) %, $p=0.2$). Likewise, the prevalence of CKD upstaging at the latest follow-up (19.5 vs. 18.7 months, $p=0.56$) did not differ between groups (43.3% vs. 42.1%, $p=0.82$) (Table-2).

In terms of secondary outcomes, there were no significant differences in intraoperative variables, including operative time, EBL, ischemia time, and intraoperative blood transfusion between the two groups. However, proteinuria was associated with higher rates of overall (26.8% vs. 16.8, $p=0.01$) and major (9.3% vs. 4.6%, $p=0.04$) postoperative complications and 30-day readmissions (Table-3).

On further analysis of postoperative complications, the specific complications, which contributed to the disparity in complication rates between groups, included postoperative cardiac complications (7.2% vs. 2.6, $p=0.02$), and haemorrhagic complications necessitating selective arterial angioembolisation (4.1% vs. 0.9%, $p=0.02$) (Table-4).

On multivariable logistic regression, after adjusting for BMI, CCI, preoperative proteinuria, tumor size, baseline eGFR, and ischemia time, significant predictors of postoperative AKI included higher BMI (OR 1.07, 95% CI 1.03-1.17, $p<0.01$), ischemia time >20 min (OR 4.86, 95% CI 2.14-11.01) $p<0.01$), and preoperative proteinuria (OR 2.4, 95% CI 1.02-5.65, $p=0.04$) (Table-5).

DISCUSSION

Despite the nephron-sparing benefits of PN, 4.9% of patients undergoing PN experience postoperative AKI (15). In turn, AKI is associated with increased morbidity and mortality (22). While preexisting CKD is one of the most common risk factor for postoperative AKI after PN, even patients with normal preoperative renal function are at risk for postoperative AKI (23). However, at present, these at-risk patients without CKD are not

Table 1 - Patient demographic and tumor characteristics.

Variables	Proteinuria		P value
	Yes N=97 (10.5%)	No N=850 (89.5%)	
Age years, (±SD)	57.1 (12.7)	57.7 (12)	0.79
Gender			**0.11**
Male, n (%)	65 (67)	499 (58.7)	
Female, n (%)	32 (33)	351 (41.3)	
Race			**<0.01**
White, n (%)	75 (77.3)	756 (89)	
Non-White, n (%)	22 (22.7)	144 (11)	
BMI, med (IQR)	31.2 (26.2-37)	29.3 (25.8-33.5)	**0.04**
ASA, med (IQR)	3 (3-3)	3 (2-3)	**<0.01**
CCI, med (IQR)	1 (0-2)	0 (0-1)	**<0.01**
Diabetes Mellitus, n (%)	29 (29.9)	154 (18.1)	**<0.01**
Hypertension, n (%)	64 (66)	451 (53.1)	**0.01**
Smoker, n (%)	13 (13.4)	123 (14.5)	0.77
Pre-Op eGFR, med (IQR)	87.2 (71.3-102.2)	88.5 (76–100.5)	0.58
Solitary kidney, n (%)	3 (3.1)	13 (1.5)	0.22
Tumor size on CT cm, med (IQR)	3.4 (2.2-4.4)	3 (2.1-4)	0.08
R.E.N.A.L score, med (IQR)	8 (6–9)	7 (6–9)	0.13
Malignant disease, n (%)	86 (88.7)	682 (80.2)	0.06

BMI = Body mass index; **CCI** = Charlson comorbidity index; **CKD** = Chronic kidney disease; **CT** = Computed tomography; **EBL** = Estimated blood loss; **eGFR** = Estimated glomerular filtration rate; **IQR** = Interquartile range; **OPN** = Open partial nephrectomy; **RPN** = Robotic partial nephrectomy; **SD** = Standard deviation

readily identifiable. Thus, there is a need for better tools to identify such patients who are more likely to experience AKI after PN.

Proteinuria has been identified as an essential component of renal dysfunction based on the most recent KDIGO guidelines (12) and appears to be a significant and independent predictor of overall survival and recurrence free survival in patients undergoing renal cancer surgery (24). Therefore, we hypothesized that preoperative proteinuria may be associated with postoperative AKI.

In this retrospective study, the prevalence of postoperative AKI was 5.1%. Some studies have reported postoperative AKI rates after PN ranging from 0.8% to 10% (13, 15, 25) varying by institution, technique, approach, data collection, and AKI criteria. In our study, we used the RIFLE classification scheme for AKI, which is generally accepted for use in the PN population (24).

We found that proteinuria was an independent risk factor for AKI in non-CKD patients undergoing PN. Patients with proteinuria had 2.4-fold higher odds of AKI than patients without proteinuria. These results are consistent with prior studies that have shown an association between proteinuria and AKI after non-renal (26-28), and renal surgeries (29). Surprisingly, in our study, pre-

Table 2 - Follow-up functional data.

Variables	Proteinuria		P value
	Yes N=97 (10.5%)	No N=850 (89.5%)	
Early postoperative functional outcomes (Primary Outcomes)			
Acute kidney injury (RIFLE), n (%)	**10 (10.3)**	**39 (4.6)**	**0.01**
Risk (R)	9 (9.3)	35 (4.1)	
Injury (I)	1(1)	4 (0.5)	
Within 1 mo. eGFR, mL/min/1.73 m^2, median (IQR)	73 (63-90)	80 (69-98)	**0.02**
Within 1 mo. % eGFR preservation, median (IQR)	83.6 (73.3-89.8)	91 (79-101)	**0.04**
Late postoperative functional outcomes			
3-mo. eGFR, mL/min/1.73 m^2, median (IQR)	76 (61-94.9)	77 (65.3-91.8)	0.98
3-mo. % eGFR preservation, median (IQR)	88 (77.3-98.4)	89 (78.2-97.5)	0.9
Follow up times, months, median (IQR)	19.5 (6.2-29.4)	18.7 (5.7-38.4)	0.56
Latest eGFR, mL/min/1.73 m^2, median (IQR)	72 (61.1–89.1)	76 (64.3–90.4)	0.12
Latest follow up % eGFR preservation, median (IQR)	85.1 (72.9–96.2)	86.9 (76.1–98.2)	0.2
CKD upstaging at last follow-up, n (%)	42 (43.3)	358 (42.1)	0.82

IQR = Interquartile range; **SD** = Standard deviation

Table 3 - Secondary outcomes.

Variables	Proteinuria		P value
	Yes N=97 (10.5%)	No N=850 (89.5%)	
Intraoperative outcomes			
Operation time, min, mean (±SD)	182 (48.8)	180 (53)	0.34
EBL, mL., med (IQR)	150 (100-300)	150 (100-250)	0.92
Ischemia time, min, mean (±SD)	20.8 (10)	20.3 (10.1)	0.68
Intraoperative transfusion, n (%)	2 (2.1)	7 (0.8)	0.23
Perioperative outcomes			
Length of stay, days, med, (IQR)	3 (2-4)	3 (2-4)	0.23
30-day readmission, n (%)	8 (8.2)	31 (3.6)	**0.03**
Postoperative transfusion, n (%)	2 (2.2)	48 (5.9)	0.22
Overall C. (Clavien-Dindo 1-5), n (%)	26 (26.8)	143 (16.8)	**0.01**
Major C. (Clavien-Dindo 3-5), n (%)	9 (9.3)	39 (4.6)	**0.04**

Table 4 - Summary of complications based on preoperative proteinuria.

	Proteinuria		P value
Complication type, % (n)	Yes N=97 (10.5%)	No N=850 (89.5%)	
Overall complications	**26 (26.8)**	**143 (16.8)**	**0.01**
Major (Clavien-Dindo 3-5)	9 (9.3)	39 (4.6)	**0.04**
Cardiac complications	**7 (7.2)**	**22 (2.6)**	**0.02**
Myocardial infarction	1 (1)	0 (0)	
Arrhythmia	4 (4.1)	16 (1.9)	
Other cardiac	2 (2.1)	6 (0.7)	
Pulmonary complications	**4 (4.1)**	**51 (6)**	**0.64**
Pneumonia	1 (1)	9 (1.1)	
DVT/PE	1 (1)	11 (1.3)	
Other pulmonary	2 (2.1)	31 (3.6)	
Genitourinary complications	**3 (3.1)**	**13 (1.5)**	**0.22**
UTI	2 (2.1)	4 (0.5)	
Urine leak	1 (1)	9 (1.1)	
Gastrointestinal complications	**5 (5.2)**	**32 (3.8)**	**0.41**
Clostridium difficil infection	1 (1)	1 (0.1)	
Ileus/small bowel obstruction	3 (3.1)	29 (3.4)	
Other gastrointestinal	1 (1)	2 (0.2)	
Wound complications	**2 (2.1)**	**18 (2.1)**	**1**
Surgical site infection	0 (0)	13 (1.5)	
Hernia	0	3 (0.4)	
Other wound	2 (2.1)	2 (0.2)	
Neurologic complications	0	2 (0.2)	1
Bleeding complications	9 (6.3)	11 (14.1)	
Postop Transfusion	2 (2.1)	48 (5.6)	0.22
Need for angioembolisation	4 (4.1)	8 (0.9)	**0.02**

operative proteinuria was not a predictor of long--term renal functional preservation. This finding contrasts a study by Krane et al. (30) and Bhindi et al. (29), and O'Donnell et al. (31) which did show an association between proteinuria and long-term risk of CKD. It is possible that our follow up was not long enough to detect a difference in long--term functional outcomes.

Our findings suggest that proteinuria detected on urine dipstick is a good predictor of postoperative AKI in non-CKD patients. Urine dipstick is quick, inexpensive, and widely available, making it a good screening test. Preoperative assessment of proteinuria may help guide preoperative patient counseling, postoperative care, and medical treatment in non-CKD PN patients.

In addition to proteinuria, longer ischemia time and higher BMI were also independent risk factors for AKI. Our study demonstrated a 4.8-fold higher risk of AKI in patients with ischemia times longer than 20 minutes. The association between longer ischemia time and increased risk of post-PN

Table 5 - Logistic regression analysis predicting AKI after PN.

Variables	Univariate			Multivariate		
	OR	95 % CI	P	OR	95 % CI	P
Age (per year)	0.99	0.97-1.01	0.3			
Race						
(White vs. Non-White)	0.66	0.3-1.45	0.4			
Gender (Male vs. Female	1.01	0.56-1.82	0.95			
BMI (per kg/m^2)	1.07	1.04-1.11	**<0.01**	1.07	1.03-1.11	**<0.01**
CCI (per unit)	1.21	0.98-1.49	**0.01**	1.19	0.95-1.48	0.12
Hypertension (yes vs.no)	1.34	0.74-2.42	0.32			
Diabetes (yes vs.no)	1.37	0.7-2.7	0.34			
Proteinuria (yes vs. no)	2.3	1.15-4.95	**0.01**	2.4	1.02-5.65	**0.04**
Tumor size (per cm)	1.28	1.11-1.47	**<0.01**	1.05	0.87-1.27	0.55
Baseline eGFR (per mL/min/1.73m^2)	1.01	0.99-1.02	0.11	1.01	0.99-1.02	0.07
Ischemia time ≤20 min.	Ref			Ref		
Ischemia time >20 min	4.63	2.13-10.4	**<0.01**	4.86	2.14-11.01	**<0.001**
EBL (per cc)	1	1-1	0.38			
IVF during surgery	1	1-1	0.57			

CI = Confidential interval; **EBL** = Estimated Blood Loss; **OR** = Odds ratio

AKI is well established in the literature (32-34). In terms of patient factors, BMI was the only independent predictor of post-PN AKI. Obesity has been identified previously as a risk factor for AKI after surgery, consistent with our results (35). The pathophysiology of obesity-associated AKI is poorly understood but may be related to comorbidities, such as DM and HTN.

Our study suggests an increased risk of overall and major complications and 30-day readmissions following PN in patients with proteinuria. This association did not persist on multivariable logistic regression analysis, suggesting that comorbid conditions, which occur commonly together with proteinuria, may be responsible for this increased morbidity. Specifically, postoperative cardiovascular complications were more common in patients with proteinuria, consistent with prior studies that have shown an association between proteinuria and cardiovascular morbidity and mortality across divergent populations (36).

Our study is not without limitations. The retrospective design is a potential source of bias, and results from this single tertiary-care center cohort may not be generalizable. While multivariable analysis was used to adjust for known risk factors for postoperative AKI, additional unmeasured factors, for which we could not adjust, may have influenced the ultimate risk of AKI. Another limitation is that urine dipstick was used rather than 24-hour urinalysis for the assessment of proteinuria. Although a 24-hour urinalysis would be the ideal study for proteinuria, it is a more expensive and cumbersome test that would not be practical in all patients undergoing PN.

CONCLUSIONS

Our results indicate that preoperative proteinuria by urine dipstick is an independent pre-

dictor of postoperative AKI after RPN in non-CKD patients. This test may be used to identify patients with occult renal dysfunction who are at increased risk for developing post-PN AKI.

Compliance with Ethical Standards

Dr. Jihad H. Kaouk is a consultant for Endocare/HealthTronics, and Intuitive. No competing financial interests exist for the other authors.

ACKNOWLEDGEMENTS

Dr. Önder Kara and Dr. Ercan Malkoç were supported by a grant for life expenses from TUBITAK: Technology and Innovation Support Programs, Directorate of the Scientific and Research Council of Turkey.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Campbell SC, Novick AC, Belldegrun A, Blute ML, Chow GK, Derweesh IH, et al. Guideline for management of the clinical T1 renal mass. J Urol. 2009;182:1271-9.
2. Sivarajan G, Taksler GB, Walter D, Gross CP, Sosa RE, Makarov DV. The Effect of the Diffusion of the Surgical Robot on the Hospital-level Utilization of Partial Nephrectomy. Med Care. 2015;53:71-8.
3. Liu JJ, Leppert JT, Maxwell BG, Panousis P, Chung BI. Trends and perioperative outcomes for laparoscopic and robotic nephrectomy using the National Surgical Quality Improvement Program (NSQIP) database. Urol Oncol. 2014;32:473-9.
4. Lane BR, Poggio ED, Herts BR, Novick AC, Campbell SC. Renal function assessment in the era of chronic kidney disease: renewed emphasis on renal function centered patient care. J Urol. 2009;182:435-43.
5. Lane BR, Fergany AF, Weight CJ, Campbell SC. Renal functional outcomes after partial nephrectomy with extended ischemic intervals are better than after radical nephrectomy. J Urol. 2010;184:1286-90.
6. Maurice MJ, Ramirez D, Malkoç E, Kara Ö, Nelson RJ, Caputo PA, et al. Predictors of Excisional Volume Loss in Partial Nephrectomy: Is There Still Room for Improvement? Eur Urol. 2016;70:413-5.
7. Chertow GM, Soroko SH, Paganini EP, Cho KC, Himmelfarb J, Ikizler TA, et al. Mortality after acute renal failure: models for prognostic stratification and risk adjustment. Kidney Int. 2006;70:1120-6.
8. Chawla LS, Eggers PW, Star RA, Kimmel PL. Acute kidney injury and chronic kidney disease as interconnected syndromes. N Engl J Med. 2014;371:58-66.
9. Go AS, Chertow GM, Fan D, McCulloch CE, Hsu CY. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. N Engl J Med. 2004;351:1296-305. Erratum in: N Engl J Med. 2008;18:4.
10. Stevens PE, Levin A; Kidney Disease: Improving Global Outcomes Chronic Kidney Disease Guideline Development Work Group Members. Evaluation and management of chronic kidney disease: synopsis of the kidney disease: improving global outcomes 2012 clinical practice guideline. Ann Intern Med. 2013;158:825-30.
11. Lane BR, Demirjian S, Derweesh IH, Riedinger CB, Fergany AF, Campbell SC. Is all chronic kidney disease created equal? Curr Opin Urol. 2014;24:127-34.
12. Ognibene A, Grandi G, Lorubbio M, Rapi S, Salvadori B, Terreni A, ET AL.F. KDIGO 2012 Clinical Practice Guideline CKD classification rules out creatinine clearance 24 hour urine collection? Clin Biochem. 2016;49:85-9.
13. Schmid M, Abd-El-Barr AE, Gandaglia G, Sood A, Olugbade K Jr, Ruhotina N, et al. Predictors of 30-day acute kidney injury following radical and partial nephrectomy for renal cell carcinoma. Urol Oncol. 2014;32:1259-66.
14. Rajan S, Babazade R, Govindarajan SR, Pal R, You J, Mascha EJ, et al. Perioperative factors associated with acute kidney injury after partial nephrectomy. Br J Anaesth. 2016;116:70-6.
15. Schmid M, Krishna N, Ravi P, Meyer CP, Becker A, Dalela D, et al. Trends of acute kidney injury after radical or partial nephrectomy for renal cell carcinoma. Urol Oncol. 2016;34:293.
16. Bellomo R, Ronco C, Kellum JA, Mehta RL, Palevsky P; Acute Dialysis Quality Initiative workgroup. Acute renal failure - definition, outcome measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. Crit Care. 2004;8:R204-12.
17. Clavien PA, Barkun J, de Oliveira ML, Vauthey JN, Dindo D, Schulick RD, et al. The Clavien-Dindo classification of surgical complications: five-year experience. Ann Surg. 2009;250:187-96.
18. Kutikov A, Uzzo RG. The R.E.N.A.L. nephrometry score: a comprehensive standardized system for quantitating renal tumor size, location and depth. J Urol. 2009;182:844-53.

19. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. Ann Intern Med. 1999;130:461-70.
20. Levey AS, Coresh J, Balk E, Kausz AT, Levin A, Steffes MW, et al. National Kidney Foundation. National Kidney Foundation practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Ann Intern Med. 2003;139:137-47. Erratum in: Ann Intern Med. 2003;139:605.
21. Kaouk JH, Khalifeh A, Hillyer S, Haber GP, Stein RJ, Autorino R. Robot-assisted laparoscopic partial nephrectomy: step-by-step contemporary technique and surgical outcomes at a single high-volume institution. Eur Urol. 2012;62:553-61.
22. Lameire NH, Bagga A, Cruz D, De Maeseneer J, Endre Z, Kellum JA, et al. Acute kidney injury: an increasing global concern. Lancet. 2013;382:170-9.
23. Hsu CY, Ordoñez JD, Chertow GM, Fan D, McCulloch CE, Go AS. The risk of acute renal failure in patients with chronic kidney disease. Kidney Int. 2008;74:101-7.
24. Zhang Z, Zhao J, Zabell J, Remer E, Li J, Campbell J, et al. Proteinuria in Patients Undergoing Renal Cancer Surgery: Impact on Overall Survival and Stability of Renal Function. Eur Urol Focus. 2016;2:616-22.
25. Tanagho YS, Kaouk JH, Allaf ME, Rogers CG, Stifelman MD, Kaczmarek BF, et al. Perioperative complications of robot-assisted partial nephrectomy: analysis of 886 patients at 5 United States centers. Urology. 2013;81:573-9.
26. Huang TM, Wu VC, Young GH, Lin YF, Shiao CC, Wu PC, et al. National Taiwan University Hospital Study Group of Acute Renal Failure. Preoperative proteinuria predicts adverse renal outcomes after coronary artery bypass grafting. J Am Soc Nephrol. 2011;22:156-63.
27. Grams ME, Astor BC, Bash LD, Matsushita K, Wang Y, Coresh J. Albuminuria and estimated glomerular filtration rate independently associate with acute kidney injury. J Am Soc Nephrol. 2010;21:1757-64.
28. James MT, Hemmelgarn BR, Wiebe N, Pannu N, Manns BJ, Klarenbach SW, et al. Glomerular filtration rate, proteinuria, and the incidence and consequences of acute kidney injury: a cohort study. Lancet. 2010;376:2096-103.
29. Bhindi B, Lohse CM, Schulte PJ, Mason RJ, Cheville JC, Boorjian SA, et al. Predicting Renal Function Outcomes After Partial and Radical Nephrectomy. Eur Urol. 2018.
30. Krane LS, Heavner MG, Peyton C, Rague JT, Hemal AK. Association of Urine Dipstick Proteinuria and Postoperative Renal Function Following Robotic Partial Nephrectomy. J Endourol. 2016;30:532-6.
31. O'Donnell K, Tourojman M, Tobert CM, Kirmiz SW, Riedinger CB, Demirjian S, Lane BR. Proteinuria is a Predictor of Renal Functional Decline in Patients with Kidney Cancer. J Urol. 2016;196:658-63.
32. Thompson RH, Lane BR, Lohse CM, Leibovich BC, Fergany A, Frank I, et al. Every minute counts when the renal hilum is clamped during partial nephrectomy. Eur Urol. 2010;58:340-5.
33. Lane BR, Gill IS, Fergany AF, Larson BT, Campbell SC. Limited warm ischemia during elective partial nephrectomy has only a marginal impact on renal functional outcomes. J Urol. 2011;185:1598-603.
34. Becker F, Van Poppel H, Hakenberg OW, Stief C, Gill I, Guazzoni G, et al. Assessing the impact of ischaemia time during partial nephrectomy. Eur Urol. 2009;56:625-34.
35. Kelz RR, Reinke CE, Zubizarreta JR, Wang M, Saynisch P, Even-Shoshan O, et al. Acute kidney injury, renal function, and the elderly obese surgical patient: a matched case-control study. Ann Surg. 2013;258:359-63.
36. Agrawal V, Marinescu V, Agarwal M, McCullough PA. Cardiovascular implications of proteinuria: an indicator of chronic kidney disease. Nat Rev Cardiol. 2009;6:301-11.

Correspondence address:
Önder Kara, MD
Glickman Urological and Kidney Institute, Cleveland Clinic
9500 Euclid Avenue, Cleveland, Ohio, 44016, USA
Fax: +1 216 636-4492
E-mail: onerkara@yahoo.com

ORIGINAL ARTICLE

Vol. 45 (5): 941-947, September - October, 2019
doi: 10.1590/S1677-5538.IBJU.2018.0198

The role of a novel decision aid to support informed decision making process in patients with a symptomatic non – lower pole renal stone < 20 mm in diameter: a prospective randomized study

Mehmet İlker Gökce [1], Cağrı Akpınar [1], Barış Esen [1], Vahid Solak [1], Ömer Gülpınar [1], Yaşar Bedük [1]

[1] *Department of Urology, Ankara University School of Medicine, Ankara, Turkey*

ABSTRACT

Objectives: To evaluate the efficacy of a novel decision aid (DA) in improving the patients' level of knowledge and decreasing decisional conflicts while deciding for SWL vs. RIRS in case of a symptomatic renal stone <2 cm.

Materials and Methods: In this prospective randomized study patients were randomized to receive either standard informing process (group 1, n=57) or DA (group 2, n=58). Level of knowledge was assessed with a questionnaire of 10 questions before and after patient informing process. Level of decisional conflict was assessed with a previously validated scoring system. Logistic regression analysis was performed to identify factors associated with adequate level of knowledge.

Results: Level of knowledge increased significantly in both groups after patient informing process. The increase was significantly more prominent in group 2 (p=0.045). Percentage of patients with adequate knowledge was also higher in group 2 (56.1%vs.74.1%, p=0.04). Mean decisional conflict scale score (higher score indicates higher decisional conflict level) was also significantly higher in group1 (14.7±14.5 vs. 10.1±13.7, p=0.045). Multivariate logistic regression analysis revealed higher education level (college degree) and use of DA as factors associated with adequate level of knowledge.

Conclusions: In the current study, The DA was shown to have a positive impact on level of knowledge and diminish the level of decisional conflict for patients with a symptomatic non-lower pole renal stone <20 mm. We recommend development and use of DAs for particular clinic scenarios to aid in education of patients and shared decision making process in stone disease clinics.

ARTICLE INFO

Keywords:
Kidney Calculi; Disease; Lithotripsy

Int Braz J Urol. 2019; 45: 941-7

Submitted for publication:
March 19, 2018

Accepted after revision:
April 28, 2018

Published as Ahead of Print:
June 15, 2018

INTRODUCTION

Stone disease is reported to have a prevalence of 8.8% in the United States and this prevalence also has a tendency increase (1). Careful evaluation and appropriate management of stone disease is crucial considering its short and long term effects on patient's quality of life and renal functions.

Both shock wave lithotripsy (SWL) and retrograde intrarenal surgery (RIRS) are recommended for the management of non - lower

pole renal stones < 20 mm in diameter by the most recent AUA and EAU guidelines (2, 3). The AUA guidelines emphasize the importance of shared decision making for this particular patient group (2).

The decision making process for management of stone disease relies on factors influenced by either the patient or the physician. In a recent study, Sarkissian et al. evaluated the factors that affect patient's preferences on choosing treatment options for management of an asymptomatic renal calculi. An important finding of this study was 56.4% of patients deferred the decision of the treatment approach to the physician (4). On the other hand, in another study, the management behaviors of urologists for a lower pole stone was investigated with a web based survey and 81.2% of the participants responded that patient's preferences were important for decision making. Therefore, involvement of patient in the decision making process should be facilitated and appropriate tools for patient education are required for this purpose (5).

Decision aids (DAs) are tools designed to educate patients on treatment options and possible outcomes. DAs have been used by the urologists especially for screening and management of prostate cancer (6, 7). We recently developed the first DA in the era of stone disease for decision making in treatment of symptomatic non - lower pole renal stones < 20 mm in diameter (8). The aim of this study is to evaluate the efficacy of this DA in improving the patient's level of knowledge and decreasing decisional conflicts in comparison with standard patient informing process in a prospective randomized manner.

MATERIALS AND METHODS

The study was approved by our Institutional Review Board (approval number: 08-428-17). In this single center randomized study, patients with symptomatic non - lower pole renal stones < 20 mm in diameter were included. Patients were included from our stone disease outpatient clinics between December 2016 and May 2017. The CONSORT statements were followed and a flow diagram is provided in Figure-1.

Inclusion criteria

1. Patients between 18-75 years of age
2. The patient should have the ability to read and write
3. The patient should have a symptomatic non - lower pole renal stones < 20 mm and planned intervention
4. The patients should consent to be included in the study

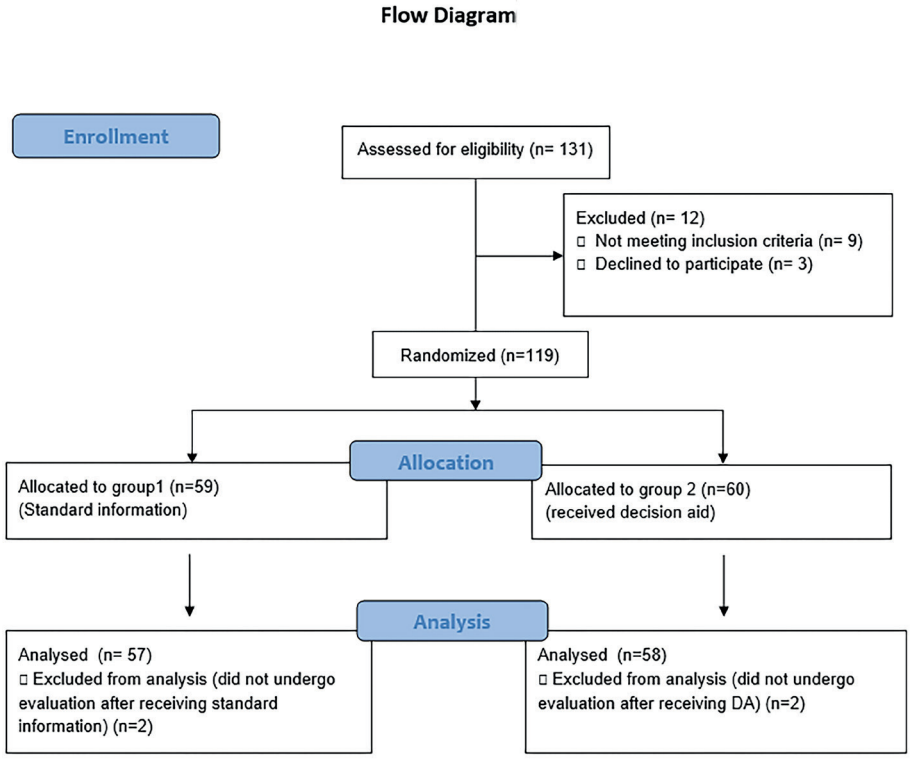
Interventions and data collection

The patients were randomized to two study groups. A computer software was used to generate random allocation sequence. The random allocation sequence was placed in preset numbered envelopes and a nurse opened the envelopes for each patient to perform randomization. The patients in group 1 received standard patient informing process. The information was provided verbally and included general information about stone disease, success and complication rates of SWL and RIRS, and advantages and disadvantages of these two techniques. All patients in this group were informed by a single physician. The patients in group 2 received the DA. The DA has been developed in accordance with the criteria of International Patient Decision Aid Standards (IPDAS) Collaboration and has been published recently (the DA is uploaded as supplementary material) (8).

All patients received a questionnaire of 10 questions (provided as supplementary material) to evaluate the level of knowledge on stone disease before receiving standard informing process or the DA. The questionnaire was provided again after receiving the informing process or DA to evaluate the change in the level of knowledge. The patients with correct answers for at least 8 of the 10 questions were accepted as having adequate level of knowledge.

After the informing processes, the subjects in both groups were asked about their decision to undergo SWL or RIRS. Additionally, they were asked to complete a ten question Decisional Conflict Scale which assessed uncertainty, whether subjects felt informed, had clarity on the information, and felt supported (9). The Decisional Conflict Scale provides a score between 0 and100. The higher score indicates higher level of decisio-

Figure 1 - Flow diagram for enrollment of the patients to the study.



nal conflict and a score of ≤ 25 was accepted as having a low decisional conflict score.

The parameters collected included age, gender, level of education, history of stone disease episodes, and history of previous interventions. The primary end point was the comparison of the change in level of knowledge in both groups and comparison of decisional conflict scores. The secondary end point was the evaluation of factors that have effect on having adequate level of knowledge.

Sample size calculation

In order to calculate the sample size, as there are no previous studies on this topic, a pilot study was conducted with 40 patients and 20 patients were provided with standard informing process and the other 20 given the DA without randomization.

After receiving the informing process, 11 of 20 (55%) patients and 16 of the 20 (80%) patients were found to have adequate level of knowledge respectively and when these values were used for effect size, at least 54 patients were required for each arm to provide a power of 80% with a significance level of 5%.

Statistical analysis

Statistical analysis was performed with SPSS ver. 20.0 (IBM Corp. Released 2011.IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.). Patient characteristics were summarized using mean ± SD or median with range for continuous variables depending on normal distribution and frequency (percentage) for categorical variables. Wilcoxon Signed-Ranks test was used to compare the level of knowledge before and after the informing process for the two groups. The Chi-square test was used to compare the categorical variables and t test or Mann-Whitney U test were used to compare the continuous variables in both groups. The percentages of patients with adequate level of knowledge and low decisional conflict scale score were also compared with Chi-

-square test. Univariate and multivariate logistic regression analysis was performed to identify factors associated with adequate level of knowledge (≥ 8 / 10 correct answers) after patient informing process. For statistical significance, p value of 0.05 was accepted.

RESULTS

The number of patients randomized to group 1 and group 2 were 59 and 60 respectively. Two patients in each group did not undergo the evaluation after informing processes and data of 57 and 58 patients were analyzed. The groups were similar for age, gender, level of education and previous history of stone disease and interventions. The results are summarized in Table-1.

Results of level of knowledge

Median (range) number of correct answers was 4 (1-9) before informing processes for both groups and significantly increased to 6 (3-10) and 8 (5-10) in group 1 (P = 0.03) and group 2 (p = 0.009) respectively. The median number of correct answers after informing process was significantly higher in group 2 compared to group 1 (6 (3-10) vs. 8 (5-10), p = 0.045). In group 1, the number of patients with adequate knowledge was 8 (14%) before informing process and significantly increased to 32 (56.1%) after informing process (p < 0.0001). Similarly, number of patients with adequate level of knowledge increased significantly after receiving the DA in group 2 (10 (17.2%) vs. 43 (74.1%), p < 0.0001)). Also, the number of patients with adequate knowledge after informing process was significantly higher in group 2 compared to group 1 (32 (56.1%) vs. 43 (74.1%), p = 0.04)).

Results of patient's decisions and decisional conflict scale

After the patient informing process, 20 of 57 (35.1%) patients of the patients in group 1 and 28 of the 58 (48.3%) of the patients in group 2 decided to undergo SWL (p = 0.15). The mean decisional conflict scale score of group 1 was significantly higher compared to group 2 (14.7 ± 14.5 vs. 10.1 ± 13.7, p = 0.045). When the groups were compared for percentages of patients with high decisional conflict (decisional conflict scale score > 25) significantly higher number of patients were detected to have high decisional conflict level. The results are summarized in Table-2.

The univariate logistic regression analysis revealed education level (college degree), previous history of stone disease, and patient informing method (use of DA) as factors associated with having adequate level of knowledge. The results of univariate analysis are summarized in Table-3. These factors are further evaluated in a multivariate model and education level (OR: 1.88, 95%CI: 1.44-3.78, p

Table 1 - Demographic characteristics and stone disease related history of the patient groups.

Parameters	Group 1 (n=57)	Group 2 (n=58)	P value
Age, mean±SD	46.5±5.8	46.2±5.9	0.88
Gender, n(%)			**0.50**
Male	33 (58.9)	30 (62.5)	
Female	24 (41.1)	28 (37.5)	
Education level, n(%)			**0.65**
Elementary School	12 (21.0)	15 (25.9)	
High school	25 (43.9)	27 (46.6)	
College degree	20 (35.1)	16 (27.5)	
Previous history of stone disease, n(%)	16 (28.1)	20 (34.4)	0.45
Previous history of intervention, n(%)	10 (17.5)	9 (15.5)	0.77

Table 2 - Comparison of the two groups for Decisional Conflict Scale scores.

Decisional Conflict Scale score	Group 1 (n=57)	Group 2 (n=58)	P value
Total score, n (%)			0.04
≤25	38 (66.7)	48 (82.8)	
>25	19 (33.3)	10 (17.2)	
Uncertainty subscale, n (%)			0.032
≤25	44 (77.2)	49 (84.5)	
>25	13 (22.8)	9 (15.5)	
Informed subscale, n (%)			0.03
≤25	37 (64.9)	48 (82.8)	
>25	20 (35.1)	10 (17.2)	
Values clarity subscale, n (%)			0.17
≤25	40 (70.2)	47 (81.1)	
>25	17 (29.8)	11 (18.9)	
Support subscale, n (%)			0.25
≤25	41 (71.9)	47 (81.1)	
>25	16 (28.1)	11 (18.9)	

= 0.03) and use of DA for patient informing (OR: 2.24, 95%CI: 1.80-4.12, p = 0.01) were identified as independent predictors of establishing adequate level of knowledge. History of stone disease was not identified as an independent factor associated with adequate level of knowledge in the multivariate analysis (OR: 1.18, 95%CI: 0.89-1.68, p = 0.17).

DISCUSSION

Shared decision making is quite important in modern medicine and active participation of patients in the decision making process is mandatory. In order to achieve this goal, the patients should have adequate level of knowledge about their condition and the possible treatment modalities. We recently developed a DA for informing patients with a symptomatic non - lower pole renal stone and in this prospective randomized study the DA was found to be beneficial to establish adequate level of knowledge and lower decisional conflict when compared with standard patient informing process.

SWL and RIRS are the two treatment modalities suggested by the EAU and AUA guidelines

Table 3 - Univariate logistic regression models for having adequate level of knowledge. The outcome variable is whether or not correctly answered at least 8 out of 10 questions (yes vs. no).

Parameters	OR	95% CI	p-value
Age	1.04	0.88 - 1.15	0.40
Gender	1.12	0.77 - 2.15	0.46
Education level (college degree vs. lower)	2.13	1.56 – 4.45	0.01
History of stone disease	1.73	1.22 - 2.43	0.03
History of intervention	1.15	0.87 - 2.53	0.34
Use of DA of patient informing	2.71	1.84 - 5.42	0.008

for management of non - lower pole renal stones < 20 mm (2, 3). The patients should understand the unique advantages and disadvantages of these conditions and participate in the shared decision making process by taking into account their personal needs. The index patient 7 in the most recent AUA guidelines presents a case of a non - lower pole renal stone < 20 mm and the final conclusion is that the decision should rely on a shared decision-making approach (2).

The decision making process of the patients has been of interest. In the study by Sarkissian et al., patients admitted to a stone clinic were provided with a hypothetical scenario of asymptomatic lower pole renal stone. The patients were suggested to select one of three options: observation, SWL, and ureteroscopy. Although the scenario in that study is quite different from the target population in the current study, the main conclusion of the study was that patients mainly rely on the physicians' choices. This result definitely emphasizes the importance of patient education (4).

Patient education on the medical conditions and benefits and limitations of the treatment options are crucial during the shared decision making process. However, this strongly depends on personal factors and standardization of this process will prevent personal bias. The physicians may have very heavy workloads and due to restricted time spent for each patient, patient education may be inadequate. DAs have potential to have benefit not only for the patients but also for the physicians and nurses as well, due to the fact that patients may be well informed before the informed consent process and ready to ask questions about the treatment choices. The DAs have potential to cover this problem as well. A Cochrane review on the use of DAs has been published in 2014 and proved the role of DAs in improving patient's level of knowledge on treatment options, and reduce the level of decisional conflict (10).

The shared decision making process has also importance from the physicians' point of view as well. In a recent study, a web based survey was conducted among urologists to investigate their choices for a small asymptomatic lower pole stone. A very important finding of this study was 81.2% of the urologists mentioned that patient's preferences are one of the two most important factors for their recommendation together with concomitant calyx dilation (5). Therefore, in order to maintain a good balance between the advantages of treatment options and patient's personal needs, standardized informing process is crucial.

We evaluated the factors that have effect on establishing adequate level of knowledge and identified patient's level of education (a college degree) and use of the DA as associated factor in the multivariate analysis. DAs were shown to increase the level of knowledge and facilitate the informed decision making process even in the under-educated populations (6). Therefore, the benefits of using DAs in stone clinics has potential to be more prominent in the under-educated patients.

The primary end point of the study was to evaluate the role of DA in increasing patient's level of knowledge and level of decisional conflict. The most important drawback of the study is the questionnaire used to evaluate level of knowledge is prepared for this study and is not validated. However, this questionnaire is prepared by taking into account the questions commonly asked by patients and during the processing of the questionnaire, 3 urologists collaborated to identify the important points. The second outcome parameter - decisional conflict scale - has already been validated with its scoring system (9). Another important limitation of the study is that the DA was prepared with the contribution of urologists from a single department. This may have effect on the content of the DA and therefore DAs prepared in a multi-institutional manner would be of greater value. Also, the perception of the DA may differ among patients from different socioeconomic and educational levels.

CONCLUSIONS

Patient education to have a sufficient level of knowledge on the treatment choices is important for a successful shared decision making process. In the current study, the DA was shown to have a positive impact on level of knowledge

and diminish the level of decisional conflict for patients with a symptomatic non-lower pole renal stone < 20 mm. We recommend development and use of DAs for particular clinic scenarios to aid in education of patients and shared decision making process in stone clinics, especially in case the patient has to intend for a treatment modality.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Scales CD Jr, Smith AC, Hanley JM, Saigal CS; Urologic Diseases in America Project. Prevalence of kidney stones in the United States. Eur Urol. 2012;62:160-5.
2. Assimos D, Krambeck A, Miller NL, Monga M, Murad MH, Nelson CP, et al. Surgical Management of Stones: American Urological Association/Endourological Society Guideline, PART II. J Urol. 2016;196:1161-9.
3. Türk C, Petřík A, Sarica K, Seitz C, Skolarikos A, Straub M, et al. EAU Guidelines on Interventional Treatment for Urolithiasis. Eur Urol. 2016;69:475-82.
4. Sarkissian C, Noble M, Li J, Monga M. Patient decision making for asymptomatic renal calculi: balancing benefit and risk. Urology. 2013;81:236-40.
5. Ates F, Zor M, Yılmaz O, Tuncer M, Ozturk M, Gurbuz C, et al. Management behaviors of the urology practitioners to the small lower calyceal stones: the results of a web-based survey. Urolithiasis. 2016;44:277-81.
6. Gökce MI, Wang X, Frost J, Roberson P, Volk RJ, Brooks D, et al. Informed decision making before prostate-specific antigen screening: Initial results using the American Cancer Society (ACS) Decision Aid (DA) among medically underserved men. Cancer. 2017;123:583-91.
7. Volk RJ, Hawley ST, Kneuper S, Holden EW, Stroud LA, Cooper CP, et al. Trials of decision aids for prostate cancer screening: a systematic review. Am J Prev Med. 2007;33:428-34.
8. Gökce Mİ, Esen B, Sancı A, Akpınar C, Süer E, Gülpınar Ö. A Novel Decision Aid to Support Informed Decision-Making Process in Patients with a Symptomatic Nonlower Pole Renal Stone <20 mm in Diameter. J Endourol. 2017;31:725-8.
9. O'Connor AM. Validation of a decisional conflict scale. Med Decis Making. 1995;15:25-30.
10. Stacey D, Légaré F, Col NF, Bennett CL, Barry MJ, Eden KB, et al. Decision aids for people facing health treatment or screening decisions. Cochrane Database Syst Rev. 2014;1:CD001431.

Correspondence address:
Mehmet Ilker Gökce, MD,
Department of Urology
Ankara University School of Medicine, Ankara
Altindag, Ankara, 06480, Turkey
Telephone: + 90 312 508-2081
E-mail: migokce@ankara.edu.tr

ORIGINAL ARTICLE

Vol. 45 (5): 948-955, September - October, 2019
doi: 10.1590/S1677-5538.IBJU.2018.0819

Computed tomography window affects kidney stones measurements

Alexandre Danilovic [1], Bruno Aragão Rocha [2], Giovanni Scala Marchini [1], Olivier Traxer [3], Carlos Batagello [1], Fabio Carvalho Vicentini [1], Fábio César Miranda Torricelli [1], Miguel Srougi [1], William Carlos Nahas [1], Eduardo Mazzucchi [1]

[1] Departamento de Urologia do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, SP, Brasil; [2] Departamento de Radiologia do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, SP, Brasil; [3] Sorbonne Université, GRC n 20 Lithiase Renale, AP-HP, Hôpital Tenon, F-75020 Paris, France. University, Paris, France

ABSTRACT

Objectives: Measurements of stone features may vary according to the non-contrast computed tomography (NCCT) technique. Using magnified bone window is the most accurate method to measure urinary stones. Possible differences between stone measurements in different NCCT windows have not been evaluated in stones located in the kidney. The aim of this study is to compare measurements of kidney stone features between NCCT bone and soft tissue windows in patients submitted to retrograde intrarenal surgery (RIRS).

Materials and Methods: Preoperative and 90th postoperative day NCCT were performed in 92 consecutive symptomatic adult patients (115 renal units) with kidney stones between 5 mm to 20 mm (< 15 mm in the lower calyx) treated by RIRS. NCCT were evaluated in the magnified bone window and soft tissue window in three axes in a different time by a single radiologist blinded for the measurements of the NCCT other method.

Results: Stone largest size (7.92±3.81 vs. 9.13±4.08; mm), volume (435.5±472.7 vs. 683.1±665.0; mm3) and density (989.4±330.2 vs. 893.0±324.6; HU) differed between bone and soft-tissue windows, respectively (p<0.0001) 5.2% of the renal units (6/115) were reclassified from residual fragments > 2 mm on soft tissue window to 0-2 mm on bone window.

Conclusion: Kidney stone measurements vary according to NCCT window. Measurements in soft tissue window NCCT of stone diameter and volume are larger and stone density is lesser than in bone window. These differences may have impact on clinical decisions.

ARTICLE INFO

Alexandre Danilovic
http://orcid.org/0000-0002-6963-6117

Keywords:
Kidney Calculi; Nephrolithiasis; Patient Outcome Assessment

Int Braz J Urol. 2019; 45: 948-55

Submitted for publication:
November 28, 2018

Accepted after revision:
March 17, 2019

Published as Ahead of Print:
June 20, 2019

INTRODUCTION

Non-contrast computed tomography (NCCT) has become the gold standard for diagnosing urinary stones (1). NCCT is able to provide stone features as size, volume and density that are relevant for making clinical decisions. Stone size is of paramount importance for spontaneous stone passage (2). Stone volume is the best predictor of operative time and is an independent predictor of stone-free status in retrograde intrarenal surgery (RIRS) for kidney stones (3, 4). Hounsfield units (HU) density is able to differentiate uric acid sto-

nes, to predict success of shockwave lithotripsy and to impact on operative time of RIRS using holmium laser lithotripsy (5-9). However, the measurements of these stone features may vary according to the NCCT technique (10).

Most data previously reported about urinary stones features were measured in soft tissue window conventional-dose NCCT (3-9). However, it has been demonstrated in distal ureteral stones that magnified soft tissue window NCCT is a poor predictor of the largest stone dimension (11) and that magnified bone window is the most accurate method to measure urinary stones in vitro and in vivo (12). The possible differences between windows of NCCT have not been evaluated in stones located in the kidney. The aim of this study is to compare kidney stone features between bone and soft tissue windows using the currently best practice protocol NCCT in patients submitted to RIRS.

MATERIALS AND METHODS

From August 2016 to August 2017, preoperative and 90th postoperative day (POD) NCCT were performed in consecutive symptomatic adult patients with kidney stones that chose to be treated by RIRS.

RIRS was offered as an option for the treatment of symptomatic kidney stones between 5mm to 20mm. We limited the option of RIRS in the lower calyx for stones up to 15mm in an attempt to maximize stone free rate and to reduce flexible ureteroscope damage (13-15). Lower calyx stones larger than 15mm were treated by percutaneous nephrolithotomy (16).

Patients with kidney malformations, ureteral stenosis, previous ipsilateral endoscopic or open kidney surgery, hydronephrosis, indwelling double J stent and contraindications for RIRS were excluded.

NCCT was performed using a 64-slice GE Lightspeed CT Scanner® (General Eletric®, USA) with a slice thickness of 1mm and radiation low-dose protocol (low tube charge current-60mAs) in patients with Body Mass Index-BMI <30Kg/m^2 and conventional protocol (160mAs) in patients with >30Kg/m^2. Low-dose NCCT is recommended for the evaluation of urinary stones in non-obese patients due to equivalent detection of urolithiasis and stone measurements comparing to conventional dose NCCT using less ionized radiation (17, 18).

Magnified (400%) NCCT were evaluated first in bone window (width, 1600HU/level, 500HU) in axial, coronal and sagittal plane and then NCCT were evaluated in soft tissue window (width, 400HU/level, 40HU) by the same radiologist blinded for the results of the measurements of the bone window NCCT (Figure-1). Postoperative NCCT stone measurements were performed in the same fashion (Figure-2).

Stone density was measured by free hand ROI determination coincident with the stone borders. Stone volume was calculated as length x width x depth x π x 0.167 (1, 19). Residual fragments were categorized as 0 when no residual fragments exists, 0-2mm and >2mm.

STATISTICAL ANALYSIS

Bone and soft tissue window NCCT results were compared using paired T-test, Wilcoxon Signed Rank. Bone and soft tissue window NCCT residual fragments were compared using McNemar-Bowker test of symmetry. Sample size was calculated based on the percentage of renal units with residual fragments more than 2mm by NCCT of 38% (20). Therefore, the sample size for a bicaudal test with significance level of 5% and test power of 95% is 115 renal units.

SAS 9.0 program® (SAS Institute Inc., Cary, NC, USA) was used with a significance level of 5%.

RESULTS

Ninety-two patients were successfully submitted to RIRS. Bilateral procedures were performed in 23 patients (25%) resulting in 115 renal units operated. Body Mass Index was 28.1±4.8, 19.0-45.5Kg/m^2 (mean±SD, range). Twenty-eight patients (35 renal units, 30.4%) were obese (BMI >30Kg/m^2) and were submitted to conventional-dose NCCT. Stone features evaluated by bone and soft tissue windows are compared in Table-1. Stone largest size, volu-

Figure 1 - Preoperative magnified (400%) NCCT bone window (ww 1600HU/wl 500HU) vs. soft tissue window (ww 400HU/ wl 40HU). A) bone window axial stone diameter, B) bone window coronal stone diameter, C) bone window sagittal stone diameter, D) bone window stone density, E) soft tissue window axial stone diameter, F) soft tissue window coronal stone diameter, G) soft tissue window sagittal diameter, H) soft tissue window stone density.

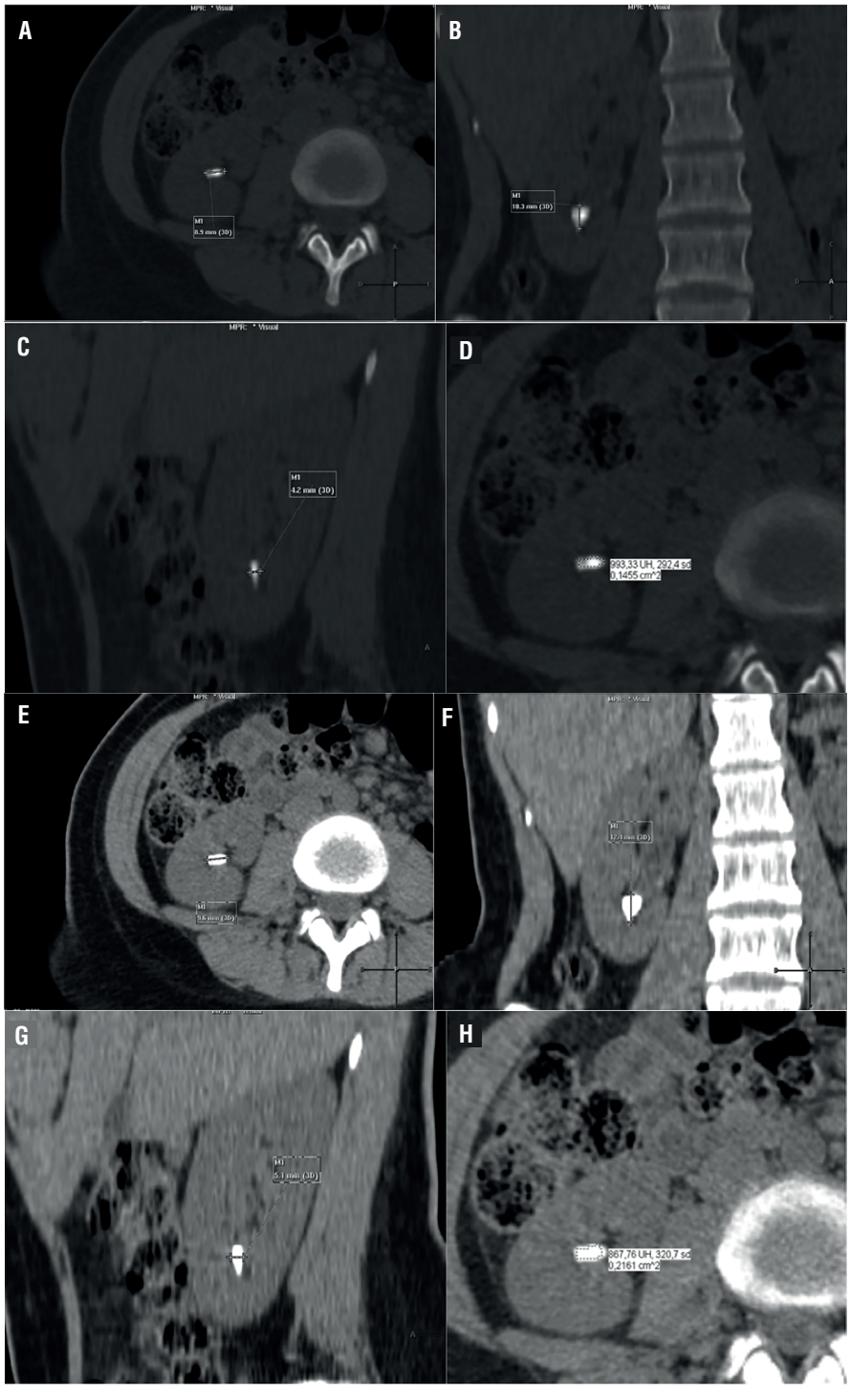


Figure 2 - Postoperative magnified (400%) NCCT bone window (ww 1600HU/wl 500HU) vs. soft tissue window (ww 400HU/wl 40HU). A) bone window axial stone diameter, B) bone window coronal stone diameter, C) bone window sagittal stone diameter, D) soft tissue window axial stone diameter, E) soft tissue window coronal stone diameter, F) soft tissue window sagittal diameter.

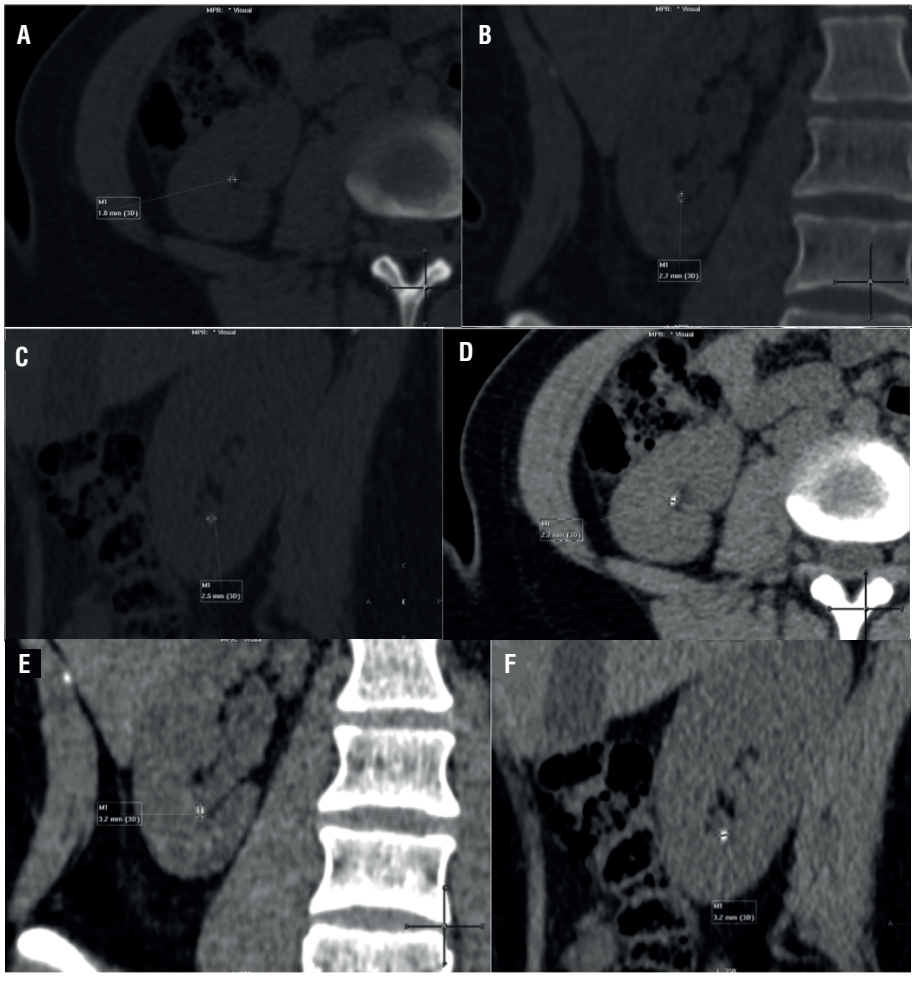


Table 1 - Comparison between pre-operative NCCT bone and soft tissue windows of the stone features of the115 renal units submitted to RIRS.

Stone features	Bone window	Soft Tissue window	p-value
Multiple stones (%)	69 (60.0)	69 (60.0)	1.000
Stone size (mean±SD, mm)	7.92±3.81	9.13±4.08	<0.0001
Stone volume (mean±SD, mm^3)	435.5±472.7	683.1±665.0	<0.0001
Stone density (mean±SD, HU)	989.4±330.2	893.0±324.6	<0.0001

me and density differed between the two methods (p <0.0001). Although residual fragments diameter was not significantly different when evaluated by NCCT using bone or soft tissue window (p=0.1116) (Table-2), 5.2% of the renal units (6/115) were reclassified from residual fragments >2mm to 0-2mm.

The 90th postoperative day bone and soft tissue window NCCT revealed one asymptomatic small subcapsular hematoma in a stone free renal unit and two asymptomatic hydronephrosis, one in a stone free renal unit and other in a renal unit with >2mm residual fragment.

DISCUSSION

Low dose NCCT is the current gold standard for the evaluation of urinary stone disease due to its lower radiation exposure (0.7-2.8mSv) than conventional dose NCCT (8-16mSv) and high pooled sensitivity of 0.966 (95% CI, 0.950-0.978) and a pooled specificity of 0.949 (95% CI, 0.920-0.970), which are equivalent to conventional dose NCCT sensitivity of 97% and specificity of 96% (17, 18). Besides, other authors found no significant difference in the measurement of stone size and HU between low dose and conventional NCCT (17, 21). However, conventional dose NCCT is still recommended for the evaluation of urinary stones in obese patient (BMI >30Kg/m^2) (1).

We used low-dose NCCT with 60mAs in non-obese patients (BMI <30kg/m^2) and NCCT with 160mAs in obese patients for this study to minimize the radiation exposure without compromising image quality (22, 23). NCCT image noise varies proportional to the value of the square root of the miliampere product. Higher noise from ultra low-dose NCCT (24) may decrease accuracy in detecting small residual fragments (<3mm) (25).

Other authors stressed the importance of standardization of making measurements on NCCT images (10). They demonstrated a larger variability for inter-reader (±1.3mm) than intra-reader (26). Narayan et al. demonstrated that stone density measurements vary depending on window, plane and ROI technique. They recommend that clinicians select a single ROI measurement technique and remain consistent to minimize variability (27). However, ROI measurement should include the periphery of the stone as we did by free hand technique to better represent the entire nature of that stone. As a result, we may better predict laser and operative time or even which laser technique (dusting, fragmentation or popcorn) is better according to stone density. A single senior radiologist evaluated all NCCT studies, in a different time, blinded for the results of the bone window NCCT stone measurements. This might have reduced the possible measurement bias.

Magnified bone window NCCT should be preferred for urinary stone evaluation due to better image quality for dense objects as it minimizes noise artifacts close to the stone limits (12, 25). In vitro study already demonstrated that soft tissue window overestimates stone size and bone window provides best accuracy (26). Clinically, it was shown that bone window allows a visual distinction between a stent and a stone (28, 29). On the other hand, urologists are more familiar with soft tissue window when looking at NCCT and most data related to stone features and NCCT were produced using soft tissue window.

In order to establish if there is a difference between kidney stone measurements in

Table 2 - Comparison between post-operative NCCT bone and soft tissue windows of the residual stone size of the115 renal units.

Residual stone size	Bone window	Soft Tissue window	p-value
0 mm, N (%)	86/115 (74.8)	86/115 (74.8)	0.1116
0-2 mm, N (%)	10/115 (8.7)	4/115 (3.5)	
> 2 mm, N (%)	19/115 (16.5)	25/115 (21.7)	

bone and soft tissue windows, we compared preoperative urinary stone features and 90 POD results in both windows. We demonstrated that preoperative bone window NCCT image produce smaller size and volume stone and bigger density stone than soft tissue window ($p < 0.0001$). These results have major clinical impact because regarding stone treatment, every millimeter counts for the decision to actively treat or not. Besides, stone-free rates of all modalities of active treatment of renal calculi are based on size, burden or volume of stone. We found differences in stone density probably because of variation in positioning their regions-of-interest due to different time of measurement as stressed by Williams Jr. (30) and to less noise in the stone surround.

Although we found no significant differences between both windows in the stone free status and complications in the follow-up evaluation, it is important to notice that in 5.2% of the renal units operated the difference in size of residual fragments was clinically relevant. According to previous studies, residual fragments >2mm are more likely to experience growth and cause disturbance to patients (16, 31-33). Therefore, the correct measurement of residual fragment is of upmost importance to plain reintervention.

Our study has several strengths. It is a prospective study using preoperative and postoperative current best practice NCCT after RIRS in patients without kidney malformations, ureteral stenosis, previous ipsilateral endoscopic or open kidney surgery, hydronephrosis or indwelling double J stent, providing more accurate results. To the best of our knowledge, it is the first study to prospectively address a comparison between NCCT bone and soft tissue windows for kidney stones.

This study has some limitations. We did not compare the real size of the intact stone to the NCCT measured size because the stones were broken during RIRS. However, other authors already proved that bone window is more accurate comparing to real distal ureteral stones (11). Also, we used two different NCCT protocols. We used low-dose NCCT in non-obese patients and conventional dose NCCT in obese patients in order to minimize the radiation exposure. Previous authors showed that low--dose NCCT did not compromise image quality (22, 23). However, we did not examine these subgroups separately. Another limitation is the single center nature of our study. Therefore, our results should be validated by other high volume centers.

CONCLUSIONS

Kidney stone measurements vary according to NCCT window. Measurements in soft tissue window NCCT of stone diameter and volume are larger and stone density is lesser than in bone window. These differences may have impact on clinical decisions.

ACKNOWLEDGEMENTS

Financial support of Fapesp No. 2014/05130-2

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Türk C, Knoll T, Petrik A, Sarica K, Skolarikos A, Straub M, et al. Guidelines on Urolithiasis. Arnhem, The Netherlands: EAU Guidelines Office; 2013.
2. Miller OF, Kane CJ. Time to stone passage for observed ureteral calculi: a guide for patient education. J Urol. 1999;162(3 Pt 1):688-90.
3. Sorokin I, Cardona-Grau DK, Rehfuss A, Birney A, Stavrakis C, Leinwand G, et al. Stone volume is best predictor of operative time required in retrograde intrarenal surgery for renal calculi: implications for surgical planning and quality improvement. Urolithiasis. 2016;44:545-50.
4. Ito H, Sakamaki K, Kawahara T, Terao H, Yasuda K, Kuroda S, et al. Development and internal validation of a nomogram for predicting stone-free status after flexible ureteroscopy for renal stones. BJU Int. 2015;115:446-51.
5. Saw KC, McAteer JA, Monga AG, Chua GT, Lingeman JE, Williams JC Jr. Helical CT of urinary calculi: effect of stone composition, stone size, and scan collimation. AJR Am J Roentgenol. 2000;175:329-32.

6. Nakada SY, Hoff DG, Attai S, Heisey D, Blankenbaker D, Pozniak M. Determination of stone composition by noncontrast spiral computed tomography in he clinical setting. Urology. 2000;55:816-9.
7. Spettel S, Shah P, Sekhar K, Herr A, White MD. Using Hounsfield unit measurement and urine parameters to predict uric acid stones. Urology. 2013;82:22-6.
8. Shah K, Kurien A, Mishra S, Ganpule A, Muthu V, Sabnis RB, et al. Predicting effectiveness of extracorporeal shockwave lithotripsy by stone attenuation value. Endourol. 2010;24:1169-73.
9. Ito H, Kawahara T, Terao H, Ogawa T, Yao M, Kubota Y, et al. The most reliable preoperative assessment of renal stone burden as a predictor of stone-free status after flexible ureteroscopy with holmium laser lithotripsy: a single-center experience. Urology. 2012;80:524-8.
10. Lidén M, Andersson T, Geijer H. Making renal stones change size-impact of CT image post processing and reader variability. Eur Radiol. 2011;21:2218-25.
11. Kishore TA, Pedro RN, Hinck B, Monga M. Estimation of size of distal ureteral stones: noncontrast CT scan versus actual size. Urology. 2008;72:761-4.
12. Eisner BH, Kambadakone A, Monga M, Anderson JK, Thoreson AA, Lee H, et al. Computerized tomography magnified bone windows are superior to standard soft tissue windows for accurate measurement of stone size: an in vitro and clinical study. J Urol. 2009;181:1710-5.
13. Danilovic A, Cavalanti A, Rocha BA, Traxer O, Torricelli FCM, Marchini GS, et al. Assessment of Residual Stone Fragments After Retrograde Intrarenal Surgery. J Endourol. 2018;32:1108-13.
14. Goldberg H, Golomb D, Shtabholtz Y, Tapiero S, Creiderman G, Shariv A, et al. The "old" 15 mm renal stone size limit for RIRS remains a clinically significant threshold size. World J Urol. 2017;35:1947-54.
15. Ozimek T, Cordes J, Wiessmeyer JR, Schneider MH, Hupe MC, Gilbert N, et al. Steep Infundibulopelvic Angle as a New Risk Factor for Flexible Ureteroscope Damage and Complicated Postoperative Course. J Endourol. 2018;32:597-602.
16. Türk C, Petřík A, Sarica K, Seitz C, Skolarikos A, Straub M, et al. EAU Guidelines on Interventional Treatment for Urolithiasis. Eur Urol. 2016;69:475-82.
17. Sohn W, Clayman RV, Lee JY, Cohen A, Mucksavage P. Low-dose and standard computed tomography scans yield equivalent stone measurements. Urology. 2013;81:231-4.
18. Niemann T, Kollmann T, Bongartz G. Diagnostic performance of low-dose CT for the detection of urolithiasis: a meta-analysis. AJR Am J Roentgenol. 2008;191:396-401.
19. Finch W, Johnston R, Shaida N, Winterbottom A, Wiseman O. Measuring stone volume - three-dimensional software reconstruction or an ellipsoid algebra formula? BJU Int. 2014;113:610-4.
20. Rippel CA, Nikkel L, Lin YK, Danawala Z, Olorunnisomo V, Youssef RF, et al. Residual fragments following ureteroscopic lithotripsy: incidence and predictors on postoperative computerized tomography. J Urol. 2012;188:2246-51.
21. Alsyouf M, Smith DL, Olgin G, Heldt JP, Lightfoot M, Li R, et al. Comparing stone attenuation in low- and conventional-dose noncontrast computed tomography. J Endourol. 2014;28:704-7.
22. Bhatt K, Monga M, Remer EM. Low-dose computed tomography in the evaluation of urolithiasis. J Endourol. 2015;29:504-11.
23. Zilberman DE, Tsivian M, Lipkin ME, Ferrandino MN, Frush DP, Paulson EK, et al. Low dose computerized tomography for detection of urolithiasis-its effectiveness in the setting of the urology clinic. J Urol. 2011;185:910-4.
24. Jin DH, Lamberton GR, Broome DR, Saaty HP, Bhattacharya S, Lindler TU, et al. Effect of reduced radiation CT protocols on the detection of renal calculi. Radiology. 2010;255:100-7.
25. Glazer DI, Maturen KE, Cohan RH, Davenport MS, Ellis JH, Knoepp US, et al. Assessment of 1 mSv urinary tract stone CT with model-based iterative reconstruction. AJR Am J Roentgenol. 2014;203:1230-5.
26. Argüelles Salido E, Aguilar García J, Lozano-Blasco JM, Subirá Rios J, Beardo Villar P, Campoy-Martínez P, et al. Lithiasis size estimation variability depending on image technical methodology. Urolithiasis. 2013;41:517-22.
27. Narayan VM, Bozorgmehri S, Ellen JH, Canales MT, Canales BK, Bird VG. Evaluating Region of Interest Measurement Strategies to Characterize Upper Urinary Tract Stones on Computerized Tomography. J Urol. 2017;197(3 Pt 1):715-22.
28. Tanrikut C, Sahani D, Dretler SP. Distinguishing stent from stone: use of bone windows. Urology. 2004;63:823-6.
29. Yoshida S, Hayashi T, Morozumi M, Osada H, Honda N, Yamada T. Three-dimensional assessment of urinary stone on non-contrast helical computed tomography as the predictor of stonestreet formation after extracorporeal shock wave lithotripsy for stones smaller than 20 mm. Int J Urol. 2007;14:665-7.

30. Williams JC Jr. Viewing windows do not alter Hounsfield units in CT scans. Urol Res. 2005;33:481-2.
31. Chew BH, Brotherhood HL, Sur RL, Wang AQ, Knudsen BE, Yong C, et al. Natural History, Complications and Re-Intervention Rates of Asymptomatic Residual Stone Fragments after Ureteroscopy: a Report from the EDGE Research Consortium. J Urol. 2016;195(4 Pt 1):982-6.
32. Kang M, Son H, Jeong H, Cho MC, Cho SY. Clearance rates of residual stone fragments and dusts after endoscopic lithotripsy procedures using a holmium laser: 2-year follow-up results. World J Urol. 2016;34:1591-7.
33. Hein S, Miernik A, Wilhelm K, Adams F, Schlager D, Herrmann TR, et al. Clinical significance of residual fragments in 2015: impact, detection, and how to avoid them. World J Urol. 2016;34:771-8.

Correspondence address:
Alexandre Danilovic, MD
Av. Dr. Eneas de Carvalho Aguiar, nº 255, 7º andar,
Sala 7175
São Paulo, SP, 05403-000, Brasil
Telephone: +55 11 2661-8080
E-mail: alexandre.danilovic@hc.fm.usp.br

ORIGINAL ARTICLE

Vol. 45 (5): 956-964, September - October, 2019
doi: 10.1590/S1677-5538.IBJU.2019.0049

Comparison of supine and prone miniaturized percutaneous nephrolithotomy in the treatment of lower pole, middle pole and renal pelvic stones: A matched pair analysis

Harun Ozdemir [1], Akif Erbin [1], Murat Sahan [1], Metin Savun [1], Alkan Cubuk [1], Ozgur Yazici [1], Mehmet Fatih Akbulut [1], Omer Sarilar [1]

[1] *Department of Urology, Haseki Traning and Research Hospital, Istanbul, Turkey*

ABSTRACT

Purpose: We aimed to compare the outcomes of supine and prone miniaturized percutaneous nephrolithotomy (m-PNL) in the treatment of lower pole, middle pole and renal pelvic stones.

Materials and Methods: 54 patients who performed supine m-PNL between January 2017 and March 2018 and 498 patients who performed prone m-PNL between April 2015 and January 2018 were included in the study. Of the 498 patients, 108 matching 1: 2 in terms of age, gender, body mass index, American Association of Anesthesiology score, stone size, stone localization and hydronephrosis according to the supine m-PNL group were selected as prone m-PNL group. The patients with solitary kidney, upper pole stone, urinary system anomaly or skeletal malformation and pediatric patients (<18 years old) were excluded from the study. The success was defined as 'complete stone clearance' and was determined according to the 1st month computed tomography.

Results: The operation time and fluoroscopy time in supine m-PNL was significantly shorter than prone m-PNL group (58.1±45.9 vs. 80.1±40.0 min and 3.0±1.7 min vs. 4.9±4.5 min, p=0.025 and p=0.01, respectively). When post-operative complications were compared according to the modified Clavien-Dindo classification, overall and subgroup complication rates were comparable between groups. There was no significant difference between the groups in terms of the success rates (supine m-PNL; 72.2%, prone m-PNL; 71.3%, p=0.902).

Conclusions: Supine m-PNL procedure is more advantageous in terms of operation time and fluoroscopy time in the treatment of lower pole, middle pole and renal pelvic stones.

ARTICLE INFO

Akif Erbin
http://orcid.org/0000-0001-7147-8288

Keywords:
Supine Position;
Nephrolithotomy, Percutaneous;
Pelvis

Int Braz J Urol. 2019; 45: 956-64

Submitted for publication:
January 26, 2019

Accepted after revision:
April 01, 2019

Published as Ahead of Print:
June 25, 2019

INTRODUCTION

The main treatment modalities in urinary system stone disease are extracorporeal shockwave lithotripsy (ESWL), ureterorenoscopy (URS), percutaneous nephrolithotomy (PNL), open and laparoscopic surgery. With the recent advances in technology, endourologic procedures (URS and PNL) among the surgical treatments have gained more popularity. The European Association of Urology (EAU) urolithiasis guideline recommends standard PNL as the first choice in the treatment of kidney stones larger than 2cm (1). Although PNL is accepted as a safe method, it can lead to

life-threatening hemorrhages. Considering that the hemorrhage in standard PNL is directly related to the instruments used, the diameters of the instruments have been reduced over the years. In this context, firstly, the miniaturized PNL (m-PNL) technique was introduced by Jackman et al. in 1988 (2). In the following years, developments have continued with defining smaller diameter systems such as ultra-m-PNL, super m-PNL and micro PNL techniques (3-5). The m-PNL is accepted as the use of 14-22Fr access sheaths by EAU Urolithiasis Guidelines Panel (6).

In PNL, the original position is accepted as 'prone'. However, PNL can be performed in classic supine or different positions such as Galdacao modification of Valdivia, lateral decubitus, lateral position modification, and reverse lithotomy (7-10). Supine PNL was introduced by Valdivia in 1987 and the first results were reported in 1998 with a series of 557 cases (11, 12). When compared with the prone position, supine position has some advantages such as easier and comfortable patient positioning, possibility of simultaneous retrograde access to kidney, lower renal pelvic pressure and easier intervention to the respiratory tract by the anesthetist (13). There are many studies in literature comparing prone PNL and supine PNL, however, almost all of them are related to standard PNL. In the present study, our purpose was to compare the outcomes of supine and prone m-PNL performed for stones located in lower pole, middle pole and renal pelvis.

MATERIALS AND METHODS

Study design

The present study was approved by the Internal Institutional Review Board. Fifty four patients who performed supine m-PNL between January 2017 and March 2018 and 498 patients who performed prone m-PNL between April 2015 and January 2018 were included in the study.

Exclusion criterias were:

- pediatric patients (<8 years old)
- patients with solitary kidney
- patients with kidney stones located in the upper pole
- patients with urinary system anomalies
- patients with skeletal malformations

Of the 498 patients, 108 matching 1: 2 in terms of age, gender, body mass index (BMI), American Association of Anesthesiology (ASA) scores, stone size, stone localization and hydronephrosis (HN) according to the supine m-PNL group were selected as prone m-PNL group. Both groups were compared in terms of demographic data (age, gender, BMI, ASA score, previous surgery and ESWL), stone characteristics (size, localization, opacity, hydronephrosis), operative data (side, operation time, fluoroscopy time, number of access, size of access sheath, nephrostomy placement, transfusion, complication) and postoperative data (hospitalization time, hemoglobin drop, transfusion, JJ stent placement, success and complication). Operation time was calculated as the time from the insertion of ureteral catheter to nephrostomy placement. The success was defined as 'complete stone clearance' and was determined according to the 1st month CT. Intraoperative complications were evaluated using the modified Stava classification system; postoperative complications were evaluated according to the modified Clavien-Dindo classification system (14, 15).

Preoperative evaluation

Written and verbal consent was obtained from all patients before the operation. Patient assessment included medical history, physical examination, complete blood count, coagulation tests, serum biochemistry, urinalysis and urine culture. Anticoagulant drugs were discontinued at least 7-10 days week before the operation. All patients were evaluated preoperatively by non-contrast computed tomography (CT). Stone size was determined by measurement of the greatest dimension. In the case of multiple calculi, the sum of the greatest dimension of each stone was calculated. All patients had sterile urine culture prior to surgery. Antibiotic prophylaxis was provided by second generation cephalosporins. The first dose was administered intravenously when anesthesia

was initiated and the second dose was given 12 hours later.

Supine m-PNL technique

Following general anesthesia, the patient was placed in the Galdakao-Modified Valdivia position. Under C-arm fluoroscopy guidance, 5 French (Fr) open end ureteral catheter was inserted retrogradely. A Foley catheter was then indwelled and the distal end of the ureteral catheter was fixed on the Foley catheter. Skin surface was marked to indicate the lower rib margin, posterior axillary line and iliac crest (Figure-1). The calyx plane to be punctured was determined by ultrasonography. Retrograde pyelography was done and an 18 gauge percutaneous access needle (Boston Scientific Corporation, Natick MA) was passed into the desired calix under fluoroscopic guidance. A 0.035 inch guidewire (Boston Scientific Corporation, Natick MA) was passed antegradely across the renal pelvis and into the ureter, upper or lower calix. The track was dilated sequentially using fascial and metallic dilators. According to stone sizes, the 15, 16.5 or 21Fr metallic sheats (Karl Storz, Tutlingen, Germany) were advanced over their metal dilators. A rigid 12Fr nephroscope (Karl Storz, Tuttlingen, Germany) was advanced through the sheath. Stone disintegration was achieved using a Holmium YAG Laser lithotripter (Sphinx, Lisa laser, USA). Flexible antegrade pyeloureteroscopy was performed if the rigid nephroscope couldn't reach to stone. Stone fragments were removed with basket catheters. At the end of the procedure, retrograde pyelography was done to assess the integrity of the pelvicaliceal system (PCS). If there was no extravasation and irrigant fluid was returning clear, no tube was left (tubeless PNL); otherwise, a nephrostomy tube was left in place.

Prone m-PNL technique

After the induction of general anesthesia, a 5Fr Ureteral catheter was placed and fixed on the Foley catheter in the lithotomy position. The patient was then repositioned in the prone position. Skin surface was marked to indicate the lower rib margin, posterior axillary line and iliac crest (Figure-2). Percutaneous access was achieved under C arm fluoroscopy guidance. The puncture was performed with an 18 gauge percutaneous access needle. Following successful puncture, a 0.035 inch guidewire was advanced through the needle into the PCS or ureter. At later stages, tract dilatation, nephroscopy, stone fragmentation, and stone retrieval were performed in a manner similar to supin m-PNL. All supine and prone procedures were performed by two experienced urologists at the tertiary referral center.

Figure 1 - Galdakao-Modified Valdivia position in supine m-PNL. The shaded area between lower rib, posterior axillary line and iliac crest shows the subcostal access location.

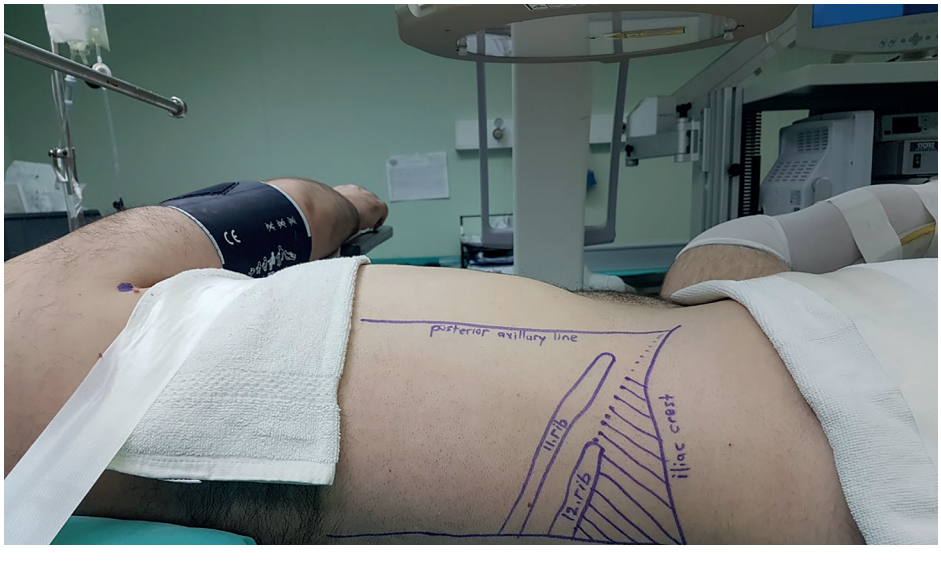
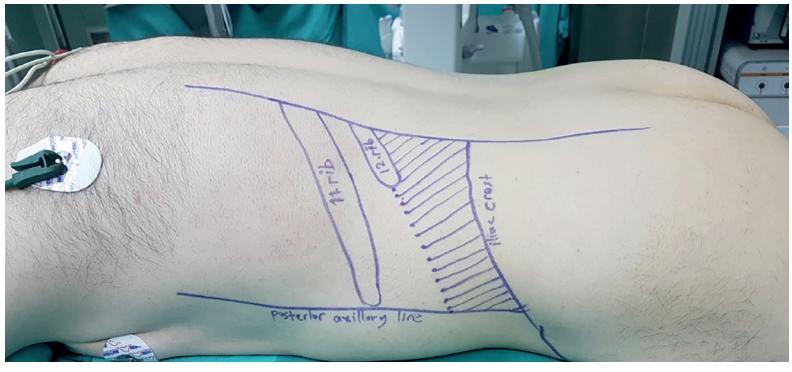


Figure 2 - Prone position. The shaded area between lower rib margin, posterior axillary line and iliac crest shows the subcostal access location in prone PNL.



Postoperative evaluation

A complete blood count and renal function tests were obtained from all patients within 6 hours after the operation. In cases with a nephrostomy tube, the tube was removed on postoperative day 1 or 2 after antegrade nephrostography revealed ureteral drainage down to the bladder. The leakage longer than 48 hours was accepted as 'prolonged urine leakage' and JJ stent was placed following CT imaging. JJ stents were removed under local anesthesia. All patients were evaluated with renal function tests and a non-contrast spiral CT 1 month after the operation.

Statistical analysis

Data were analyzed by using Statistical Package for the Social Sciences software package version 20 (SPSS Inc., Chicago, IL, USA). Quantitative data were expressed as mean±std values on tables and categorical data were expressed with frequency (n) and percentages (%). The distribution of the variables was measured by the Kolmogorov Smirnov test. Independent t test was used to compare independent groups. Pearson Correlation test was used to examine the relationship between variables. Pearson Chi-Square and Fisher Exact tests were used to compare the categorical data. The data were analyzed at 95% confidence level and the threshold for statistical significance was accepted as $p < 0.05$ for all analyses.

RESULTS

Demographic data and stone characteristics are shown in Table-1. Age, sex, BMI, ASA score, stone size, stone localization and HN grade were similar between the groups because of fact that '1: 2 matched pair' was performed.

Operative data are summarized in Table-2. The operation time and fluoroscopy time in supine m-PNL was significantly shorter than prone m-PNL group (58.1±45.9 vs. 80.1±40.0 min and 3.0±1.7 min vs. 4.9±4.5 min, p=0.025 and p=0.01, respectively). While the rate of tubeless PNL was 37% in supine m-PNL group, it was 17.6% in prone m-PNL group (p=0.006). None of the patients had intercostal or upper pole access. There was no significant difference between the groups in terms of the intraoperative complications classified according to Satava.

Post-operative complications and outcomes are summarized in Table-3. When post-operative complications were compared according to the modified Clavien-Dindo classification, overall and subgroup complication rates were comparable between groups. Grade-4 complications (angioembolization and urosepsis) were observed in 3 patients in both groups. The hospitalization time was similar and there was no significant difference between the groups in terms of the success rates (supine m-PNL; 72.2%, prone m-PNL; 71.3%, p=0.902). When success was separately evaluated as single stone and multicaliceal stone, there was no significant difference.

Table 1 - Demographic data and stone characteristics.

	Supine m-PNL (n=54)	Prone m-PNL (n=108)	*p*
Sex (female/male) *	15/39	38/70	0.343
Age (years)*	43.4±11.9	44.0±13.4	0.813
BMI (kg/m^2)*	27.3±3.9	26.9±4.1	0.609
ASA score*	1.2±0.5	1.1±0.4	0.645
Previous ESWL / surgery			0.553
ESWL	9 (16.7%)	27 (25.0%)	
URS	2 (3.7%)	6 (5.6%)	
PNL	6 (11.1%)	16 (14.8%)	
Open Surgery	3 (5.6%)	6 (5.6%)	
Stone opacity (opaque / non-opaque)	50/4	94/14	0.289
Stone localization*			0.821
Lower calyx	8 (14.8%)	19 (17.6%)	
Middle calyx	0	0	
Upper calyx	2 (3.7%)	4 (3.7%)	
Pelvis	23 (42.6%)	51 (47.2%)	
Multiple calyx	21 (38.9%)	34 (31.5%)	
Stone size (mm)*	25.8±7.9	24.8±5.6	0.886
Hydronephrosis (mild/severe)*	43/11	85/23	0.891

* Matching parameters (1:2 scenario)

DISCUSSION

In the literature, there is only one retrospective study comparing supine m-PNL and prone m-PNL (16). In our study, supine m-PNL and prone m-PNL were compared using '1: 2 match pair analysis' in terms of success and complications. In the present study, general complication rates were similar in both group. Urosepsis was seen in one patient in the supine m-PNL group. Supin PNL provides the lower renal pelvic pressures. This is accepted as a protective factor for urosepsis (17). However, the presence of urosepsis in the supine m-PNL group suggested that the patient and operative factors (diabetes mellitus and long operation time) rather than surgical technique were effective in this patient.

In PNL, the pleura and the colon are the most injured organs. In the literature, supine and prone PNL have different numbers for colon injury. In the supine position, intestines will be more anteriorly displaced and this condition will reduce the risk of colonic injury (18). In a comparative study using the CROES database, colon injury was found to be similar in both groups (3.4% and 3.3%, p=0.95) (19). However, in the randomized prospective studies, no colonic injuries have been reported in the supine PNL (19-21). In our study, no organ injuries were detected in both groups. In the supine m-PNL group, no upper pole access was performed due to positional difficulty in patients. Antegrade or retrograde flexible ureterorenoscope were used in cases where upper pole access was required. Samely, in the unique study comparing supine m-PNL and prone m-PNL in the literature, no upper pole access was performed in the supine group (16).

Table 2 - Operative data.

	Supine m-PNL (n=54)	Prone m-PNL (n=108)	*p*
Operation side (right / left)	27/27	51/57	*0.739*
Operation time (min)	58.1+45.9	80.1+40.0	*0.025*
Fluoroscopy time (min)	3.0±1.7	4.9±4.5	*0.013*
Amplatz sheath size			*0.076*
15 Fr	19 (35.2%)	21 (19.4%)	
16.5 Fr	20 (37.0%)	55 (50.9%)	
21 Fr	15 (27.8%)	32 (29.6%)	
Access			*0.065*
Lower pole	48 (88.9%)	85 (78.7%)	
Middle pole	6 (11.1%)	13 (12.0%)	
Multiple access	0	10 (9.3%)	
Tubuless procedure	20 (37.0%)	19 (17.6%)	*0.006*
Intraoperative complication			*0.677*
Satava grade 1a	2 (3.7%)	4 (3.7%)	
Satava grade 2a	1 (1.9%)	5 (4.6%)	

In the meta-analysis including two randomized trials, it was reported that there was no statistically significant difference between supine standard PNL and prone standard PNL in terms of success rates (83.5% vs. 81.6%, respectively) (22). However, in another current meta-analysis, it was reported that prone standard PNL had significantly higher success rates than supine standard PNL (77.7% vs. 74.4%, p=0.0001). In the study, this difference was thought to be due to the fact that the nephroscope mobility was better in the prone PNL and that it was difficult to perform the upper pole access in the supine PNL (17). In the study comparing supine m-PNL and prone m-PNL, 54 and 126 patients were performed via supine m-PNL and prone m-PNL; the stone-free rates were 74.1% and 76.2%, respectively (16). Our study also confirmed that supine m-PNL and prone m-PNL were not superior to each other in terms of success.

We concluded that prone PNL procedure has a longer operation time than supine PNL. This difference is due to the time for repositioning the patient in prone PNL. In the meta-analysis study, it was reported that supine standard PNL had the advantage of an average operation time of 18 min and this difference was statistically significant (17). This result was also confirmed by a prospective randomized trial (21). In the study comparing supine m-PNL and prone m-PNL, operation times were 55 min and 82 min in supine m-PNL and prone m-PNL, respectively (16). In our study, the difference in operation time between supine and prone m-PNL was of average 22 minutes.

Because of the fact that it has some advantages in terms of cardiovascular, respiratory and anesthesia application, supine is a more accepted position by anesthetists than prone. There is a risk of the endotracheal tube being removed

Table 3 - Postoperative complications and outcomes.

	Supine m-PNL (n=54)	Prone m-PNL (n=108)	*p*
Clavien - Dindo classification			*0.452*
Grade 0	38 (70.4%)	76 (70.4%)	
Grade 1	5 (9.3%)	17 (15.7%)	
Grade 2	1 (1.9%)	5 (4.6%)	
Grade 3a	2 (3.7%)	3 (2.8%)	
Grade 3b	5 (9.3%)	4 (3.7%)	
Grade 4	3 (5.6%)	3 (2.8%)	
Double-J stent placement	1 (1.9%)	4 (3.7%)	*0.521*
Fever	3 (5.6%)	7 (6.5%)	*0.817*
Hematocrit drop (gr/dL)	3.9±3.3	3.2±3.0	*0.376*
Transfusion	4 (7.4%)	6 (5.6%)	*0.644*
Angioembolization	2 (3.7%)	1 (0.9%)	*0.216*
Urosepsis	1 (1.9%)	2 (1.8%)	*0.214*
Hospitalization time (hour)	56.3±62.5	66.0±37.2	*0.401*
Overall success	39 (72.2%)	77 (71.3%)	*0.902*
Success in isolated calyx stones	22 (66.7%)	53 (71.6%)	*0.605*
Success in multiple calyx stones	17 (81.0%)	24 (70.6%)	*0.391*

during the positioning to prone and the possibility of intervention to airway becomes limited after the patient is positioned. Furthermore, in the prone position, the risks of nerve tension, musculoskeletal injuries and visual impairment due to increased ocular pressure are more likely (23, 24). These risks are clinically insignificant in patients at low risk (ASA 1/2) groups (25). Despite its significant disadvantages, the prone position is used more often by surgeons. The reason for this is that surgeons are more accustomed to prone position. In the supine position, the surgeon can comfortably sit during the operation, and x-ray exposure is reduced because puncture and dilation of the nephrostomy tract are quite perpendicular to the body and the operator's hands are outside the fluoroscopic field. Furthermore, by rotating the legs into the lithotomy position, combined antegrade and retrograde procedures can effectively be performed in the supine position. This represents the main advantage of this procedure because it combines the benefits of percutaneous and ureteroscopic intrarenal surgery in selected cases of contemporary treatment of bilateral stones (26).

Although the present study is a 1: 2 match pair analysis study, it has some limitations. The main limitations of the present study is its retrospective nature and the relatively small sample size. Thus, large-scale randomized trials should be encouraged to be designed, so that the above conclusions can be verified with an increased statistical power. Secondly, we did not match stone compositions for comparison. Theoretically, SFR could be affected by the differences in stone components between the two groups. Thirdly, we excluded the patients with skeletal malformations and with kid-

ney stones located in the upper pole. As surface area used in prone m-PNL is extended, performing an access to the upper calyx is easier than supine m-PNL. In patients with wide hips and thin calices, it can be more difficult or even impossible to reach the upper calyx with a rigid nephroscope in supine position. So, the patients with upper pole stones were excluded from the study. Stone treatment in patients with skeletal deformity can be a serious problem for urologists. Skeletal deformities make both conventional and minimal invasive surgical interventions difficult. In these patients, it may be necessary to perform stone treatment by giving different positions other than supine or prone position. Also, for these patients, PNL may not always be the appropriate option. Instead, open surgery, laparoscopic-assisted PNL or f-URS may be more suitable options. Because of these reasons, the patients with skeletal deformity were excluded from our study. Another limitation of our study is that Guy's stone score is not included. This system includes some parameters such as the presence of upper pole stone, anatomical abnormalities (calyceal diverticulum) and skeletal deformities (spina bifıda, spinal injury). So, we were unable to use the Guy's score in the present study.

CONCLUSIONS

In the treatment of lower pole, middle pole and renal pelvic stones, supine m-PNL and prone m-PNL procedures have similar success rates. There is no significant difference in terms of general complication rates. However, supine PNL is more advantageous in terms of operation and fluoroscopy times.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Türk C, Petřík A, Sarica K, Seitz C, Skolarikos A, Straub M, et al. EAU Guidelines on Diagnosis and Conservative Management of Urolithiasis. Eur Urol 2016;69:468-74.
2. Jackman SV, Docimo SG, Cadeddu JA, Bishoff JT, Kavoussi LR, Jarrett TW. The "mini-perc" technique: a less invasive alternative to percutaneous nephrolithotomy. World J Urol. 1998;16:371-4.
3. Desai J, Solanki R. Ultra-mini percutaneous nephrolithotomy (UMP): one more armamentarium. BJU Int. 2013;112:1046-9.
4. Zeng G, Wan S, Zhao Z, Zhu J, Tuerxun A, Song C, et al. Super-mini percutaneous nephrolithotomy (SMP): a new concept in technique and instrumentation. BJU Int. 2016;117:655-61.
5. Desai MR, Sharma R, Mishra S, Sabnis RB, Stief C, Bader M. Single-step percutaneous nephrolithotomy (microperc): the initial clinical report. J Urol. 2011;186:140-5.
6. Ruhayel Y, Tepeler A, Dabestani S, MacLennan S, Petİík A, Sarica K, et al. Tract Sizes in Miniaturized Percutaneous Nephrolithotomy: A Systematic Review from the European Association of Urology Urolithiasis Guidelines Panel. Eur Urol. 2017;72:220-35.
7. Cracco CM, Scoffone CM. ECIRS (Endoscopic Combined Intrarenal Surgery) in the Galdakao-modified supine Valdivia position: a new life for percutaneous surgery? World J Urol. 2011;29:821-7.
8. Kerbl K, Clayman RV, Chandhoke PS, Urban DA, De Leo BC, Carbone JM. Percutaneous stone removal with the patient in a flank position. J Urol. 1994;151:686-8.
9. Lehman T, Bagley DH. Reverse lithotomy: modified prone position for simultaneous nephroscopic and ureteroscopic procedures in women. Urology. 1988;32:529-31.
10. Papatsoris AG, Zaman F, Panah A, Masood J, El-Husseiny T, Buchholz N. Simultaneous anterograde and retrograde endourologic access: "the Barts technique". J Endourol. 2008;22:2665-6.
11. Valdivia Uría JG, Lachares Santamaría E, Villarroya Rodríguez S, Taberner Llop J, Abril Baquero G, Aranda Lassa JM. [Percutaneous nephrolithectomy: simplified technic (preliminary report)]. Arch Esp Urol. 1987;40:177-80.
12. Valdivia Uría JG, Valle Gerhold J, López López JA, Villarroya Rodriguez S, Ambroj Navarro C, Ramirez Fabián M, et al. Technique and complications of percutaneous nephroscopy: experience with 557 patients in the supine position. J Urol. 1998;160(6 Pt 1):1975-8.
13. Scoffone CM, Cracco CM. Invited review: the tale of ECIRS (Endoscopic Combined IntraRenal Surgery) in the Galdakao-modified supine Valdivia position. Urolithiasis. 2018;46:115-23.
14. Satava RM. Identification and reduction of surgical error using simulation. Minim Invasive Ther Allied Technol. 2005;14:257-61.
15. Dindo D, Demartines N, Clavien PA. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. Ann Surg. 2004;240:205-13.

16. Tokatlı Z, Gokce MI, Süer E, Saİlam R. Supine or prone position for mini-PNL procedure: does it matter. Urolithiasis. 2015;43:261-4.
17. Yuan D, Liu Y, Rao H, Cheng T, Sun Z, Wang Y, et al. Supine Versus Prone Position in Percutaneous Nephrolithotomy for Kidney Calculi: A Meta-Analysis. J Endourol. 2016;30:754-63.
18. Boon JM, Shinners B, Meiring JH. Variations of the position of the colon as applied to percutaneous nephrostomy. Surg Radiol Anat. 2001;23:421-5.
19. Valdivia JG, Scarpa RM, Duvdevani M, Gross AJ, Nadler RB, Nutahara K, et al. Supine versus prone position during percutaneous nephrolithotomy: a report from the clinical research office of the endourological society percutaneous nephrolithotomy global study. J Endourol. 2011;25:1619-25.
20. Duty B, Okhunov Z, Smith A, Okeke Z. The debate over percutaneous nephrolithotomy positioning: a comprehensive review. J Urol. 2011;186:20-5.
21. De Sio M, Autorino R, Quarto G, Calabrò F, Damiano R, Giugliano F, et al. Modified supine versus prone position in percutaneous nephrolithotomy for renal stones treatable with a single percutaneous access: a prospective randomized trial. Eur Urol. 2008;54:196-202.
22. Liu L, Zheng S, Xu Y, Wei Q. Systematic review and meta-analysis of percutaneous nephrolithotomy for patients in the supine versus prone position. J Endourol. 2010;24:1941-6.
23. Edgcombe H, Carter K, Yarrow S. Anaesthesia in the prone position. Br J Anaesth. 2008;100:165-83.
24. Patel RM, Okhunov Z, Clayman RV, Landman J. Prone Versus Supine Percutaneous Nephrolithotomy: What Is Your Position? Curr Urol Rep. 2017;18:26.
25. Al-Dessoukey AA, Moussa AS, Abdelbary AM, Zayed A, Abdallah R, Elderwy AA, et al. Percutaneous nephrolithotomy in the oblique supine lithotomy position and prone position: a comparative study. J Endourol. 2014;28:1058-63.
26. Giusti G, Proietti S, Pasin L, Casiraghi GM, Gadda GM, Rosso M, et al. Simultaneous Bilateral Endoscopic Manipulation for Bilateral Renal Stones. Urology. 2016;94:265-9.

Correspondence address:
Akif Erbin, MD
Department of Urology,
Haseki Traning and Research Hospital, Istanbul,
Turkey
Phone: + 90 506 543 1062
Fax: + 90 212 529 4400
E-mail: akiferbin@hotmail.com

ORIGINAL ARTICLE

Vol. 45 (5): 965-973, September - October, 2019
doi: 10.1590/S1677-5538.IBJU.2018.0781

Comparison of the outcomes of laparoscopic pyeloplasty with and without concomitant pyelolithotomy

Mustafa Kadihasanoglu [1], Ugur Yucetas [1], Emre Karabay [1], Erkan Sönmezay [1]

[1] *Department of Urology, Istanbul Training & Research Hospital, Istanbul, Turkey*

ABSTRACT

Objective: We aimed to evaluate the results of laparoscopic pyeloplasty with concomitant pyelolithotomy and compare results with a cohort of patients undergoing laparoscopic pyeloplasty without pyelolithotomy.
Materials and Methods: We retrospectively reviewed records of 43 patients undergoing transperitoneal laparoscopic Anderson-Hynes dismembered pyeloplasty between December 2012 and July 2018 at our department. Eighteen patients (42%) underwent laparoscopic pyeloplasty with concomitant pyelolithotomy. The results of patients with renal stones were compared with 25 matched patients undergoing laparoscopic pyeloplasty without concomitant renal stones. Demographic data, operative and stone parameters were compared between the groups.
Results: The groups were similar regarding to demographic characteristics. All operations were completed laparoscopically with no conversions to open surgery. In 3 cases without renal stones and 15 cases with renal stones, transposition of the ureter due to crossing vessels was performed. The mean stone size was 13±5.24 mm, and the median number of stones was 1 (1-18). The success of laparoscopic pyeloplasty with and without pyelolithotomy was 93.3% and 92.9%, respectively, as confirmed by negative diuretic renogram at postoperative 3rd months. Overall stone-free rate after laparoscopic pyelolithotomy was 93.3%. Mean operative time was 222.6765.71 minutes vs. 219.11±75.63 minutes for the pyeloplasty with concomitant pyelolithotomy vs. pyeloplasty, respectively (p=0.88).
Conclusions: Laparoscopic pyeloplasty with concomitant pyelolithotomy is a safe and effective intervention with associated good cosmetic results and high stone-free rates without significant increase in operative time or complications.

ARTICLE INFO

Mustafa Kadihasanoglu
https://orcid.org/0000-0001-5109-5319

Keywords:
Laparoscopy; Pyeloform [Supplementary Concept]; Cakut [Supplementary Concept]

Int Braz J Urol. 2019; 45: 965-73

Submitted for publication:
December 25, 2018

Accepted after revision:
May 06, 2019

Published as Ahead of Print:
May 30, 2019

INTRODUCTION

Ureteropelvic junction (UPJ) obstruction is the most frequently seen congenital abnormality of the upper urinary tract, with an incidence of 1 per 500 live births screened by routine antenatal ultrasound. A 70-fold increased risk of developing renal stones in UPJ obstruction has been estimated by Husmann et al. (1), due to stasis and infection associated with upper urinary tract obstruction.

The coexistence of UPJ obstruction and renal stone may worsen symptoms such as pain and fever, throughout the course of upper and lower urinary tract infections. The presence of nephrolithiasis in patients with UPJ obstruction

is an unquestionable indication for pyeloplasty with pyelolithotomy. Urologists face a therapeutic dilemma in patients with renal stones and UPJ obstruction, because of the selection of the appropriate treatment, because the extraction of stones from the renal pelvis is suitable at the time of dismembering the ureter from the pelvis; however, calyceal stones in peripheral calyxes with tight ostia are not very easy to treat. Although concurrent performance of open pyeloplasty and pyelolithotomy remains the gold standard therapy for the patients with UPJ obstruction and renal stones, multiple minimally invasive methods have been used for the surgical management of this coexistence. Laparoscopic transperitoneal pyeloplasty, which was introduced by Schuessler et al. in 1993 to minimize the morbidity associated with open pyeloplasty, is a preferred technique to open procedure for the outcomes (2). Recently, percutaneous endopyelotomy with nephrolithotomy, and laparoscopic pyeloplasty with concomitant pyelolithotomy are the preferred minimally invasive procedures for the coexistence of UPJ obstruction and nephrolithiasis (3, 4). The aim of this study was to compare the outcomes of laparoscopic pyeloplasty with and without pyelolithotomy.

MATERIALS AND METHODS

Between December 2012 and July 2018, 43 patients (43 renal units) with symptomatic UPJ obstruction that underwent laparoscopic repair and completed at least 3 months of postoperative follow-up were included in this retrospective study. Fifteen of these patients had concomitant non-obstructing pelvic or calyceal stones. We evaluated 25 men and 18 women with a mean age 33.63±12.7 years (range, 11-57 years). They were symptomatic prior to diagnosis, and presented with flank pain on the side of stone. Stones were on right side in 11, on the left side in 4 patients. Many patients had associated symptoms, such as hematuria or recurrent urinary tract infection. The patients were divided in two groups based on surgical techniques applied as follows: only laparoscopic pyeloplasty (Group-1; n=25), laparoscopic pyeloplasty with concomitant pyelolithotomy (Group-2; n=18).

The preoperative diagnosis of the UPJ obstruction, and the number, size and location of stones were determined by a combination of helical computerized tomography (CT) with and without contrast, intravenous pyelography (IVP), and diethylenetriaminepentaacetic acid (DTPA) diuretic renogram. Obstruction was defined as half-time more than 20 minutes after diuretic on renal scan, and as delayed nephrogram and/or excretion with hydronephrosis on radiological examinations. Indication of operation was made based on the presence of obstruction, clinical presentation of patient, and radiological findings. None of our patients had a history of previous surgical procedures for UPJ obstruction. All of patients were first-time stone formers with no history of stones or stone-related operations. The patients were subjected to transperitoneal laparoscopic Hynes-Anderson dismembered pyeloplasty and, if indicated, concomitant pyelolithotomy. No patients had intrarenal lithotripsy procedures.

The patient's charts were reviewed retrospectively to analyze grade of preoperative hydronephrosis (according to Society of Fetal Urology), number and size of stones, presence of crossing vessels, the duration of hospital stay, estimated blood loss, operative time, and anastomosis time. The operative time was defined as the time from first skin incision to last skin suture.

After surgery, patients were evaluated with a kidney-ureter-bladder film and renal ultrasound before discharge to check for residual stones. All patients were evaluated with DTPA scan and IVP after 3 months of surgery, and with ultrasound and DTPA scan annually. Success was defined as resolution of preoperative symptoms and hydronephrosis on diuretic renogram. Intraoperative complications were classified according to the Satava classification system (5), and postoperative complications were grouped based on the Clavien-Dindo grading system (6).

The pneumoperitoneum was established with a Veress needle positioned in the umbilicus and maintained at 12-15mm Hg throughout the procedure after induction of general anesthesia. The patient was placed in a modified 45º lateral decubitus position with the affected side up. After the abdomen was insufflated, a standard three-

-port transperitoneal technique was performed to maximize the working space and anatomical orientation. An 11mm trocar was placed in umbilicus for the camera in slim patients and children. In regular patients, 11mm trocar was placed lateral and superior to the umbilicus at the lateral border of the rectus abdominalis muscle. Five and 12mm trocars were placed at the midclavicular line, at the spino-umbilical line and subcostally, respectively. An additional 5mm port was used if needed.

After achieving pneumoperitoneum, the peritoneal incision along the white line of Toldt, division of the renocolic ligament, and the reflection of the colon medially off the kidney were performed using standard laparoscopic techniques in order to provide clear exposure of the ureter and renal pelvis. After sharp and blunt dissection of the renal pelvis, and making it free from adjacent structures, an initial pyelotomy incision was performed, in order to remove all renal and calyceal stones. The initial incision for mini-pyelotomy was made with the decision to perform Anderson-Hynes dismembered pyeloplasty, because this incision was incorporated into the final pyeloplasty. This incision was just long enough to use laparoscopic grasper of flexible cystoscope. The extraction of stones was accomplished by using laparoscopic graspers, and irrigation for flushing out smaller stones in the calices. If the stones could not be removed by these techniques, flexible cystoscope was placed through 12mm trocar with irrigation, and stone extraction was performed under direct vision using nitinol basket. Intraoperative fluoroscopy was used to confirm the clearance of stones. After the pyelolithotomy, by extending initial pyelotomy, redundant pelvic tissue was removed, the UPJ was circumferentially transected, and the ureter was spatulated at the lateral border towards the lower pole of the kidney to a sufficient length. In case of a crossing vessel, the same technique was used, and prior to the initiation of the anastomosis the ureter was transposed to the anterior of the vessel. A classic Anderson--Hynes pyeloplasty was performed using two running 4-0 polyglycolic-acid sutures for both anterior and posterior anastomosis. Intracorporeal knot tying was performed in a free-hand fashion. After the completion of the posterior wall suturing, a guidewire was inserted through a trocar in the ureter reaching the bladder, and a 4.7F double J (DJ) stent was placed in an antegrade fashion. After completing the anastomosis, a drain was finally passed through one of the port side in the retroperitoneum posterior to the anastomosis.

The urethral catheter was removed on first day of operation and the drain was removed when drain output was less than 50mL, after which was removed and the patient was discharged. The DJ stent was removed by cystoscopy after 6 weeks.

For statistical analyses, SPSS 14 (SPSS Inc., Chicago, IL, USA) was used. All data were expressed as the mean±standard deviation or median (interquartile range). The distribution of continuous variables was evaluated according to the Shapiro-Wilk normality test. If the distribution was normal, Student-t test was used for statistical analysis; if the distribution was not normal, Mann-Whitney U test was used. The categorical variables were analyzed by Fisher-exact test (two-tailed) or chi-square. A P value less than 0.05 was considered statistically significant.

RESULTS

The patients with renal stones were older than the patients without kidney stone (26.9410.94 vs. 41.07±12.73 years, p=0.004). The groups were similar regarding to other demographic characteristics and preoperative renal function (Table-1). In 15 patients of group-1, and 3 patients of group-2, transposition of the ureter was performed due to crossing vessel (p=0.03).

The mean operative time was 222.67±65.71 minutes in patients with kidney stones, and in those without renal calculi it was 219.11±75.63 minutes (p=0.88) (Table-2). The mean nephrolithotomy time was 30.67±13.1 minutes in patients with kidney stones. There was no statistically significant difference in

Table 1 - Baseline demographics and clinical characteristics.

	Group 1 (n=28)	Group 2 (n=15)	p
Age [years, mean±SD, (range)]	29.64±10.94 (11-53)	41.07±12.73 (24-57)	**0.004**
Gender [male/female, n (%)]	15 (53.6) / 13 (46.4)	10 (66.7) / 5 (33.3)	0.41
BMI [kg/m^2, mean±SD, (range)]	24.37±5.23 (18.03-4297)	27.40±5.38 (23.46-40.89)	0.15
Side [L/R, n (%)]	9 (32.1)/19 (67.9)	4 (26.7)/11 (73.3)	0.71
Grade of hydronephrosis [n (%)]			**0.69**
Grade 1	0 (0)	0 (0)	
Grade 2	12 (42.9)	6 (40)	
Grade 3	12 (42.9)	8 (53.3)	
Grade 4	4 (14.2)	1 (6.7)	
Crossing vessel [n (%)]	15 (53.6)	3 (20)	**0.03**
Preoperative split renal function [%, mean±SD, (range)]	39.11±12.14 (14-55)	39.27±10.35 (16-52)	0.97
$T_{1/2}$ [minutes, mean±SD, (range)]	30.75±12.18 (18-64)	27.67±7.88 (17-40)	0.38

anastomosis time between groups (59.64±20.23 vs. 52.33±11.78, p=0.21). There were no perioperative complications, and no patient required conversation to open surgery. The estimated blood loss was similar in both groups (p=0.71) (Table-2). The median hospitalization time and time to remove drain were significantly longer in group-2 than in group-1 (p=0.005 and p=0.005, respectively) (Table-2) (Figures 1 and 2).

The median number of stones removed was 1 (range 1-18) and the mean stone size was 13±5.24mm (range, 6-28mm) (Table-3). Most of these stones were located in lower calyces (Table-3). Flexible nephroscope and nitinol basket were used to extract the stones. The stones were collected in specimen retrieval bag and extracted at the end of the surgery.

The mean follow-up of patients with and without renal stones was not statistically different between groups (p=0.66) (Table-2). All patients became stone-free at 3 months, and with no evidence of residual stone on imaging after operation. At the 3rd month after operation the success of operation was assessed objectively with diuretic renogram. These diuretic renograms revealed normal drainage in 26/28 patients in group-1 and in 14/15 patients in group-2.

DISCUSSION

The surgical treatment of kidney stones in the last 25 years has developed from primarily an open surgery to include non-invasive shock wave lithotripsy (SWL) and several minimally invasive treatment modalities. SWL, flexible ureteroscopy, and percutaneous nephrolithotomy (PCNL) have markedly reduced the morbidity of treating kidney stones. Nonetheless, renal stone patients needing simultaneous treatment of UPJ obstruction might still require open procedures, because they cannot be treated with SWL. The success rate of open pyeloplasty and pyelolithotomy has been reported as 90% (7). However, open surgery has several handicaps including unfavorable cosmetic results due to long incision, substantial postoperative pain because of the lombotomy incision,

Table 2 - Operative characteristics of patients.

	Group 1 (n=28)	Group 2 (n=15)	p
Mean operative time [minutes, mean±SD, (range)]	219.11±75.63 (110-420)	222.67±65.71 (90-360)	0.88
Anastomosis time [minutes, mean±SD, (range)]	59.64±20.23 (30-120)	52.33±11.78 (30-65)	0.21
Renal Pelvis Reduction [n (%)]	8 (28.6)	3 (20)	0.54
Estimated blood loss [mL, median (IQR), (range)]	50 (38) (10-150)	50 (60) (10-100)	0.71
Drain stay time days, [median (IQR), (range)]	3 (1) (2-7)	4 (1) (2-5)	**0.005**
Hospital stay [days, median (IQR), (range)]	4 (1) (3-7)	5 (3) (3-8)	**0.005**
Stent time [days, mean±SD, (range)]	42.54±12.30 (30-92)	45.80±18.37 (30-103)	0.69
Success [n (%)]	26 (92.9)	14 (93.3)	0.73
Follow-up [months, median (IQR), (range)	12 (26) (3-52)	14 (23) (3-54)	0.66
Postoperative split renal function at 3th months [%, median (IQR), (range)]	41 (17) (14-50)	41 (15) (17-48)	0.97
Postoperative $T_{1/2}$ [minutes, mean±SD, (range)]	16.75±4.53 (8-26)	16.93±2.76 (12-23)	0.89

Figure 1 - Distribution of postoperative duration of hospital stay between laparoscopic pyeloplasty and laparoscopic pyeloplasty with pyelolithotomy.

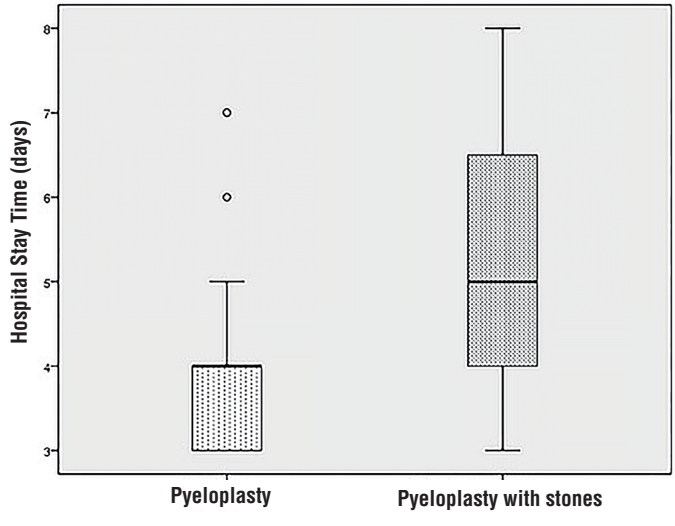


Figure 2 - Distribution of time to remove drain between laparoscopic pyeloplasty and laparoscopic pyeloplasty with pyelolithotomy.

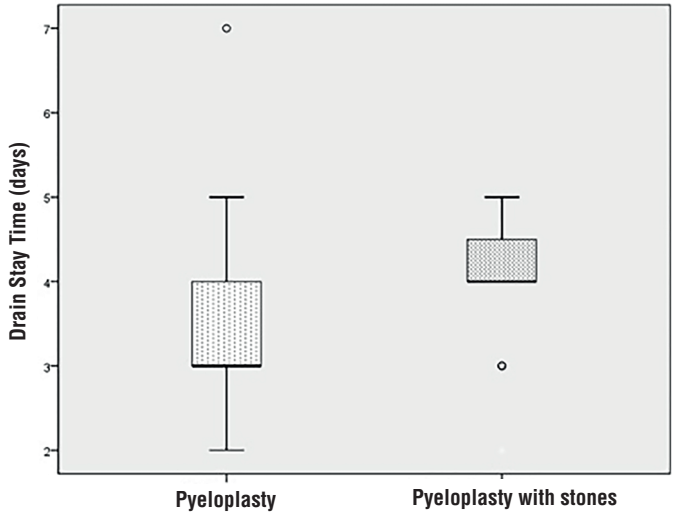


Table 3 - Sstone characteristics of patients.

Variables	
Mean stone diameter [mm, mean±SD, (range)]	13.00±5.24 (6-28)
Number of stones removed [median(IQR), (range)]	1 (1) (1-18)
Stone location [n(%)]	
Renal pelvis	3 (20)
Middle calyx	3 (20)
Lower calyx	9 (60)
Mean lithotomy time [minutes, mean±SD, (range)]	30.67±13.1 (5-50)
Using flexible nephroscope for lithototmy [n(%)]	12 (80)
Stone free [n(%)]	14 (93.3)

and prolonged hospitalization and convalescence times. Hence, open surgery for renal stones with UPJ obstruction has diminished because the introduction of minimally invasive surgery.

Several minimal endoscopic interventions have been evolved to reduce the unfavorable drawbacks of open pyeloplasty and pyelolithotomy. Until recently, antegrade endopyelotomy with concomitant PCNL was the minimally invasive treatment of choice with success rate of 56-90%, but much lower than open surgery (8-13). However, it is not indicated to perform antegrade endopyelotomy with PCNL in some cases of severe hydronephrosis, crossing vessels, strictures longer than 2cm, renal failure, bleeding disorder, and extended periureteral inflammation (14). In our series, we detected significant crossing vessels with helical CT in 15 patients without renal stones and 3 patients with renal stones. Another significant disadvantage of PCNL performed with antegrade endopyelotomy is requiring an upper pole access which may cause pleural excursion. Also, its lower success rate is another disadvantage in comparison with laparoscopic and robotic surgery (15).

The development of technology allowed for the combination of the benefits of minimally invasive surgery with the higher success rate of open procedures by presenting laparoscopic pyeloplasty and pyelolithotomy (2, 16). The first dismembered laparoscopic pyeloplasty series was presented by Schuessler et al. in 1993 (2), and its success rate was between 96-98% (16-19). In addition, it has been shown that laparoscopic pyeloplasty is an efficient procedure as a salvage operation in cases of failed endopyelotomies (12). The feasibility and safety of laparoscopic pyeloplasty with concomitant pyelolithotomy was supported by several studies that reported excellent stone-free rates and functional results, ranging between 90-100% (Table-4) (4, 20-27). However, these studies included small number of patients because of the rarity of the combination of UPJ obstruction and renal stone. Lusuardi and Janetschek suggested that laparoscopic pyelolithotomy may be offered as first choice treatment when variations are involved, such as UPJ obstruction (28). Therefore, the European Association of Urology Guidelines recommend laparoscopy for stones with UPJ obstruction (29). However, while laparoscopic pyeloplasty and pyelolithotomy have very important advantages, the major drawbacks of laparoscopic surgery are longer operative time and a steep learning curve (4).

This study presents the author's first experiences with laparoscopic pyeloplasty and pyelolithotomy, which seems to be a safe and efficient procedure for patients presenting with UPJ obstruction and concomitant kidney stones. Our result showed no significant impact of concomitant pyelolithotomy on operative time, estimated blood loss, anastomosis time, complications, and success rates when pyeloplasty was performed simultaneously with pyelolithotomy. Despite of approximately 30 minutes for stone extraction, the operative time of laparoscopic pyeloplasty with pyelolithotomy was similar to laparoscopic pyeloplasty without pyelolithotomy. None of patients required conversion to open surgery. Our conversion rate is similar to other series ranging between 0-5.4 (14, 19). However, the mean hospitalization time was longer in patients with renal stones (p=0.016). The mean hospital stay of

Table 4 - Contemporary series of pyeloplasty with concomitant pyelolithotomy.

	#renal units (n)	#stones [n (range)]	Stone size (mean mm)	Operative time (minutes)	EBL (mL)	Hospital stay (days)	Pyeloplasty success rate (%)	Stones-free rate (%)	Follow-up (months)
Ramakumar	20	1* (1-28)	N/A	276	145	3.4	90	90	12
Ball	7	2.5 (1-4)	10.3	N/A	N/A	N/A	100	85.7	8.5
Srivastava	20	2* (1-12)	15	168	69.5	4.9	90	100	34
Stein	15	6.2 (1-21)	5.8	174	53.3	1.6	93.3	80	5.4
Rivas	12	N/A	1.53*	N/A	N/A	N/A	91.6	100	N/A
Stravodimos	13	8.2 (1.32)	8.7	218.8	N/A	7	100	84.6	30.2
Naitoh	4	1.6 (1-4)	11.5	277	9.5	N/A	N/A	100	N/A
Kouriefs	6	(1-6)	1-20	180 (150-220)*	50 (50-100)	2*	100	100	(18-87)
Nambirajan	1	1	2	N/A	N/A	N/A	100	100	N/A
Present study	18	1* (1-18)	13	222.67	50*	5*	93.3	100	14*

*: median

our patients is similar to those previously published in the literature (Table-4).

Currently, the general consensus on the follow-up after laparoscopic pyeloplasty is to perform a diuretic renogram at the postoperative 3rd month. An unobstructed drainage in diuretic renogram and/or IVP is accepted as success (30). In our study, diuretic renogram at the postoperative 3rd month demonstrated that $T_{1/2}$ decreased from 23.18 to 15.8 in patients without renal stones (p=0.034), and from 24.55 to 16.45 in patients with renal stones (p=0.038). Moreover, there was not any difference between the groups in relation to the reduction of $T_{1/2}$. Because the most of the failure of pyeloplasty occurs usually within the first postoperative year, and an unobstructed 3-month renogram is followed by unobstructed 1-year renogram, the follow-up of the patients undergoing pyeloplasty is not necessary after the first year (30, 31). However, several studies demonstrated that the long-term success rates of endopyelotomy and pyeloplasty are worse than previously reported (32, 33). Most failures occur within the first 2 years for pyeloplasty and endopyelotomy and there are still failures as follow-up continues (33). In our study, the median follow-up of the patients with and without renal stones were 11 and 12 months, respectively, and these are not different from the other series. Therefore, we still follow-up our patients accordingly with annual ultrasound, although our success rate was over 90% in both groups. These findings indicate the laparoscopic pyeloplasty with concomitant pyelolithotomy is a highly effective procedure for UPJ obstruction and renal stones. Also, the presence of renal stones in hydronephrotic kidney due to the UPJ obstruction does not affect the success and complication rates of laparoscopy.

The stone-free rate of our operations was 93.3%. This rate is similar to the rates of previous studies (Table-4). The most important factors influencing the stone-free rate of these operations are the number, size, and location of stones (24). If the patient has a small single stone in renal pelvis, the rate of being stone-free is higher than the patients with multiple, large calyceal stones. The median number of stones in our patients was 1, and the mean size of these stones was 13mm. These results are in accordance with the results of the studies in the literature (4, 27). However, our stone size is slightly larger than the stones of the other studies (22, 24, 27). Our mean operative times of both types of operations are in accordance with the other results presented in the literature (4, 24).

The limitation of this study is its retrospective nature. However, the number of patients is one of the highest in the literature (Table-4). Another importance of this study is that the data were collected from a single institution.

CONCLUSIONS

Laparoscopic pyeloplasty with concomitant pyelolithotomy is an effective and safe alternative to open surgery for patients with renal stones and UPJ obstruction. The addition of pyelolithotomy might prolong the operation and hospitalization time. However, it does not affect the success or complication rates.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Husmann DA, Milliner DS, Segura JW. Ureteropelvic junction obstruction with concurrent renal pelvic calculi in the pediatric patient: a long-term followup. J Urol. 1996;156(2 Pt 2):741-3.
2. Schuessler WW, Grune MT, Tecuanhuey LV, Preminger GM. Laparoscopic dismembered pyeloplasty. J Urol. 1993;150:1795-9.
3. Desai RA, Assimos DG. Role of laparoscopic stone surgery. Urology. 2008;71:578-80.
4. Ramakumar S, Lancini V, Chan DY, Parsons JK, Kavoussi LR, Jarrett TW. Laparoscopic pyeloplasty with concomitant pyelolithotomy. J Urol. 2002;167:1378-80.
5. Satava RM. Identification and reduction of surgical error using simulation. Minim Invasive Ther Allied Technol. 2005;14:257-61.
6. Dindo D, Demartines N, Clavien PA. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of survey. Ann Surg. 2004;240:205-13.
7. Scardino PT, Scardino PL. Obstruction at the ureteropelvic junction. In: Bergman H, Hrsg. The Ureter. 2nd. Aufl. New York: Springer-Verlag; 1981; p. 697.

8. Motola JA, Badlani GH, Smith AD. Results of 212 consecutive endopyelotomies: an 8-year followup. J Urol. 1993;149:453-6.
9. Cassis AN, Brannen GE, Bush WH, Correa RJ, Chambers M. Endopyelotomy: review of results and complications. J Urol. 1991;146:1492-5.
10. Meretyk I, Meretyk S, Clayman RV. Endopyelotomy: comparison of ureteroscopic retrograde and antegrade percutaneous techniques. J Urol. 1992;148:775-82.
11. Berkman DS, Landman J, Gupta M. Treatment outcomes after endopyelotomy performed with or without simultaneous nephrolithotomy: 10-year experience. J Endourol. 2009;23:1409-13.
12. Knudsen BE, Cook AJ, Watterson JD, Beiko DT, Nott L, Razvi H, et al. Percutaneous antegrade endopyelotomy: long-term results from one institution. Urology. 2004;63:230-4.
13. Desai MM, Desai MR, Gill IS. Endopyeloplasty versus endopyelotomy versus laparoscopic pyeloplasty for primary ureteropelvic junction obstruction. Urology. 2004;64:16-21.
14. Eden CG. Minimally invasive treatment of ureteropelvic junction obstruction: a critical analysis of results. Eur Urol. 2007;52:983-9.
15. Atug F, Castle EP, Burgess SV, Thomas R. Concomitant management of renal calculi and pelvi-ureteric junction obstruction with robotic laparoscopic surgery. BJU Int. 2005;96:1365-8.
16. Kavoussi LR, Peters CA. Laparoscopic pyeloplasty. J Urol. 1993;150:1891-4.
17. Jarrett TW, Chan DY, Charambura TC, Fugita O, Kavoussi LR. Laparoscopic pyeloplasty: the first 100 cases. J Urol. 2002;167:1253-6.
18. Moon DA, El-Shazly MA, Chang CM, Gianduzzo TR, Eden CG. Laparoscopic pyeloplasty: evolution of a new gold standard. Urology. 2006;67:932-6.
19. Inagaki T, Rha KH, Ong AM, Kavoussi LR, Jarrett TW. Laparoscopic pyeloplasty: current status. BJU Int. 2005;95(Suppl 2):102-5.
20. Ball AJ, Leveillee RJ, Patel VR, Wong C. Laparoscopic pyeloplasty and flexible nephroscopy: simultaneous treatment of ureteropelvic junction obstruction and nephrolithiasis. JSLS. 2004;8:223-8.
21. Srivastava A, Singh P, Gupta M, Ansari MS, Mandhani A, Kapoor R, et al. Laparoscopic pyeloplasty with concomitant pyelolithotomy--is it an effective mode of treatment? Urol Int. 2008;80:306-9.
22. Stein RJ, Turna B, Nguyen MM, Aron M, Hafron JM, Gill IS, et al. Laparoscopic pyeloplasty with concomitant pyelolithotomy: technique and outcomes. J Endourol. 2008;22:1251-5.
23. Rivas JG, Alonso Y Gregorio S, Sánchez LC, Guerin Cde C, Gómez AT, Togores LH, et al. Approach to kidney stones associated with ureteropelvic junction obstruction during laparoscopic pyeloplasty. Cent European J Urol. 2014;66:440-4.
24. Stravodimos KG, Giannakopoulos S, Tyritzis SI, Alevizopoulos A, Papadoukakis S, Touloupidis S, et al. Simultaneous laparoscopic management of ureteropelvic junction obstruction and renal lithiasis: the combined experience of two academic centers and review of the literature. Res Rep Urol. 2014;6:43-50.
25. Naitoh Y, Kawauchi A, Kamoi K, Soh J, Hongo F, Okihara K, et al. Nephrolithotomy performed concurrently with laparoendoscopic single-site pyeloplasty. Urology. 2014;83:243-6.
26. Kouriefs C, Georgiades F, Grange P. Stones First! A Gas Pyelo-nephroscopy Strategy for Laparoscopic Pyeloplasty and Renal Stone Extraction. Urology. 2017;109:206-9.
27. Nambirajan T, Jeschke S, Albqami N, Abukora F, Leeb K, Janetschek G. Role of laparoscopy in management of renal stones: single-center experience and review of literature. J Endourol. 2005;19:353-9.
28. Lusuardi L, Janetschek G. Indications and outcomes of laparoscopic uretero-renal stone surgery. Curr Opin Urol. 2011;21:161-5.
29. Bultitude M, Smith D, Thomas K. Contemporary Management of Stone Disease: The New EAU Urolithiasis Guidelines for 2015. Eur Urol. 2016;69:483-4.
30. Pohl HG, Rushton HG, Park JS, Belman AB, Majd M. Early diuresis renogram findings predict success following pyeloplasty. J Urol. 2001;165(6 Pt 2):2311-5.
31. Psooy K, Pike JG, Leonard MP. Long-term followup of pediatric dismembered pyeloplasty: how long is long enough? J Urol. 2003;169:1809-12; discussion 1812; author reply 1812.
32. Dimarco DS, Gettman MT, McGee SM, Chow GK, Leroy AJ, Slezak J, et al. Long-term success of antegrade endopyelotomy compared with pyeloplasty at a single institution. J Endourol. 2006;20:707-12.
33. Yanke BV, Lallas CD, Pagnani C, McGinnis DE, Bagley DH. The minimally invasive treatment of ureteropelvic junction obstruction: a review of our experience during the last decade. J Urol. 2008;180:1397-402.

Correspondence address:
Mustafa Kadihasanoglu, MD
Department of Urology
Istanbul Training and Research Hospital
Fatih, Istanbul, Turkey
Telephone: + 90 212 459-6000
E-mail: kadihasanoglu@gmail.com

ORIGINAL ARTICLE

Vol. 45 (5): 974-980, September - October, 2019
doi: 10.1590/S1677-5538.IBJU.2019.0026

Prevalence of enuresis and its impact in quality of life of patients with sickle cell disease

Alana de Medeiros Nelli [1], Flávia Cristina de Carvalho Mrad [2], Mateus de Andrade Alvaia [1], Heros Aureliano Antunes da Silva Maia [1], Carina Oliveira Silva Guimarães [3], Evanilda Souza de Santana Carvalho [3], Cristiano Mendes Gomes [3], José Murillo Bastos Netto [4,5,6], José de Bessa Junior [1,3]

[1] Departamento de Cirurgia Universidade Estadual de Feira de Santana, Feira de Santana, BA, Brasil; [2] Departamento de Pediatria, Unidade de Nefrologia Pediátrica, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brasil.; [3] Programa de Pós-Graduação em Saúde Coeltiva, Universidade Federal de Juiz de Fora, Juiz de For a, MG, Brasil; [4] Departamento de Cirurgia, Faculdade de Ciências Médicas e da Saúde de Juiz de Fora; [5] Faculdade de Ciências Médicas e da Saúde de Juiz de Fora, MG, Brasil; [6] Hospital e Maternidade Terezinha de Jesus de Juiz de Fora, Juiz de Fora, MG, Brasil

ABSTRACT

Introduction: Evidence indicates an increase in the prevalence of enuresis in individuals with sickle cell disease. The present study aims to evaluate the prevalence and impact of enuresis on quality of life in individuals with sickle cell disease.
Materials and Methods: This cross-sectional study evaluated individuals with sickle cell disease followed at a reference clinic, using a questionnaire designed to evaluate the age of complete toilet training, the presence of enuresis and lower urinary tract, and the impact on quality of life of these individuals.
Results: Fifty children presenting SCD (52% females, mean age ten years) were included in the study. Of those, 34% (17/50) presented as HbSC, 56% with HbSS (28/50), 2% Sα-thalassemia (1/5) and 8% the type of SCD was not determined. The prevalence of enuresis was 42% (21/50), affecting 75% of subjects at five years and about 15% of adolescents at 15 years of age. Enuresis was classified as monosymptomatic in 33.3% (7/21) and nonmonosymptomatic in 66.6% (14/21) of the cases, being primary in all subjects. Nocturia was identified in 24% (12/50), urgency in 20% (10/50) and daytime incontinence 10% (5/50) of the individuals. Enuresis had a significant impact on the quality of life of 67% of the individuals.
Conclusion: Enuresis was highly prevalent among children with SCD, and continues to be prevalent throughout early adulthood, being more common in males. Primary nonmonosymptomatic enuresis was the most common type, and 2/3 of the study population had a low quality of life.

ARTICLE INFO

Flávia Cristina de Carvalho Mrad
http://orcid.org/0000-0002-8072-2091

Keywords:
Quality of Life; Sickle Cell Trait; Enuresis

Int Braz J Urol. 2019; 45: 974-80

Submitted for publication:
January 10, 2019

Accepted after revision:
May 06, 2019

Published as Ahead of Print:
August 01, 2019

INTRODUCTION

Sickle cell disease (SCD) is an autosomal recessive hereditary disease in which hemoglobin S is present (1, 2). According to the type of alteration present in hemoglobin, SCD can be classified in different clinical forms: homozygous form SS (referred to as sickle cell anemia-HbSS), and heterozygous forms, represented by associations of HbS with other hemoglobin defects (SC, HbS/

β0 thalassemia, HbS/β + thalassemia, S/α thalassemia) (1). SCD is the most common congenital hemoglobinopathy and affects mainly Africans or their descendants in America, being responsible for more than 300.000 live births per year (3, 4). In Brazil, 60.000 to 100.000 cases of the disease are currently estimated. SCD requires multi-professional approach for early diagnosis and management, due to its physical, psychological and socioeconomic impact, with high morbidity and mortality (1).

Lower urinary tract symptoms (LUTS) are also common in children occurring in about 14.7 to 21.8% (5, 6). LUTS is characterized by abnormal urine storage and/or bladder emptying in the absence of urinary tract infections, neurological or anatomical abnormalities (7, 8). Enuresis is both a symptom and a condition of intermittent incontinence that occurs during periods of sleep after the age of five years. According to symptoms, enuresis is classified as monosymptomatic, when no other symptom is present and non-monosymptomatic when associated with LUTS (8). Prevalence of enuresis in children aged 6 to 13 years varies from 9.5% to 12.9% (9, 10). Enuresis has adverse emotional and social effects that affect children's quality of life (11).

The prevalence of enuresis in children with SCD is approximately 32% (12, 13) and its etiopathogenesis is still controversial (14). It has been related to nocturnal polyuria induced by hyposthenuria (13, 15, 16) and low functional bladder capacity (14, 17). Hyposthenuria is one of the earliest organic manifestations of SCD occurring as early as 12 months of age (13, 18). However, in a more recent study, Eneh et al. have shown that enuresis in children with SCD appears not to be related to hyposthenuria, but to other causal factors that apply to the general population (14).

The present cross-sectional study was conducted to estimate the prevalence of enuresis in individuals with SCD and its impact in the quality of life. We have hypothesized that enuresis is more prevalent in children, adolescents and young adults with SCD than described for general population, and that it greatly impacts the quality of life of this population.

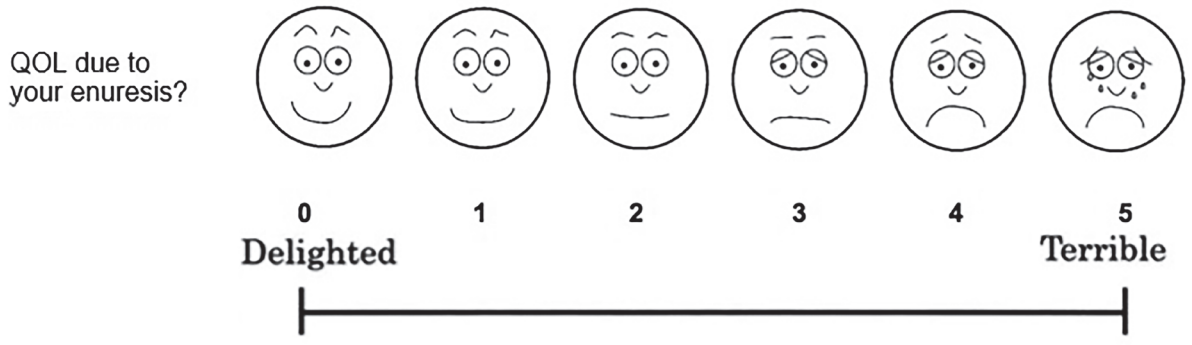
MATERIALS AND METHODS

This cross-sectional study was carried out from July 2016 to September 2017. During this period, 50 consecutive patients (children, adolescent, and young adults) with SCD, aged 5 to 24 years, who regularly attended the regional outpatient reference center for SCD, were evaluated. Patients with current urogenital disorder, current use of medications or diseases known to interfere with bladder or sphincter function, such as urinary tract infection and severe intellectual disability, and those not yet toilet trained were not included in the study.

A questionnaire was developed for this study and applied to the participants and/or their caregivers. Questions related to sociodemographic characteristics, hemoglobin electrophoresis for detect different hemoglobin genotypes, age of acquisition of complete toilet training, presence of LUTS and enuresis (including frequency and classification: monosymptomatic or non-monosymptomatic) were addressed. The Visual Analogue Scale (VAS), adapted from Ushijima et al., 2006 (19), with appropriate facial expressions throughout the VAS was used to analyze the quality of life. The VAS used in this study was a 10cm line ranging from delighted at the left end of the line up to terrible at the right end of the line. The subjects were asked to assign a score of 0 to 5 according to the intensity with which the enuresis affected their well-being with faces graphic expressing each note. The higher the score, the more uncomfortable the impact and the subject was in a situation (19, 20) (Figure-1).

Quantitative variables, continuous or ordinal, were described by measures of central tendency (mean or median) and the respective dispersion measures (standard deviation, interquartile range or minimum and maximum values). Nominal or qualitative variables were described for their absolute values, percentages or proportions. To compare the differences of continuous variables, we used the Student t-test or the Mann-Whitney test. For comparison of categorical data, we used the chi-square test and its variants. The association between the parameters studied was expressed by the prevalence ratio (Odds Ratio). A 95% confi-

Figure 1 - Visual Analog Scale.



Visual Analogue Scale (VAS) adapted from Ushijima et al., 2006

VAS questionnaire to assess discomfort or satisfaction regarding patient's quality of life (QOL). How would you rate your discomfort with enuresis? The face scales were demostrated to help understanding, includinf: 1) laughing face to represent delighted or pleased above left end of VAS, 2) smiling face to represent mostly satisfied above left side to center of VAS, 3) face with neutral expression to represent neither satisfied and dissatisfied above center of VAS, 4) face in trouble to represent mostly dissatisfied above righr side to center of VAS and 5) crying face to represent unhappy or terrible above right end of VAS.

dence intervals were used as measures of precision of the results and p values less than 0.05 ($p < 0.05$) were considered significant. In the analysis, we used computational statistical software (GraphPad Prism, version 8.0.0, GraphPad Software, San Diego-CA, USA).

The study was approved by the institution ethics committee, (CEP-UEFS #1.440.239) and all participants or legal representative that agreed in participate in the study signed a free and informed consent.

RESULTS

In this study, a total of 50 patients with SCD were evaluated. The mean age at enrollment was of 10 years [7-15], being 52% (26/50) female. SCD genotypes diagnosed by hemoglobin electrophoresis at alkaline pH are shown in Table-1.

Of the 50 individuals evaluated, enuresis was identified in 42% (21/50) of the cases, affecting 75% of the subjects at five years and about 15% of the adolescents at 15 years of age (Figure-2). Enuresis was classified as monosymptomatic in 33.3% (7/21) and non-monosymptomatic in 66.6% (14/21) of the cases, being primary in 100% of all subjects. In 62% of subjects frequency, enuresis episodes were more than three times a week. Nocturia was identified in 24% (12/50), urgency in 20% (10/50) and daytime incontinence 10% (5/50) of the individuals.

Enuresis appears to be more common in males (OR=1.89 [0.59-5.38]), but no statistical significance was found (p=0.395). Toilet training daytime was finished before completing two years of age in 32% (16/50) of patients, 24% (12/50) between two and three years, 20% (10/50) between three and four years, 14% (7/50) between four and five. The other five did not remember when toilet training was completed. No correlation was found between the age of completing toilet training daytime and the prevalence of enuresis.

Enuresis had a negative impact on the quality of life of those affected. Asymptomatic patients had significantly higher scores in VAS than symptomatic ones (9.26 ± 0.92 and

Table 1- General characteristics of the study population (n=50).

	Description	Percentage
Gender	Female	52 (26/50)
	Male	48 (24/50)
Age (years)	6-11	58(29/50)
	12-18	30(15/50)
	19-24	12(06/50)
Genotype	SS	58(28/50)
	SC	34(17/50)
	Sα-thalassemia	02(01/50)
	Undetermined	08 (04/50)

7.22±3.44, respectively- p=0.015). According to VAS, 67% (14/21) of the study subjects considered that enuresis had a serious impact on their quality of life.

DISCUSSION

The present study is in agreement with previous studies that have shown a significantly increase in the prevalence of enuresis in subjects with SCD (13, 15, 17, 21), as evidenced in 42% of the population studied herein. A recent study recruited 243 children with SCD aged between 5 and 17 years and showed a prevalence of 49.4% of enuresis versus 29.6% in the control group (22). The inclusion of patients as old as 24 years of age is justified as these studies have shown that enuresis remains prevalent in adolescents and young adult with SCD (12, 16, 22).

Although some studies have reported hyposthenuria as the main determinant of enuresis in individuals with sickle cell disease (23), recent studies have not shown this association (14).

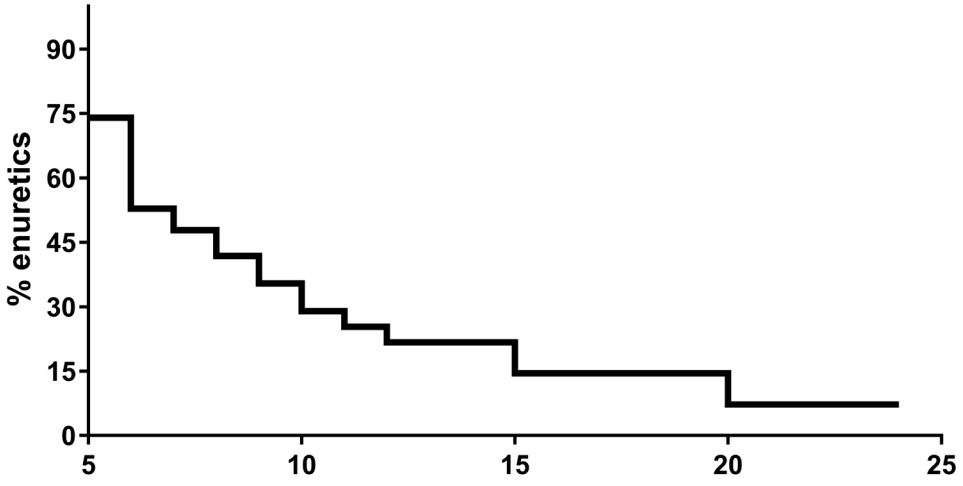
In the present study, there was a higher prevalence of monosymptomatic enuresis (66%) and all cases were primary enuresis. This finding is in agreement with Portacarrero et al. who showed that children and adolescents with SCD had non-monosymptomatic and primary enuresis in 58% and 86% of the cases, respectively. Another recent study also demonstrated an increased prevalence of primary non-monosymptomatic enuresis in SCD patients (24).

It is important to note that 15% of adolescents at 15 years of age had enuresis. These results are also similar to those of Portacarrero et al. that demonstrated the prevalence of 21% of enuresis in adolescents with sickle cell disease (15-18 years of age) (12). They are also compatible with findings from Field et al. and Esezobor et al. who documented persistently high rates of enuresis of 18% and 25% among adolescents over 14 years of age, respectively (22, 25). On the contrary, studies in healthy population have shown that the prevalence of enuresis decreases with age to around 1 to 3% at 15 years (11, 26). Therefore, the well-established decline in the prevalence of enuresis with age was less pronounced in individuals with sickle cell disease.

Although Mabiala et al. documented a higher prevalence of enuresis in females with SCD (27), our findings are in agreement with the majority of other studies (12, 16, 22) that have found a higher prevalence of enuresis in males. The reason for this preponderance in males is still unclear. No correlation between age of acquisition diurnal urinary control and the presence of enuresis and other lower urinary tract symptoms, as in Down syndrome patients was found (7).

An increased prevalence of urgency and

Figure 2 - Prevalence of enuresis according to age.



daytime incontinence was present in this series as well as in other series (15). Portacarrero et al. demonstrated 33% of urgency and 23% of daytime incontinence in SCD (12). In addition to these LUTS, 24% of subjects in the present study had nocturia. Enuresis and nocturia are common in children with SCD being reported to be present in 68 to 79% of the patients (15, 23, 25) Further prospective studies are needed to clarify the pathophysiological mechanisms underlying the urinary manifestations of SCD.

In this study, enuresis negatively affected the quality of life in 67% of the individuals. SCD is characterized by systemic complications including thoracic syndrome, chronic lung disease, cardiomyopathy, splenic infarction, chronic liver disease, bone infarction, osteomyelitis and depression (28, 29). All these complications, with prolonged hospitalizations and frequent chronic pain, significantly reduce quality of life (29, 30). Although quality of life of all patients included in the study may be compromised by SCD, those presenting enuresis had an even worse score when compared to non-enuretic ones, showing that enuresis negatively impact quality of life, as previously demonstrated by Savaser et al. for general population (11).

This study has several limitations small sample size and absence of a control group, family history of enuresis was not investigated, the main complications of SCD, which may have negative impact patient quality of life, have not been addressed and presence of intestinal constipation did not was evaluated.

CONCLUSIONS

Enuresis was highly prevalent among children with SCD, and continues to be prevalent throughout early adulthood, especially in males generating a negative impact on quality of life. In all cases, enuresis was primary enuresis, and in the majority it was non-monosymptomatic. These findings are important to alert the parents and professionals involved in the follow-up of these patients about the need for diagnosing and treating this condition.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Azar S, Wong TE. Sickle Cell Disease: A Brief Update. Med Clin North Am. 2017;101:375-93.

2. Lobitz S, Telfer P, Cela E, Allaf B, Angastiniotis M, Backman Johansson C, et al. Newborn screening for sickle cell disease in Europe: recommendations from a Pan-European Consensus Conference. Br J Haematol. 2018;183:648-60.
3. Serjeant G. World Sickle Cell Day: Lessons for India. Indian J Med Res. 2017;145:705-7.
4. Ware RE, de Montalembert M, Tshilolo L, Abboud MR. Sickle cell disease. Lancet. 2017;390:311-23.
5. Mrad FCC, Figueiredo AA, Bessa J Jr, Bastos Netto JM. Prolonged toilet training in children with Down syndrome: a case-control study. J Pediatr (Rio J). 2018;94:286-92.
6. Vaz GT, Vasconcelos MM, Oliveira EA, Ferreira AL, Magalhães PG, Silva FM, et al. Lima EM. Prevalence of lower urinary tract symptoms in school-age children. Pediatr Nephrol. 2012;27:597-603.
7. Austin PF, Bauer SB, Bower W, Chase J, Franco I, Hoebeke P, et al. The standardization of terminology of lower urinary tract function in children and adolescents: update report from the Standardization Committee of the International Children's Continence Society. J Urol. 2014;191:1863-1865.e13.
8. Austin PF, Bauer SB, Bower W, Chase J, Franco I, Hoebeke P, et al. The standardization of terminology of lower urinary tract function in children and adolescents: Update report from the standardization committee of the International Children's Continence Society. Neurourol Urodyn. 2016;35:471-81.
9. Sarici H, Telli O, Ozgur BC, Demirbas A, Ozgur S, Karagoz MA. Prevalence of nocturnal enuresis and its influence on quality of life in school-aged children. J Pediatr Urol. 2016;12:159.e1-6.
10. Srivastava S, Srivastava KL, Shingla S. Prevalence of monosymptomatic nocturnal enuresis and its correlates in school going children of Lucknow. Indian J Pediatr. 2013;80:488-91.
11. Savaser S, Kizilkaya Beji N, Aslan E, Gozen D. The Prevalence of Diurnal Urinary Incontinence and Enuresis and Quality of Life: Sample of School. Urol J. 2018;15:173-9.
12. Portocarrero ML, Portocarrero ML, Sobral MM, Lyra I, Lordêlo P, Barroso U Jr. Prevalence of enuresis and daytime urinary incontinence in children and adolescents with sickle cell disease. J Urol. 2012;187:1037-40.
13. Wolf RB, Kassim AA, Goodpaster RL, DeBaun MR. Nocturnal enuresis in sickle cell disease. Expert Rev Hematol. 2014;7:245-54.
14. Eneh CI, Ikefuna AN, Okafor HU, Uwaezuoke SN. Nocturnal enuresis in school-aged children with sickle-cell anemia: Any relationship with hyposthenuria? Niger J Clin Pract. 2017;20:215-20.
15. Claudino MA, Fertrin KY. Sickling cells, cyclic nucleotides, and protein kinases: the pathophysiology of urogenital disorders in sickle cell anemia. Anemia. 2012;2012:723520.
16. Eneh CI, Okafor HU, Ikefuna AN, Uwaezuoke SN. Nocturnal enuresis: prevalence and risk factors among school-aged children with sickle-cell anaemia in a South-east Nigerian city. Ital J Pediatr. 2015;41:66.
17. Tewari S, Rees DC, Hannemann A, Gbotosho OT, Al Balushi HW, Gibson JS. Nocturnal enuresis and K+ transport in red blood cells from patients with sickle cell anemia. Haematologica. 2016;101:e469-e472.
18. Ware RE, Rees RC, Sarnaik SA, Iyer RV, Alvarez OA, Casella JF, et al. Renal function in infants with sickle cell anemia: baseline data from the BABY HUG trial. J Pediatr. 2010;156:66-70.e1.
19. Esezobor CI, Akintan P, Nwaogazie U, Akinwunmi E, Temiye E, Akinsulie A, et al. Enuresis in children and adolescents with sickle cell anaemia is more frequent and substantially different from the general population. PLoS One. 2018;13:e0201860.
20. Ushijima S, Ukimura O, Okihara K, Mizutani Y, Kawauchi A, Miki T. Visual analog scale questionnaire to assess quality of life specific to each symptom of the International Prostate Symptom Score. J Urol. 2006;176:665-71.
21. Preciado-Estrella DA, Kaplan SA, Iturriaga-Goyón E, Ramón-Trejo E, Mayorga-Gómez E, Auza-Benavides A. International Prostate Symptom Score and Gea Visual Analogue Scale® comparison for evaluating lower urinary tract symptoms. Rev Mex Urol 2017; 77:372-382.
22. Ekinci O, Celik T, Ünal Ş, Oktay G, Toros F, Ozer C. Nocturnal enuresis in sickle cell disease and thalassemia major: associated factors in a clinical sample. Int J Hematol. 2013;98:430-6. Noll JB, Newman AJ, Gross S. Enuresis and nocturia in sickle cell disease. J Pediatr. 1967;70:965-7.
23. Noll JB, Newman AJ, Gross S. Enuresis and nocturia in sickle cell disease. J Pediatr. 1967;70:965-7.
24. Elawad Ahmed F.: Nocturnal Enuresis in Children and Adolescent with Sickle cell Anemia. Med Surg Urol. 2017; 6:119.

25. Field JJ, Austin PF, An P, Yan Y, DeBaun MR. Enuresis is a common and persistent problem among children and young adults with sickle cell anemia. Urology. 2008;72:81-4.
26. Ozden C, Ozdal OL, Altinova S, Oguzulgen I, Urgancioglu G, Memis A. Prevalence and associated factors of enuresis in Turkish children. Int Braz J Urol. 2007;33:216-22.
27. Mabiala Babela JR, Loumingou R, Pemba-Loufoua A, Londjongo W, Nzingoula S, Senga P. [Enuresis in children with sickle cell disease]. Arch Pediatr. 2004;11:1168-72.
28. Wang MX, Pepin EW, Verma N, Mohammed TL. Manifestations of sickle cell disease on thoracic imaging. Clin Imaging. 2018;48:1-6.
29. Graves JK, Hodge C, Jacob E. Depression, Anxiety, and Quality of Life In Children and Adolescents With Sickle Cell Disease. Pediatr Nurs. 2016;42:113-9.
30. Lim CS, Karlson C, Edmond SN, Welkom JS, Osunkwo I, Cohen LL. Emotion-Focused Avoidance Coping Mediates the Association Between Pain and Health-Related Quality of Life in Children With Sickle Cell Disease. J Pediatr Hematol Oncol. 2019;41:194-201.

Correspondence address:
Flavia Cristina de Carvalho Mrad, MD
Departamento de Pediatria,
Unidade de Nefrologia Pediátrica,
Universidade Federal de Minas Gerais,
Av Alfredo Balena, nº 190, Belo Horizonte
MG, 30130-100, Brasil
E-mail: flaviacarvalhomrad@gmail.com

ORIGINAL ARTICLE

Vol. 45 (5): 981-988, September - October, 2019
doi: 10.1590/S1677-5538.IBJU.2019.0176

Assessment of long term outcomes after buccal mucosal graft urethroplasty: the impact of chronic kidney disease

Manoj Kumar [1], Ajay Aggarwal [1], Siddharth Pandey [1], Samarth Agarwal [1], Satya Narayan Sankhwar [1]

[1] *Department of Urology, King George's Medical University, Lucknow, INDIA.*

ABSTRACT

Objectives: To compare and assess various outcomes and success of buccal mucosal graft urethroplasty (BMGU) in patients with CKD versus patients having normal renal function.

Material and Methods: This was a retrospective, single centre study, during period 2013 to 2017. Patients were grouped into two groups. Group 1 had patients with estimated Glomerular Filtration Rate (eGFR)>60mL/min/1.73m2 while group 2 had patients with eGFR <60mL/min/1.73m^2. eGFR was calculated according to the MDRD equation. The two groups were compared with regard to various outcomes like length, location of stricture, technique of graft placement, intra-operative blood loss (haemoglobin drop), duration of hospital stay, post-operative complications and recurrence.

Results: A total of 223 patients were included in study with group 1 had 130 patients and group 2 had 93 patients. Mean age of patients with CKD were higher (47.49 years versus 29.13 years). The mean follow-up period was comparable between both groups (23.29 months and 22.54 months respectively). Patients with CKD had more post-operative Clavien Grade 2 or higher complications (p=0.01) and a greater recurrence rates (p<0.001) than in non-CKD patients. On multivariate analysis, age and CKD status was significant predictor of urethroplasty success (p=0.004) (OR= 14.98 (1.952-114.94, 95% CI).

Conclusions: CKD patients are more prone to post-operative complications in terms of wound infection, graft uptake and graft failure and higher recurrence rates following BMGU.

ARTICLE INFO

Ajay Aggarwal
http://orcid.org/0000-0003-3157-7067

Keywords:
Oral Mucosal Absorption; Renal Insufficiency, Chronic; Kidney Glomerulus

Int Braz J Urol. 2019; 45: 981-8

Submitted for publication:
March 11, 2019

Accepted after revision:
May 31, 2019

Published as Ahead of Print:
August 01, 2019

INTRODUCTION

Urethral stricture is one of the primeval and challenging diseases in urological practice. It may lead to a spectrum of conditions ranging from bothersome lower urinary tract symptoms to Fournier's gangrene and death. It's incidence rate is as high as 0.6% in some susceptible populations (1). Corpus spongiosal or urethral epithelial injury may result in urothelial scar formation and stricture disease. The primary aetiologies of urethral stricture disease are idiopathic, trauma, iatrogenic (instrumentation) and infections. Urethroplasty for the management of stricture has been proven highly successful along with its durability and cost-effectiveness (2). End-to-end anastomotic urethroplasty is considered the "gold standard" procedure with 90-95% success rate. But, it is best reserved for bulbar urethral strictures ≤2cm long (3). For more complex strictures, substitution urethroplasty techniques are used with acceptable success rate of approximately 90%. Buccal Mucosal Graft Urethroplasty (BMGU) is a well-established procedure with acceptable results for management of such complex strictures.

Renal impairment affect wound healing due to elevated uraemia toxins which inhibit granulation tissue formation and neovascularisation. This lead to impaired wound healing (4). The success of substitution urethroplasty mainly depend upon proper uptake of graft which may be hampered in patients of Chronic Kidney Disease(CKD). Various studies in literature analysed success of urethroplasty in normal population, but none of them reported outcome in CKD patients. The hypothesis of our study is that patients with normal renal function would have better wound healing and urethroplasty success rate than CKD patients. In our study, our aim was to assess and report the outcome and success of BMGU in moderate and severe chronic kidney disease patients.

MATERIALS AND METHODS

We retrospectively analysed our departmental database

Inclusion criteria: Patients who underwent BMGU between January 2013 to December 2017 Exclusion criteria: Patients who had history of previous perineal surgical procedure, radiotherapy and having multiple strictures.

In the above mentioned period, 252 patients underwent BMGU, out of which 223 met inclusion criteria. Patient were grouped into CKD patients (Grade 3 or higher, KDIGO criteria (5)) (eGFR <60mL/min/1.73m^2) and non-CKD (eGFR >60mL/min/1.73m^2) patients using MDRD equation (6). Approval was taken from the institutional ethical committee before study (1443/Ethics/R.cell-18).

Patient's age, eGFR, duration of presentation, other comorbidities like diabetes and hypertension, addiction like smoking, previous procedures like Optical Internal Urethrotomy (OIU) or Sequential Dilation (SqD), location, length, site and aetiology of stricture, post-operative haemoglobin drop, hospital stay after surgery were recorded. Post-operative complications (Clavien-Dindo Classification (CDC)) were also recorded.

SURGICAL PROCEDURE

All the surgeries were performed by two consultants of the urology department (MK and SNS), well versed with the technique used. All surgeries were carried out under regional anaesthesia. There were two teams involved in most surgeries: one for harvestation of buccal mucosa and the other team for perineal dissection. Buccal mucosa graft was harvested under local anaesthesia (7). Dorsal/dorso-lateral onlay BMGU was carried out using technique described by Singh et al. (8) whereas ventral onlay BMGU was done using technique described by Wessels (9).

Prophylactic intravenous antibiotic (third generation cephalosporin or ampicillin and gentamicin) was given at time of induction of anaesthesia. The oral pack was removed in the evening of the day of surgery and cold liquids allowed orally. First dressing of perineal site was done 48 hrs after surgery and then daily thereafter. Patients were discharged with Foley in situ and oral antibiotics (fluoroquinolones) were prescribed till catheter was in situ. Foley catheter was removed after 4-6 weeks.

FOLLOW-UP

In follow-up, clinical history and uroflowmetry with post void residual urine were performed at 3-month intervals for initial year and then yearly thereafter. Urethroplasty success was defined as Qmax greater than 15mL/sec. Urethroplasty failure/recurrence was declared when post-operative intervention was needed such as OIU/SD or redo-urethroplasty due to obstructive symptoms reported by patient followed by abnormal urethrogram/urethroscopy.

DEFINITION OF CKD

Estimated glomerular filtration rate (eGFR) was calculated using MDRD equation (eGFR/mL/min/1.73m^2=186*[SCr]-1.154*[age]-0.203*[0.742 for female]). As per National Kidney Foundation KDOQI TM, CKD was defined as eGFR <60mL/min/1.73m^2 (10).

STATISTICAL ANALYSIS

Data were compiled and entered on MS Office Excel 2016 spreadsheet. Analysis was done using SPSS (version 23.0; IBM, USA). The paired t-

-test was used for continuous variables, to detect the level of significance between baseline and follow--up data. The unpaired t test was used to detect the difference between the two intervention arms. The categorical data were analysed by Fisher's exact test. P <0.05 was considered significant.

RESULTS

Patients were categorised into two groups. Group-1 contained patients with eGFR more than and equal to 60mL/min/1.73m^2 and Group-2 had patients with eGFR less than 60mL/min/1.73m^2. The mean age of patients in group-2 was significantly higher (47.49±11.71 years) than in group-1 (29.13±12.39 years) (p <0.001). Patients with CKD presented early (17.68±12.13 months) as compared with non-CKD patient (23.97±16.56 months) (p=0.01). The mean follow-up period in groups-1 and 2 was 23.29 months and 22.54 months respectively. Most of strictures in both groups were idiopathic in nature (67.7% and 57% respectively). Other aetiologies of stricture and previous procedures like optical internal urethrotomy (OIU)/sequential dilation (SqD) in both groups are shown in Table-1.

Intra-operatively, majority of strictures in both groups were bulbar/penobulbar in location versus pan-anterior urethral strictures (Table-1). The mean length of stricture in groups 1 and 2 was 3.85±1.52 and 4.19±1.47cm respectively (p=0.10). Around 85% patients in group 1 and 65% patients in group 2 had dorsal/dorso-lateral placement of buccal mucosal graft. In rest of patients, ventral onlay placement was done. Post--operative haemoglobin drop was more in CKD patients (1.44±0.66gm/dL) as compared in non--CKD patients (1.26±0.73gm/dL), but there was no statistical difference (p=0.054). The mean length of hospital stay was also comparable between both groups (Table-1).

83.9% and 47.3% patients in group 1 and group 2 respectively had no complications (Clavien-Dindo Classification, CDC-0) post-operatively, (p=0.07, OR=0.59 (0.33-1.05),95% confidence interval). CKD patients had significantly more CDC ≥2 complications than in non-CKD patients and were statistically significant (p=0.01). Also, urethroplasty failure/recurrence rate was significantly higher in CKD patients, (p <0.001) (Table-1). On multivariate regression analysis for risk factors associated with stricture recurrence, age and CKD were found to be significantly associated with stricture recurrence (Table-2).

DISCUSSION

Urethral stricture management is a demanding surgery in urological field. Before the emergence of newer techniques using flaps and grafts, the results of management of long-term strictures were poor. In 1972, Orandi successfully used penile skin flap in one stage urethroplasty for anterior urethral strictures (11). Humby et al. in 1941 demonstrated use of buccal mucosal graft for urethral construction (12). Nowadays, buccal mucosa is considered as ideal urethral substitute for reconstruction due to its ease of harvesting, wet environment and early ingrowth and graft survival.

CKD is defined as a persistent reduction in GFR to below 60mL/min/1.73m^2 for three months or the presence of haematuria, proteinuria/microalbuminuria and radiologic/histologic changes in the kidneys. Renal impairment has been known to affect wound healing. Heller et al. demonstrated association of chronic kidney disease with impaired wound healing and as an independent risk factor for incisional hernia development (13). Research data on mice also showed deteriorating effect of CKD on wound healing. This is mediated by the disruption of keratinization mechanics, delayed granulation, and large epithelial gap. The veiled chronic inflammatory state and low rate of neovascularization and cellular proliferation also contributed to poor wound healing (14, 15).

In our study, mean age of patients who had CKD was significantly higher than non-CKD patients (47.49 year's vs. 29.13 years, p <0.001). This was mostly caused by diabetes and hypertension, which are most common causes of chronic kidney disease worldwide and more prevalent in elderly age group. Different studies in literature evaluated various predictors of urethroplasty success. One such study done by Singh et al. concluded that age of patient do not predict outcome

Table 1 - Patient characteristics and demographic profile.

	eGFR>60(n=130)	eGFR<60 (n=93)	P value
Pre-operative parameters			
Age(years)(mean ± SD)	29.13 ± 12.39	47.49 ± 11.71	**<0.001**
BMI(kg/m²)	23.21 ± 3.23	22.78 ± 4.12	0.76
Duration of presentation(months) (mean ± SD)	23.97 ± 16.56	17.68 ± 12.13	**0.01**
Follow up period(months) (mean ± SD)	23.29 ± 8.16	22.54 ± 6.14	0.45
Co-morbidities			
Diabetes(%)	11(8.46)	10(9.3)	-
Hypertension(%)	10(13.0)	40(43.0)	-
Etiology of stricture			
Traumatic(%)	7(5.3)	0(0)	**0.02**
Iatrogenic(%)	27(20.8)	40(43)	**<0.001**
Idiopathic(%)	88(67.7)	53(57)	0.10
Inflammatory(%)	8(6.2)	0(0)	**0.01**
Previous procedure			
(OIU)/ (SqD) (%)	46(35.4)	25(26.9)	0.17
Intraoperative parameters			
Length of stricture(cm) (mean ± SD)	3.85 ± 1.52	4.19 ± 1.47	0.10
<2cm, n(%)	26(20)	3(3.2)	-
2-4cm, n(%)	62(47.7)	56(60.2)	-
>4 cm, n(%)	42(32.3)	34(36.6)	-
Location of stricture			
Pan anterior(%)	24(18.5)	8(8.6)	**0.03**
Bulbar/ penobulbar(%)	106(81.5)	85(91.4)	
Graft Placement			
Dorsal/Dorso-lateral onlay(%)	111(85.4)	60(64.5)	-
Ventral onlay(%)	19(14.6)	33(35.5)	-
Post-operative parameters			
Haemoglobin drop (gm%)(mean ± SD)	1.26 ± 0.73	1.44 ± 0.66	0.054
Hospital stay(days) (mean ± SD)	9.4 ± 4.08	9.09 ± 1.71	0.438
Clavien Dindo			
0(%)	109(83.9)	44(47.3)	**0.07**
1(%)	9(6.9)	2(2.1)	0.22
≥ 2(%)	12(9.2)	47(50.5)	**0.01**
Recurrence(%)	6 (4.6)	37(39.78)	**< 0.001**
Recurrence in stricture ,n (%)			
<2cm	0(0)	0(0)	-
2-4cm	6(100)	20(54)	**<0.001**
>4cm	0(0)	17(46)	<0.001

eGFR = estimated Glomerular Filtration rate; **BMI =** Body Mass Index; **SD =** Standard deviation; **OIU =** Optical Internal Urethrotomy; **SqD =** Sequential Dilation.

Table 2 - The multivariate logistic regression analysis for risk factors associated with stricture recurrence.

Risk factor	P value	OR (95% CI)
Age	**0.029**	1.067(1.003-1.135)
Pan Anterior	0.125	0.238(0.019-2.914)
Bulbar/Penobulbar	0.432	0.642(0.212- 0.988)
CDC-0	0.298	0.243(0.023-2.615)
CDC-≥ 2	0.083	6.431(0.614-67.36)
CKD	**0.004**	14.98(1.952-114.94)

CDC = Clavien- Dindo Complication; **CKD** = Chronic Kidney Disease

after urethroplasty (16). However, in our study, on multivariate logistic regression analysis for risk factors associated with stricture recurrence, age was a significant factor (p=0.029), OR=1.067 (1.003-1.135, 95% CI) (Table-2). Various studies in literature mentioned obesity as a predicting factor for stricture recurrence (17, 18). In our study both groups had comparable body mass index.

Patients with normal GFR had longer duration of presentation of stricture (23.97+16.56 months) that was statistically significant as compared with CKD patients (17.68+12.13 months, p=0.01). The most possible explanation for this finding is that CKD patients have more frequent hospital visits and check-ups, during which stricture might be detected early.

In our study, patients with hypertension were more in number in group 2, owing to the likely impact of HTN on eventual CKD. However, diabetic patients were almost equal in both groups. Our study was under-powered to assess the impact of HTN and DM as independent variable on the success and outcomes of BMGU in both groups.

Almost 90% of strictures in non-CKD patients and 100% in CKD patients, were idiopathic or iatrogenic in nature. Stein et al. (19) looked retrospectively at 2.589 patients who underwent urethroplasty procedures from 2000 to 2011 in the USA, Italy, and India. They also concluded similar results regarding aetiology of stricture as found in our study. The reason for higher percentage of iatrogenic strictures in CKD patients (43%) as compared to non-CKD patients (20.8%) is not known. Almost 36% and 27% patients in group 1 and group 2 respectively had previous history of surgical procedures (OIU/SqD). Singh et al. (16) studied 58 patients with post-traumatic posterior urethral stricture who underwent anastomotic urethroplasty. They concluded that previous failed procedures significantly decrease the success of subsequent anastomotic urethroplasty. In another study, Barbagli et al. (20) retrospectively reviewed a series of 93 patients comparing patients with primary repair versus patients with prior history of urethrotomy and underwent secondary treatment. They concluded that failed urethrotomy does not affect the long-term result of surgical repair. In our study, both groups were comparable in terms of previous procedures.

Long length of strictures(>4cm) are usually associated with increased risk of recurrence (21). In our study, mean length of stricture in both groups were comparable (3.85+1.52 vs. 4.19+1.47cm respectively, p=0.10). Around one third of patients had stricture >4cm in both groups (Table-1). Patients with CKD had more number of patients with bulbar/peno-bulbar strictures (p=0.03) in comparison with pan-urethral strictures. The reason for this finding is unknown. Meeks et al. (22) described surgical outcomes in patients with kidney and kidney-pancreas transplants after urethroplasty for stricture or fistula disease. Around 80% patients in that study had mid-bulbar/peno-bulbar strictures and only 20% had penile urethral strictures.

Various techniques of BMGU have been mentioned in literature, including dorsal onlay, ventral onlay, lateral onlay and combined techniques, all having success rates above 90%. Among

all, dorsal/dorso-lateral onlay and ventral onlay techniques are the most commonly used (23, 24). In our study, most of the cases in both groups (85.4% and 64.5% respectively) used dorsal/dorsolateral onlay technique followed by ventral onlay technique depending on the site of stricture and surgeon discretion. Pathak et al. (25), conducted retrospective analysis on 112 patients who underwent BMGU for non-traumatic long segment bulbar urethral strictures. They compared long- term outcomes of BMGU by placing the graft ventrally, dorso-laterally and dorsally. The author found that the overall success rate for BMG augmentation urethroplasty is equal for all techniques. Other studies reported similar success rates of 85-97% and 83-94% for dorsal and ventral BMGU, respectively (26, 27). In our study, patients with CKD had significantly more ventral onlay placement of buccal mucosa and non-CKD patients had more of dorsal/dorsolateral graft placement (Table-1). However, the decision of graft position was solely based on surgeon's choice.

Patients with CKD are more prone for blood loss during any surgery. Platelet dysfunction is the main factor responsible. Various factors like anaemic state of patient, dialysis per se, delayed clearance of medications and anticoagulation used during dialysis also play some role in causing impaired haemostasis in these patients. Platelet dysfunction occurs due to impaired platelet aggregation and impaired platelet-vessel wall interaction. The normal platelet response to vessel wall injury include platelet activation, recruitment, adhesion, and aggregation which are impaired in chronic renal failure. A systematic review and meta-analysis done by Acidello et al. (28), screened 9376 citations from multiple databases, concluded that chronic kidney disease is associated with significant perioperative bleeding but rarely required reoperation. In our study, mean haemoglobin drop in CKD patients was higher, however, statistically comparable with patients having normal renal function (1.26±0.73 and 1.44±0.66g/dL respectively, p=0.054).

Both groups of patients had statistically comparable length of hospital stay (9.4±4.08 and 9.09±1.71days, p=0.43). Patients in our study usually come from far areas to our tertiary care centre and they prefer to stay in hospital till their suture removal, explaining the long length of stay in hospital.

Around 84% patients in non-CKD group had no post-operative complications (Clavien--Dindo=0) as compared to only 47% patients in CKD group. We also observed significantly higher CDC ≥2 complications in patients with CKD (50.5% vs. 9.2%, p=0.01) (Table-1). These complications included surgical site infection, urinary fistula, graft contracture and graft failure. In patients with stricture length less than 2cm, none of them had recurrence (Table-1). Patients with stricture length between 2 to 4cm and more than 4cm had significantly higher recurrence rate in group-2. It implied that stricture length did not affect BMGU success. Overall recurrence rate was also significantly higher (4.6% vs. 39.78%, p=<0.001) in CKD patients versus patients with normal renal function on bivariate analysis. On multivariate analysis also, CKD appeared as significant risk factor for stricture recurrence (p=0.004) (OR=14.98 (1.952-114.94, 95% CI) (Table-2). Mori et al. (29) reported an overall complication rate of 19.2% and a revision rate of 10.3% in 78 patients who underwent multistage reconstruction for complex anterior urethral strictures. The revision rate in contemporary series ranges from 6.7% to 59% in patients with normal renal function, and our results are in line with literature (30). The possible reason for more complications and urethroplasty failure rate in CKD patients is due to delayed vascularisation and graft uptake leading to delayed wound healing and ultimately graft failure (15).

The strength of our study lies on its simplicity and the fact that in best of our knowledge, till date, no study in literature compared different outcomes following BMGU between CKD patients and patients with normal renal function.

Limitations of our study include: (i) retrospective analysis (ii) follow-up limited to 30 months. Longer follow-up is required to determine if stricture recurrence rates between the two groups changes over time. (iii) Data on patients having haemodialysis is lacking.

CONCLUSIONS

Buccal mucosal graft urethroplasty is an established procedure for stricture urethra with overall success rate of around 94%. However,

in patients with CKD, overall success rate drops down to 70% with more post-operative complications. Comorbidities like diabetes, length, location, duration of stricture and technique of graft placement do not affect outcomes in CKD patients.

ACKNOWLEDGEMENTS

I acknowledge the cooperation of residents of Urology department of King George's medical university who participated in data collection and evaluation of the patient. We also appreciate the commitment and compliance of the patient who reported the required data.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Santucci RA, Joyce GF, Wise M. Male urethral stricture disease. J Urol. 2007;177:1667-74.
2. Rourke KF, Jordan GH. Primary urethral reconstruction: the cost minimized approach to the bulbous urethral stricture. J Urol. 2005;173:1206-10.
3. Jezior JR, Schlossberg SM. Excision and primary anastomosis for anterior urethral stricture. Urol Clin North Am. 2002;29:373-80.
4. Maroz N, Simman R. Wound Healing in Patients With Impaired Kidney Function. J Am Coll Clin Wound Spec. 2014;5:2-7.
5. Kellum JA, Lameire N, Aspelin P, Barsoum RS, Burdmann EA, Goldstein SL, et al. Kidney disease: improving global outcomes (KDIGO) acute kidney injury work group. KDIGO clinical practice guideline for acute kidney injury. Kidney international supplements. 2012; 2: 1-138.
6. Kuan Y, Hossain M, Surman J, El Nahas AM, Haylor J. GFR prediction using the MDRD and Cockcroft and Gault equations in patients with end-stage renal disease. Nephrol Dial Transplant. 2005;20:2394-401.
7. Goel A, Dalela D, Sinha RJ, Sankhwar SN. Harvesting buccal mucosa graft under local infiltration analgesia--mitigating need for general anesthesia. Urology. 2008;72:675-6.
8. Singh BP, Pathak HR, Andankar MG. Dorsolateral onlay urethroplasty for anterior urethral strictures by a unilateral urethral mobilization approach. Indian J Urol. 2009;25:211-4.
9. Wessells H. Ventral onlay graft techniques for urethroplasty. Urol Clin North Am. 200;29:381-7.
10. Nelson RG, Tuttle KR. The new KDOQI clinical practice guidelines and clinical practice recommendations for diabetes and CKD. Blood Purif. 2007;25:112-4.
11. Orandi A. One-stage urethroplasty: 4-year followup. J Urol. 1972;107:977-80.
12. Humby G, Higgins TT. A one‌istage operation for hypospadias. British journal of surgery. 1941; 29: 84-92.
13. Heller A, Westphal SE, Bartsch P, Haase M, Mertens PR. Chronic kidney disease is associated with high abdominal incisional hernia rates and wound healing disturbances. Int Urol Nephrol. 2014;46:1175-81.
14. Seth AK, De la Garza M, Fang RC, Hong SJ, Galiano RD. Excisional wound healing is delayed in a murine model of chronic kidney disease. PLoS One. 2013;8:e59979.
15. Kursh ED, Klein L, Schmitt J, Kayal S, Persky L. The effect of uremia on wound tensile strength and collagen formation. J Surg Res. 1977;23:37-42.
16. Singh BP, Andankar MG, Swain SK, Das K, Dassi V, Kaswan HK, et al. Impact of prior urethral manipulation on outcome of anastomotic urethroplasty for post-traumatic urethral stricture. Urology. 2010;75:179-82.
17. Chapman D, Kinnaird A, Rourke K. Independent Predictors of Stricture Recurrence Following Urethroplasty for Isolated Bulbar Urethral Strictures. J Urol. 2017;198:1107-12.
18. Breyer BN, McAninch JW, Whitson JM, Eisenberg ML, Master VA, Voelzke BB, et al. Effect of obesity on urethroplasty outcome. Urology. 2009;73:1352-5.
19. Stein DM, Thum DJ, Barbagli G, Kulkarni S, Sansalone S, Pardeshi A, et al. A geographic analysis of male urethral stricture aetiology and location. BJU Int. 2013;112:830-4.
20. Barbagli G, Palminteri E, Lazzeri M, Guazzoni G, Turini D. Long-term outcome of urethroplasty after failed urethrotomy versus primary repair. J Urol. 2001;165(6 Pt 1):1918-9.
21. Kinnaird AS, Levine MA, Ambati D, Zorn JD, Rourke KF. Stricture length and etiology as preoperative independent predictors of recurrence after urethroplasty: A multivariate analysis of 604 urethroplasties. Can Urol Assoc J. 2014;8:E296-300.
22. Meeks JJ, Gonzalez CM. Urethroplasty in patients with kidney and pancreas transplants. J Urol. 2008;180:1417-20.
23. Andrich DE, Mundy AR. What is the best technique for urethroplasty? Eur Urol. 2008;54:1031-41.
24. Barbagli G, Palminteri E, Guazzoni G, Montorsi F, Turini D, Lazzeri M. Bulbar urethroplasty using buccal mucosa grafts placed on the ventral, dorsal or lateral surface of the urethra: are results affected by the surgical technique? J Urol. 2005;174:955-7.

25. Pathak HR, Jain TP, Bhujbal SA, Meshram KR, Gadekar C, Parab S. Does site of buccal mucosa graft for bulbar urethra stricture affect outcome? A comparative analysis of ventral, dorso-lateral and dorsal buccal mucosa graft augmentation urethroplasty. Turk J Urol. 2017;43:350-4.
26. Kane CJ, Tarman GJ, Summerton DJ, Buchmann CE, Ward JF, O'Reilly KJ, et al. Multi-institutional experience with buccal mucosa onlay urethroplasty for bulbar urethral reconstruction. J Urol. 2002;167:1314-7.
27. Andrich DE, Leach CJ, Mundy AR. The Barbagli procedure gives the best results for patch urethroplasty of the bulbar urethra. BJU Int. 2001;88:385-9.
28. Acedillo RR, Shah M, Devereaux PJ, Li L, Iansavichus AV, Walsh M, et al. The risk of perioperative bleeding in patients with chronic kidney disease: a systematic review and meta-analysis. Ann Surg. 2013;258:901-13.
29. Mori R, Wood H, Angermeier K. PD3-01 Multistage buccal mucosa graft urethroplasty for complex anterior urethral strictures. J Urol. 2014;191:e19-e20.
30. Meeks JJ, Erickson BA, Gonzalez CM. Staged reconstruction of long segment urethral strictures in men with previous pediatric hypospadias repair. J Urol. 2009;181:685-9.

Correspondence address:
Ajay Aggarwal, MD
Department of Urology,
King George's Medical University
Lucknow, India
Fax: + 91 522 2369841
E-mail: drajayaggarwal2004@gmail.com

ORIGINAL ARTICLE

Vol. 45 (5): 989-998, September - October, 2019
doi: 10.1590/S1677-5538.IBJU.2019.0070

Macroplastique for women with stress urinary incontinence secondary to intrinsic sphincter deficiency

Timothy F. Carroll [1], Alana Christie [1], Melissa Foreman [1], Gaurav Khatri [1], Philippe E. Zimmern [1]

[1] *University of Texas Southwestern Medical Center, TX, USA*

ABSTRACT

Objective: To evaluate the subjective and objective outcomes of Macroplastique® (MPQ) in women with stress urinary incontinence (SUI) secondary to intrinsic sphincter deficiency (ISD).

Materials and Methods: Following Institutional Review Board (IRB) approval, charts of non-neurogenic women with SUI secondary to ISD who underwent MPQ injection and had 6 months minimum follow-up were reviewed from a prospectively maintained database. Patients were divided into 3 groups: Naïve (Group I), Prior Anti-Incontinence Surgery (Group II), and combined Prior Bulking Agent and Anti-Incontinence Surgery (Group III). Data collected included SUI self-report, Urogenital Distress Inventory (UDI-6) Question 3, and VAS Quality of Life (QoL) Questionnaire scores at baseline and in follow-up. Three-dimensional ultrasound (3DUS) evaluated volume/configuration of MPQ. Success was defined after the last MPQ injection as a UDI-6 Question 3 score of 0 (dry) or 1, and no reoperation for SUI.

Results: From 2011-2017, 106 of 142 women met study criteria. At a median follow-up of 20 months (mean=26 months; range: 6-71), success rate was 41% for Group I, 40% for Group II, and 65% for Group III (p = 0.22). QoL scores were significantly improved over baseline in all groups. There was no significant difference in clinical outcome between the asymmetrical and symmetrical group on 3DUS. The completely dry rate was highest in Group III at 29%, compared to 4% for Group I and 15% for Group II (p = 0.05).

Conclusion: Macroplastique® improved subjective and objective outcome measures for SUI secondary to ISD as both a primary and secondary treatment option in women.

ARTICLE INFO

Philippe E. Zimmern
http://orcid.org/0000-0002-7612-2042

Keywords:
Urinary Incontinence; Urinary Sphincter, Artificial; Women

Int Braz J Urol. 2019; 45: 989-98

Submitted for publication:
February 02, 2019

Accepted after revision:
May 06, 2019

Published as Ahead of Print:
August 01, 2019

INTRODUCTION

Macroplastique® (Cogentix Medical, Orangeburg, New York, USA) (MPQ) is a bulking agent used in the treatment of stress urinary incontinence (SUI) secondary to intrinsic sphincter deficiency (ISD). MPQ has been available since 1991 in Europe, but has only been used at our institution since 2011 when Collagen (Contigen™) was no longer available. The material is composed of both the resorbed carrier, polyvinylpyrrolidone, and the permanent silicone-like elastomer, polydimethylsiloxane beads.

While Collagen was generally regarded as a safe and efficacious injectable, less literature is available on MPQ outcomes and the factors that may predict its long-term success. In a randomized controlled trial comparing MPQ to Collagen, Ghoniem and colleagues reported that MPQ injection resulted in a statistically significant 12% increase in dry/cure rate over the group receiving Collagen (1). A few series have reported MPQ suc-

cess rates between 40-85% over more than 2 years using a variety of outcome measures including patient self-report, urodynamics, and Stamey grades (2, 3). Our group has only examined the short-term outcome of MPQ and reported a success rate of 75% in 59 women followed for a mean of 9 months (4).

In this report, as we did for Collagen in the past (4-6), we examined our longer term experience with MPQ using subjective and objective (three-dimensional ultrasound (3DUS)) outcome criteria as well as studied factors that could predict success over time in order to better counsel patients with bothersome incontinence interested in a minimally invasive approach.

MATERIALS AND METHODS

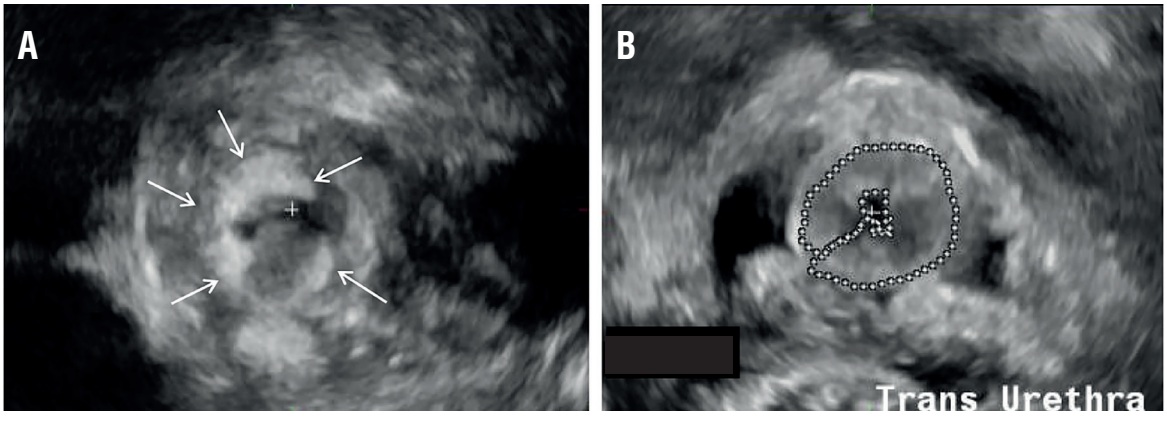
After Institutional Review Board approval, a review from a prospectively maintained database of women treated with MPQ injection was performed. Included in this study were women with bothersome stress urinary incontinence due to ISD and with a radiologically proven well-supported urethra. Excluded were those with follow-up <6 months, a neurogenic bladder, or an indwelling suprapubic tube.

The diagnosis of ISD was based on several criteria including: a positive supine stress test, a well-supported urethra as confirmed by standing cystourethrogram revealing minimal difference in urethral angle between rest and straining (7), and Valsalva leak point pressure (VLPP) obtained during urodynamic studies performed according to the Urinary Incontinence Treatment Network protocol (8). Similar to prior studies on Collagen, we did not use a specific cutoff for VLPP to diagnose ISD, although a higher VLPP may indicate a less severe form of ISD (9, 10).

The same urologist (PZ) performed MPQ injection on an outpatient basis under light anesthesia (monitored anesthesia care or laryngeal mask) using a 21F Wolf cystoscope with a 30° lens. Following our experience with Collagen, injections were made transurethrally at the 3 and 9 o'clock positions at the mid-urethral level. MPQ tracked around the urethra both superiorly and inferiorly as confirmed by subsequent 3DUS (Figure-1). A total of 5mL MPQ was generally injected, typically with 2.5mL at each injection site. Patients who failed their voiding trial (complete retention or voiding less than half of bladder capacity) were discharged home with a small urethral catheter (12Fr-14Fr) for 24 hours.

As in our prior early study on MPQ, women were grouped into 3 different categories: Naïve (Group I), previous anti-incontinence surgery (Group II), or prior anti-incontinence procedure and bulking agent (Group III) (4). Prior anti-incontinence surgeries included: bladder neck suspension, autologous sling, removal of a prior synthetic sling/urethrolysis, and/or associated prolapse repair addressing the anterior and apical compartments (e.g. suspensions involving the anterior wall, sacrocolpopexy, and apical suspension).

Figure 1 - (+)=Urethral lumen; 3DUS=Three-dimensional ultrasound. 3DUS reveals asymmetric MPQ configuration (A) compared to a symmetrical "horseshoe configuration" (B). MPQ is indicated by either arrows (A) or as outlined (B).



Subjective outcomes were evaluated through patient self-report of improvement, question 3 of the validated Urogenital Distress Inventory--Short From (UDI-6) ("Do you experience, and if so, how much are you bothered by leakage related to physical activity, coughing, or sneezing?"; 0=not at all, 1=slightly, 2=moderately, 3=greatly), and the VAS Quality of Life Questionnaire (QoL) ("With regards to the impact your bladder condition has on your life, how would you describe your current quality of life?" with answers ranging from 0=pleased to 10=terrible) (11). These subjective outcome measures were recorded both at baseline (pre-injection) and at each follow-up visit. Women who did not return after 6 months for follow-up were administered the same questionnaires (self-report, UDI-6, QoL) over a structured telephone interview by a neutral investigator not involved in the care of these patients.

Three dimensional ultrasound (3DUS) evaluations were performed at 6-8 weeks postoperatively and at 1-2 year intervals thereafter. These measurements were made using the Philips IU22 ultrasound system (Philips Healthcare, Bothell, WA) with endovaginal 3D 9-3V end-fire mechanical probe with automatic 3-D multi-planar image acquisition. 3DUS evaluations were performed with the patient in the dorsal lithotomy position with a moderately distended bladder. MPQ was easy to identify as it is very echogenic. Volume calculations were made using the stacked contour method. As in our prior study on Collagen injection (12), the radiology report contained information on whether the MPQ configuration was symmetrical (circumferential/horseshoe shape of MPQ around the urethra) or asymmetrical (MPQ lacking in one area) (Figure-1). The ultrasound examination and volume/configuration measurements were originally obtained by an ultrasound technician at the time of evaluation, then re-measured by the same senior sonographer (MF) for the whole study. The images were then reviewed by a radiologist for final interpretation. The sonographer and radiologist were unaware of patient outcomes. In addition, our senior sonographer (MF) randomly chose to re-read 10 studies to determine her intra-rater reliability, and had a Pearson's correlation at 0.999 (95% CI 0.993-0.999).

The primary outcome of the study, "success", was defined after the last MPQ injection as a UDI-6 Question 3 score of 0 or 1 and no surgical reoperation for SUI. A repeat MPQ injection was not considered failure. Secondary outcome variables included VAS QoL score as well as patient self-report of improvement. Additionally, patients were considered "dry/cure" with a self-report of 100% improvement or a UDI-Question 3 score of 0 were observed.

STATISTICAL METHODS

Descriptive statistics were provided as medians, means, standard deviations, and ranges for continuous factors, and frequencies and percentages for categorical factors. We used the Chi-square test to determine if categorical factors were significantly associated with prior incontinence treatment history or with success versus failure of the MPQ, and the Student *t*-test for continuous factors. Differences in questionnaire results were compared between pre-MPQ and last follow-up using the paired *t*-test. All analyses were completed at the 0.05 significance level using SAS 9.4 (SAS Institute Inc., Cary, NC).

RESULTS

From 2011-2017, 106 of 142 women met study criteria, including 16 who completed phone interviews. Of the 36 excluded, 30 were lost to follow-up, 5 had a neurogenic bladder, and 1 had a suprapubic tube. There were no serious adverse events in our series, including no systemic complications observed.

Mean age was highest in Group III (73 years), while BMI was not significantly different amongst the groups. The majority of patients in each group only had 1 injection, while the remainder had 2 or more within the study period at a mean interval time of 9 months between first and second injection. Pre-injection urodynamic findings were not different amongst the groups with the exception of Qmax, which was highest in Group I (19.6mL/sec) (p=0.03).

At a median follow-up of 20 months (mean=26 months; range: 6-71 months), the suc-

cess rate was 45% with minimal differences between the 3 groups (41% for Group I, 40% for Group II, and 65% for Group III (p=0.22)). Group III had a significantly higher success rate after the first MPQ injection (59%) compared to Group I (37%) and Group II (25%) (p=0.03). Of 26 women with follow-up over 3 years, 58% met success criteria as did 75% of 8 women with follow-up of 5 years or more. The completely dry/cure rate was 4% for Group I, 15% for Group II, and 29% for Group III (p=05) (Table-1). Improvements in UDI-6 Question 3 scores were not significantly different across groups.

Table-2 details an analysis of factors that could potentially predict success. Overall, transient retention rate was 45% after the last MPQ injection. Retention after final MPQ and the number of MPQ injections were near statistical sig-

Table 1 - Patient characteristics by prior incontinence treatment history.

	Naive (n=28)	Prior Surgery (n=61)	Prior Collagen± Surgery (n=17)	p
Age, years (range)	65.4±8.3 (45-81)	63.4±11.1 (37-92)	72.9±7.9 (59-88)	0.0036
BMI	25.8±5.3	28.8±6.7	27.7±5.8	0.1202
Gravida	2.5±1.3	2.9±1.3	2.7±1.4	0.3514
Parity	1.9±1	2.5±1.2	2.6±1.4	0.0618
Hysterectomy				
No	6 (21%)	10 (16%)	3 (18%)	0.8312
Yes	22 (79%)	51 (84%)	14 (82%)	
HRT				
No	13 (46%)	33 (54%)	9 (53%)	0.8296
Yes	15 (54%)	28 (46%)	8 (47%)	
VLPP, cm H_2O (range)	62.6±32.1 (20-160)	61.7±30.9 (17-140)	46.6±27.0 (16-88)	0.4179
Qmax, mL/sec (range)	19.6±8.9 (4.2-41)	16.1±8.5 (5.8-50)	12.6±6.0 (3-24)	0.0296
Years between 1st and 2nd MPQ	0.5±0.3	0.8±0.6	1.0±0.7	0.3072
Retention after 1st MPQ	10 (36%)	25 (41%)	10 (59%)	0.3243
Retention after 2nd MPQ	2 (33%)	12 (55%)	1 (25%)	0.5014
Retention after final MPQ	10 (36%)	29 (48%)	9 (53%)	0.4672
Completely dry	1 (4%)	9 (15%)	5 (29%)	0.0517
Success after first MPQ	10 (37%)	14 (25%)	10 (59%)	0.0331
Success after final MPQ	11 (41%)	23 (40%)	11 (65%)	0.2229

BMI=Body Mass Index; **HRT=**Hormone Replacement Therapy; **VLPP=**Valsalva Leak Point Pressure; **Qmax=**Maximum urinary flow rate.

Patient characteristics according to the respective group based on prior incontinence treatment history. Age and Qmax were significantly different at baseline. Success after first MPQ injection and completely dry rate after final MPQ injection are highest in Group III (p=0.03 and 0.05, respectively).

Table 2 - Patient characteristics by success after final Macroplastique.

	Failed Macroplastique (n=56)	Successful Macroplastique (n=45)	No UDI-6 Q3, no reoperation (n=5)	p
Age, years (range)	63.4±10.7 (37-85)	68.1±9.9 (46-92)	64.8±9.6 (57-78)	0.1203
BMI	28.5±7.3	26.9±4.8	27.8±5.0	0.8449
Pregnancy	2.8±1.4	2.7±1.2	2.0±1.6	0.5333
Parity	2.4±1.2	2.3±1.2	2.0±1.4	0.9090
Hysterectomy				
No	11 (20%)	7 (16%)	1 (20%)	0.9142
Yes	45 (80%)	38 (84%)	4 (80%)	
HRT				
No	27 (48%)	25 (56%)	3 (60%)	0.7146
Yes	29 (52%)	20 (44%)	2 (40%)	
UDI6 Q3 (0-3)	2.7±0.5	2.6±0.7	2.3±1.5	0.8973
QoL Score (0-10)	8.3±2.2	7.3±2.5	9.8±0.5	0.0412
Retention after 1st MPQ	19 (34%)	24 (53%)	2 (40%)	0.1383
Retention after 2nd MPQ	10 (43%)	5 (56%)	-	0.6989
Retention after final MPQ	20 (36%)	26 (58%)	2 (40%)	0.0796
Volume voided	272.9±155.8	197.6±124.3	281.8±119.0	0.4148
Post-void residual	29.8±70.0	7.8±21.4	11.3±22.5	0.0968
Median VLPP, cm H_2O	55	60	40	0.9884
Mean VLPP (range)	60.1±30.2 (17-160)	60.0±31.0 (16-140)	64.0±48.7 (32-120)	0.9790
VLPP <60, cm H_2O	23 (55%)	13 (46%)	2 (67%)	0.7174
VLPP ≥60, cm H_2O	19 (45%)	15 (54%)	1 (33%)	
Qmax, mL/sec (range)	15.4±8.4 (4.2-50)	17.7±8.7 (3-41)	17.2±8.0 (10-30)	0.3276
Pdet Qmax, cm H_2O (range)	15.6±11.6 (0-45)	13.0±9.8 (0-40)	7.6±2.5 (5-10)	0.3407
Number of injections				
1	32 (57%)	34 (76%)	5 (100%)	0.0705
2	19 (34%)	11 (24%)	0 (0%)	
3	5 (9%)	0 (0%)	0 (0%)	

UDI-6 Q3=UDI-6 Question 3; **QoL=**Quality of Life; **VLPP=**Valsalva Leak Point Pressure; **Qmax=**Maximum urinary flow rate; **Pdet Qmax**=Detrusor pressure at Qmax

Details an analysis of factors predicting success in patients receiving MPQ. Retention after first MPQ injection and number of injections approach significance. Baseline QoL score is highest (worst) in those without UDI-6 Question 3 after MPQ injection but also with no reinjection to indicate failure.

nificance in predicting success at last follow-up (p=0.07). Pre-operative urodynamic values did not show a significant relationship with MPQ success, including VLPP, which was also not associated with UDI-6 Question 3 scores when ranked from 0-3 (p=0.54). The pre-injection UDI-6 Question 3 scores were not different between the success and failure groups, arguing against the possibility that ISD severity influenced our MPQ outcomes. QoL scores at baseline were significantly lower in the success group. Five patients with missing UDI-6 Question 3 score but all other complete data were separated from this analysis (Table-2).

Table-3 illustrates the differences in UDI-6 and QoL scores with each group from pre-injection to last follow-up. A statistically significant improvement was observed with Groups I and II in total UDI-6 score. Groups I and II also reported significant improvement in UDI-6 Question 2 (urgency). Each group experienced significant improvement in UDI-6 Question 3 score (stress). Only Group I experienced significant improvement in UDI-6 Question 5 (ability to empty), although Group II approached significance (p=0.07). QoL scores were significantly improved over baseline in all groups. Of 19 women with low baseline UDI-6

Table 3 - Changes in UDI-6 and QoL questionnaire responses by prior incontinence treatment history.

	n	Pre-MPQ Mean	Last FU Mean	Mean difference (95% CI)	p
UDI-6 Total Score (0-36)					
Naive	17	10.5±4.2	6.6±4.2	-3.4 (-5.5, -1.3)	0.0030
Prior surgery	41	11.9±3.3	8.2±4.7	-3.7 (-5.2, -2.3)	<0.0001
Prior collagen±surgery	10	8.9±2.5	6.3±4.5	-2.0 (-6.2, 2.2)	0.3048
UDI-6 Q2 UUI (0-3)					
Naive	20	2.0±1.2	1.1±1.1	-0.6 (-1.2, 0.01)	0.0535
Prior surgery	48	2.4±0.9	1.8±1.1	-0.6 (-0.9, -0.3)	0.0008
Prior collagen±surgery	13	2.4±0.9	1.6±1.2	-0.3 (-1.1, 0.5)	0.4156
UDI-6 Q3 SUI (0-3)					
Naive	22	2.8±0.4	1.9±1.0	-0.8 (-1.3, -0.4)	0.0010
Prior surgery	49	2.6±0.7	1.7±1.0	-0.9 (-1.2, -0.6)	<0.0001
Prior collagen±surgery	14	2.4±0.6	1.2±1.0	-0.9 (-1.6, -0.3)	0.0094
UDI-6 Q5 Empty (0-3)					
Naive	20	0.8±1.2	0.3±0.7	-0.5 (-0.9, 0.02)	0.0583
Prior surgery	45	1.0±1.1	0.7±1.0	-0.3 (-0.6, 0.03)	0.0702
Prior collagen±surgery	14	0.6±0.9	0.7±0.9	0.2 (-0.5, 0.9)	0.5328
Quality of Life Score (0-10)					
Naive	14	7.1±2.5	4.4±3.2	-2.1 (-3.8, -0.3)	0.0256
Prior surgery	45	8.6±2.0	4.6±3.4	-3.9 (-4.9, -2.9)	<0.0001
Prior collagen±surgery	12	6.5±2.6	3.9±2.8	-2.0 (-4.1, 0.1)	0.0580

UDI-6=Urogenital Distress Inventory; **QoL**=Quality of Life questionnaire; **FU**=Follow-Up; **UUI**=Urge Urinary Incontinence; **Q**=Question; **SUI**=Stress Urinary Incontinence

Question 2 scores of 0-1, only 6 reported increases in score over time after MPQ, indicating a low incidence of de novo urgency/urge incontinence symptomatology.

Women with a symmetric distribution of MPQ at last follow-up had higher rates of both completely dry/cure (18% of symmetric vs. 6% of asymmetric) and success (48% of symmetric vs. 33% of asymmetric), although these differences were not statistically significant (p=0.22 and 0.19, respectively). Symmetry was not associated with post-injection UDI-6 Question 3 or QoL scores. After controlling for time since last MPQ injection and total number of injections, no association between volume and success was found (p=0.83).

In the "prior surgery" group (n=61), 13 had prior prolapse repair alone, including sacrocolpopexy (3), anterior vaginal wall suspension (4) and anterior-posterior repair (4). Of the 48 who had prior incontinence surgery, 17 had an autologous fascial sling and 31 a sub-urethral synthetic sling release. No difference was noted in retention rate immediately post-MPQ injection (42% in both groups), or in success rate (41% after sling placement, 39% after sling release).

Among the 56 failures (defined as those that did not meet criteria for "success"), 32 failed after 1 injection, 20 after 2 injections, and 4 after 3 injections. Ten of fifty-six failures proceeded to autologous sling (8) or artificial urinary sphincter (AUS) placement (2) at a mean follow-up interval of 8 months after the last MPQ injection. Four of these 10 patients proceeded to autologous sling after the first injection, while the remaining six patients had two or three injections before sling or AUS insertion. Kaplan-Meier survival analysis based on failure criteria revealed a gradual decline in questionnaire scores over years and a latency in time to receiving fascial sling/AUS after injection (Figure-2).

DISCUSSION

Our study examined the outcome of MPQ injection using subjective and objective outcome measures with a success rate of 45% after the last MPQ injection at a median of 20 months, and with minor variance amongst our 3 MPQ indication subgroups. In a subset of women with longer follow-up at 3 and 5 years, the success rates remained comparable.

Success rates for MPQ at different time periods are variable in the literature (Table-4) and differ based on definition of success and the tools to define outcomes. A meta-analysis by Ghoniem and Miller reported a success rate of 64% at 18

Figure 2 - The red line represents the 10 patients who proceeded to Sling/AUS after last MPQ injection. The remaining three lines represent the entire cohort.

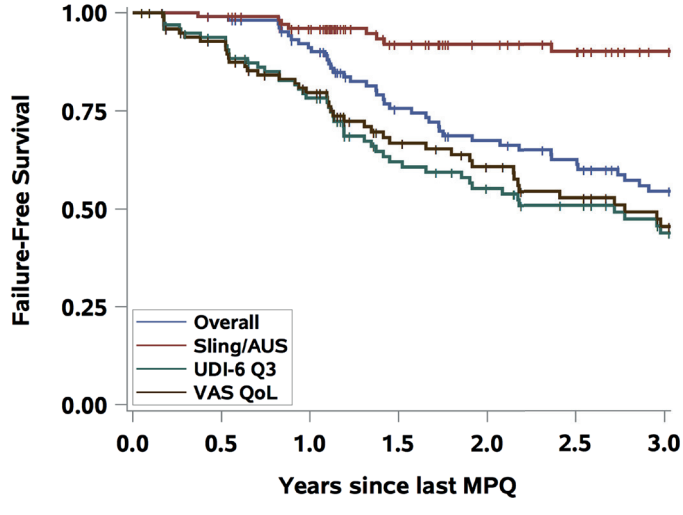


Table 4 - Success Rates in the Literature.

Study	Study Design	Method of Determining Success	N	Follow-Up (months)	Dry Rate (%)	Improvement Rate (%)
Ghoniem et al. (1)	Randomized, single-blind	Stamey Grade	122	12	36.9	61.5
Ghoniem et al. (2)	Case Series	Stamey Grade	67	24	67	84
Ghoniem and Miller (13)	Meta-Analysis	Variable	Variable	>18	36	64
Harriss et al. (20)	Case Series	Self-Report	40	36	40	18
Maher et al. (21)	RCT*	Self-Report	22	12	n/a	60
Plotti et al. (22)	Case Series	Voiding Diary/Stress Test	24	12	42	42
Radley et al. (18)	Case Series	Self-Report	56	19	20	59
Rosenfeld et al. (4)	Case Series	Questionnaires/Self-Report	59	9	19	75
Serati et al. (19)	Prospective Cohort	Questionnaires/Stress Test	85	36	47	49
Tamanini et al. (3)	Prospective Cohort	Urodynamic Testing	15	60	40	33
Zullo et al. (14)	Prospective Cohort	Voiding Diary/Stress Test	61	60	18	39

***RCT=**Randomized Controlled Trial

months, although the analyzed reports used different methods of determining outcomes (13). Longer-term findings have been reported by Tamanini and colleagues who used post-operative VLPP to establish a cure/improvement rate of 73% in 15 women followed for 60 months (3). Zullo and colleagues reported on a series of 61 women with a success rate of 57% at a follow-up point of 60 months (14), with success defined as cure or improvement in voiding symptoms (measured with voiding diary and cough stress test).

Group III had the highest completely dry/cure rate (29%) and overall success rate (65%) after final MPQ injection, despite the latter value not reaching statistical significance. This experience parallels that of Gumus and colleagues, whose report compared MPQ outcomes using I-QOL, IIQ-7, and UDI-6 questionnaires in 35 women with and without history of anti-incontinence procedures (15). This group also found that at a median follow-up of 58 months, women receiving MPQ after failed prior SUI surgery were more satisfied with the outcome of MPQ injection.

Factors predicting success after MPQ injection were studied. First, a repeat MPQ injection led to a nearly 25% increase in success over

time. Second, transient post-operative retention, although not statistically significant, was considerably higher in the success group (58%) than in the failure group (36%). Post-operative urinary retention is reportedly found in approximately 6-32% of women receiving MPQ injection (13), while the rate in our series was 45% after final injection. Third, we analyzed volume and configuration of MPQ by 3DUS, a technology employed in different specialties that is well suited to image the vaginal space (16, 17). However, its use as a follow-up tool after MPQ injection has only recently been described and therefore, its role as an outcome predictor has not been fully evaluated yet. A prior study of Collagen injection at our institution in 46 women with a mean follow-up of 14 months who were evaluated with 3DUS 4-12 weeks after injection found a correlation between a positive clinical outcome and a symmetrical/circumferential configuration of Collagen (12). These results have since been corroborated by Radley and colleagues who reported that in 9 women followed for 19 months after MPQ injection, a good clinical outcome was seen with MPQ completely surrounding the urethra compared to a poor outcome in 3 women with incomplete urethral encirclement (18).

Although not studied in our series, Serati and colleagues found that increased surgeon's skill and lack of prior radical pelvic surgery significantly correlated with success in 85 women injected with MPQ and followed for 3 years (19). Notable differences with the present study include the lack of 3DUS data as well as the decision to not offer reinjection should patients require further anti-incontinence treatment after the first MPQ injection.

Strengths of our study included several ISD groups based on prior SUI treatment history, a relatively large sample size, and a mid-term follow-up including SUI self-reporting and validated questionnaires. In addition, this study utilized 3DUS as an objective outcome measure. Our efforts to reach all participants over the phone to optimize our long-term results and limit our loss to follow-up data were met with limitations, as in any real-life practice study. Slight differences across our three groups at baseline could also have had an impact as they varied in age and Q-max.

CONCLUSIONS

MPQ resulted in improved UDI-6 scores, QoL scores, and self-reported continence as both a primary and secondary treatment option in women with SUI secondary to ISD. MPQ may be particularly valuable in those with more extensive prior anti-incontinence treatments. Factors that could predict success such as repeat MPQ injection, immediate post-MPQ retention, and injection configuration/volume by 3DUS will have to be tested over longer term follow-up.

ABBREVIATIONS

MPQ = Macroplastique
SUI = Stress Urinary Incontinence
ISD = Intrinsic Sphincter Deficiency
3DUS = Three-Dimensional Ultrasound
UDI = Urogenital Distress Inventory
QoL = Quality of Life
VLPP = Valsalva Leak Point Pressure
AUS = Artificial Urinary Sphincter

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Ghoniem G, Corcos J, Comiter C, Bernhard P, Westney OL, Herschorn S. Cross-linked polydimethylsiloxane injection for female stress urinary incontinence: results of a multicenter, randomized, controlled, single-blind study. J Urol. 2009;181:204-10.
2. Ghoniem G, Corcos J, Comiter C, Westney OL, Herschorn S. Durability of urethral bulking agent injection for female stress urinary incontinence: 2-year multicenter study results. J Urol. 2010;183:1444-9.
3. Tamanini JT, D'Ancona CA, Netto NR. Macroplastique implantation system for female stress urinary incontinence: long-term follow-up. J Endourol. 2006;20:1082-6.
4. Emily C.Rosenfeld, aAlana Christie, Chasta D.Bacsu, Philippe E.Zimmerna. Macroplastique outcome in women with stress urinary incontinence secondary to intrinsic sphincteric deficiency. Urological Science, 2016,27, 258-62.

5. Poon CI, Zimmern PE, Wilson TS, Defreitas GA, Foreman MR. Three-dimensional ultrasonography to assess long-term durability of periurethral collagen in women with stress urinary incontinence due to intrinsic sphincter deficiency. Urology. 2005;65:60-4.
6. Bacsu CD, Cunningham C, Christie A, Zimmern PE. Durability of collagen injection for stress urinary incontinence in women proven by transvaginal 3-dimensional ultrasound. Female Pelvic Med Reconstr Surg. 2015;21:25-9.
7. Walsh LP, Zimmern PE, Pope N, Shariat SF; Urinary Incontinence Treatment Network. Comparison of the Q-tip test and voiding cystourethrogram to assess urethral hypermobility among women enrolled in a randomized clinical trial of surgery for stress urinary incontinence. J Urol. 2006;176:646-9.
8. Zimmern P, Nager CW, Albo M, Fitzgerald MP, McDermott S; Urinary Incontinence Treatment Network. Interrater reliability of filling cystometrogram interpretation in a multicenter study. J Urol. 2006;175:2174-7.
9. Haab F, Zimmern PE, Leach GE. Female stress urinary incontinence due to intrinsic sphincteric deficiency: recognition and management. J Urol. 1996;156:3-17.
10. McGuire EJ, Fitzpatrick CC, Wan J, Bloom D, Sanvordenker J, Ritchey M, et al. Clinical assessment of urethral sphincter function. J Urol. 1993;150(5 Pt 1):1452-4.
11. Uebersax JS, Wyman JF, Shumaker SA, McClish DK, Fantl JA. Short forms to assess life quality and symptom distress for urinary incontinence in women: the Incontinence Impact Questionnaire and the Urogenital Distress Inventory. Continence Program for Women Research Group. Neurourol Urodyn. 1995;14:131-9.
12. Defreitas GA, Wilson TS, Zimmern PE, Forte TB. Three-dimensional ultrasonography: an objective outcome tool to assess collagen distribution in women with stress urinary incontinence. Urology 2003;62:232-6.
13. Ghoniem GM, Miller CJ. A systematic review and meta-analysis of Macroplastique for treating female stress urinary incontinence. Int Urogynecol J. 2013;24:27-36.
14. Zullo MA, Plotti F, Bellati F, Muzii L, Angioli R, Panici PB. Transurethral polydimethylsiloxane implantation: a valid option for the treatment of stress urinary incontinence due to intrinsic sphincter deficiency without urethral hypermobility. J Urol. 2005;173:898-902.
15. Gumus II, Kaygusuz I, Derbent A, Simavli S, Kafali H. Effect of the Macroplastique Implantation System for stress urinary incontinence in women with or without a history of an anti-incontinence operation. Int Urogynecol J. 2011;22:743-9.
16. Athanasiou S, Khullar V, Boos K, Salvatore S, Cardozo L. Imaging the urethral sphincter with three-dimensional ultrasound. Obstet Gynecol. 1999;94:295-301.
17. Shobeiri SA, White D, Quiroz LH, Nihira MA. Anterior and posterior compartment 3D endovaginal ultrasound anatomy based on direct histologic comparison. Int Urogynecol J. 2012;23:1047-53.
18. Radley SC, Chapple CR, Mitsogiannis IC, Glass KS. Transurethral implantation of macroplastique for the treatment of female stress urinary incontinence secondary to urethral sphincter deficiency. Eur Urol. 2001;39:383-9.
19. Serati M, Soligo M, Braga A, Cantaluppi S, Coluccia AC, Di Dedda MC, et al. Efficacy and safety of polydimethylsiloxane injection (Macroplastique(®)) for the treatment of female stress urinary incontinence: results of a series of 85 patients with ≥3 years of follow-up. BJU Int. 2019;123:353-9.
20. Harriss DR, Iacovou JW, Lemberger RJ. Peri-urethral silicone microimplants (Macroplastique) for the treatment of genuine stress incontinence. Br J Urol. 1996;78:722-5; discussion 726-8.
21. Maher CF, O'Reilly BA, Dwyer PL, Carey MP, Cornish A, Schluter P. Pubovaginal sling versus transurethral Macroplastique for stress urinary incontinence and intrinsic sphincter deficiency: a prospective randomised controlled trial. BJOG. 2005;112:797-801.
22. Plotti F, Zullo MA, Sansone M, Calcagno M, Bellati F, Angioli R, et al. Post radical hysterectomy urinary incontinence: a prospective study of transurethral bulking agents injection. Gynecol Oncol. 2009;112:90-4

Correspondence address:
Philippe E. Zimmern, MD
UT Southwestern Medical Center,
5323 Harry Hines Blvd
Dallas, TX 75390-9110, USA
Fax: +1 214 648-8786
E-mail: philippe.zimmern@utsouthwestern.edu

ORIGINAL ARTICLE

Vol. 45 (5): 999-1007, September - October, 2019
doi: 10.1590/S1677-5538.IBJU.2019.0103

Intermediate-term outcomes of laparoscopic pectopexy and vaginal sacrospinous fixation: a comparative study

Bahar Sariibrahim Astepe [1], Aybike Karsli [1], Işil Köleli [2], Orhan Seyfi Aksakal [3], Hasan Terzi [1], Ahmet Kale [1]

[1] Department of Obstetrics and Gynecology, Kocaeli Derince Training and Research Hospital, Kocaeli, Turkey; [2] Department of Obstetrics and Gynecology, Inönü University Medicine Faculty, Malatya, Turkey; [3] Urogynecology Clinics, Zekai Tahir Burak Training and Research Hospital, Ankara, Turkey

ABSTRACT

Objective: To compare the intermediate-term follow-up results of laparoscopic pectopexy and vaginal sacrospinous fixation procedures.
Materials and Methods: Forty-three women who had vaginal sacrospinous fixations(SSF) using Dr. Aksakal's Desta suture carrier and 36 women who had laparoscopic pectopexies were re-examined 7 to 43 months after surgery. The PISQ-12 and P-QOL questionnaires were answered by all of the women.
Results: The apical descensus relapse rates did not differ between the groups (14% in the SSF vs. 11.1% in the pectopexy group). The de novo cystocele rates were higher in the SSF group (25.6% in the SSF vs. 8.3% in the pectopexy group). There were no significant differences in the de novo rectocele numbers between the groups. The treatment satisfaction rates were high in both groups (93% in the SSF vs. 91.7% in the pectopexy group), which was not statistically significant. Moreover, the postoperative de novo urge and stress urinary incontinence rates did not differ; however, the postoperative sexual function scores (PISQ-12) (36.86±3.15 in the SSF group vs. 38.21±5.69 in the pectopexy group) were better in the pectopexy group. The general P-QOL scores were not significantly different between the surgery groups.
Conclusion: The vaginal sacrospinous fixation maintains its value in prolapse surgery with the increasing importance of native tissue repair. The new laparoscopic pectopexy technique has comparable positive follow-up results with the conventional sacrospinous fixation procedure.

ARTICLE INFO

Bahar Sarıibrahim Astepe
http://orcid.org/0000-0002-9012-4802

Keywords:
Pelvic Organ Prolapse;
Hand-Assisted Laparoscopy;
Treatment Outcome

Int Braz J Urol. 2019; 45: 999-1007

Submitted for publication:
February 11, 2019

Accepted after revision:
May 31, 2019

Published as Ahead of Print:
July 10, 2019

INTRODUCTION

A pelvic organ prolapse (POP) is defined as a herniation of the pelvic organs to or beyond the vaginal walls, and can significantly affect a woman's daily activities and sexuality. The surgical approaches to the POP treatment vary, including vaginal, open abdominal, laparoscopic, and robotic methods. The decision regarding the surgical route depends on the surgeon's experience, the need for repairing other pelvic organ defects, and the coexistence of urinary incontinence.

In 2016, the US Food and Drug Administration (FDA) reclassified the use of surgical mesh for transvaginal POP surgery as a class 3 procedure (i.e., high risk) (1). Since this FDA reclassification, native tissue repair in vaginal surgery and laparoscopic procedures has been gaining more

importance. In vaginal surgery, native tissue repair eliminates the mesh-related complications, such as mesh erosion and infection. This is particularly important for women at a high risk of mesh erosion (e.g., women who smoke, are immunosuppressed, or have uncontrolled diabetes mellitus).

With the promising advantages of laparoscopic surgery, such as shorter hospitalization, shorter recovery time, less postoperative pain, early mobilization, and fewer scars (2), minimal invasive surgery for POP treatment has become more significant. In 1993, Joshi VM. described a new technique for uterine suspension to pectineal ligament bilaterally with mersile tape through a Cherney incision (3). In 2010, Banerjee and Noe described a laparoscopic pectopexy operation for obese patients using the iliopectineal ligament for the vaginal vault or cervical stump suspension (4). To our best knowledge, we have yet to find literature in the area of postoperative results comparing laparoscopic pectopexy (LPP) and vaginal sacrospinous fixation procedures (VSSF). In the present study, we aimed to compare the intermediate-term outcomes of the pectopexy technique and conventional sacrospinous fixation in our clinic.

MATERIALS AND METHODS

This retrospective observational study was based on the postoperative results of women who had undergone uterovaginal or vaginal vault prolapse surgery. Forty-three women who had VSSF and 36 women who had LPP between January 2014 and June 2018 at the S.B.U Kocaeli Derince Education and Research Hospital Obstetrics and Gynecology Clinics were gynecologically re-evaluated between 15 June and 30 December 2018.

Preoperatively, all of the patients provided urogynecological histories and underwent routine physical examinations, cough stress tests, perineal ultrasonography, and Bonney tests. The POP (apical, anterior and posterior wall prolapsus) was staged according to the Pelvic Organ Prolapse Quantification (POP-Q) system. Those patients with stage 2 or greater uterovaginal/vaginal cuff prolapses according to the POP-Q system underwent surgery (vaginally or laparoscopically) were included in the study. Patients with stage 2 uterine / vaginal cuff prolapsus were recommended to have surgery if they had complaints of bulging symptoms. Young women with fertility desire and who had had hysteropexy procedures were not included in the study. Women who had had surgery for malignancy suspicion or pelvic inflammatory disease were not included in the study. Those patients with stress urinary incontinence and positive cough stress tests underwent anti-incontinence surgery (transobturator tape or tension free vaginal tape) along with the POP surgery. The hospital database was evaluated for any perioperative and postoperative complication records. All of the methods and definitions used in the study conformed with the standards recommended by the International Urogynecological Association and the International Continence Society (5). Ethical approval for this study was obtained from the S.B.U Kocaeli Derince Training and Research Hospital Management and Training Commission. The clinical trials registration ID is NCT03663959.

All of the patients received telephone calls and those patients that could be reached at phone were invited for a gynecological re-examination. All but one patient in the vaginal surgery group came in for a gynecological check. All patients gave written informed consent for the scientific use of their evaluation results, operation videos and images in this study. We conducted a telephone interview with the one patient not agreeing to come for the check, and we learned that she had had a relapse 6 months after the operation and she was unsatisfied with the surgery. In the postoperative re-evaluation between 15 June and 30 December 2018, all of the women were examined in the lithotomy position for apical, anterior, and posterior compartment descensus. Stage 2 or greater apical descensus or a cystocele or rectocele according to the POP-Q system were accepted as postoperative relapses. All of the patients answered the Pelvic Organ Prolapse / Urinary Incontinence Sexual Questionnaire (PISQ-12) and the Prolapse Quality of Life (P-QOL) questionnaire. The Turkish validations of the PISQ-12 and P-QOL were conducted by Cam et al. (6, 7). The PISQ-12 is a self-administered questionnaire that evaluates the sexual function of women with pelvic organ prolapse or urinary incontinence. Higher scores

show good sexual functioning of women. The P--QOL evaluates the impact of the urogenital prolapsus on the quality of life in women. A high total score indicates a worsening of quality of life of women with pelvic organ prolapsus.

All of the women were asked about de novo urge urinary incontinence and de novo stress urinary incontinence. In addition, each patient's satisfaction with the surgery was asked and recorded.

The vaginal surgery group underwent a right sacrospinous fixation using Dr. Aksakal's Desta suture carrier (Figure-1) with two permanent sutures, which combined the sacrospinous ligament and vaginal cuff fascia. The Desta suture carrier was developed for deep pelvic surgery,

Figure 1 - Dr. Aksakal's Desta suture carrier.

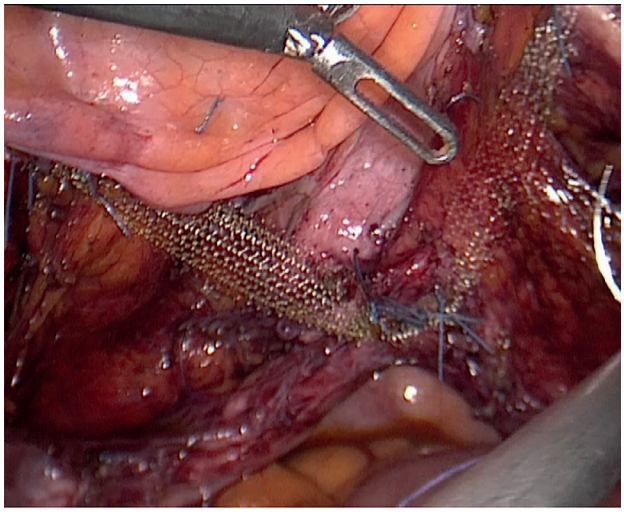
and the suture depth can be easily adjusted. After the vaginal hysterectomy, under the vaginal cuff mucosa, we created a tunnel through the spinous process with a straight tool. After passing the rectovaginal pillars, the perirectal space was entered, and the ischial spine was palpated. With the help of an index finger placed on the spinous process, we placed two permanent sutures 1.5-2 cm medial onto the spinous process on the sacrospinous ligament and iliococcygeus muscle complex using the Desta suture carrier. Next, the permanent sutures were combined with the pubocervicovaginal and rectovaginal fascia under the vaginal cuff mucosa. Those patients with stress urinary incontinence underwent transobturator tape or tension free vaginal tape procedures. Those patients with lateral defect cystoceles underwent paravaginal repairs that were performed using late absorbable sutures that combined the arcus tendineus fasciae pelvis and pubocervi covaginal fascia.

In vaginal surgery, in stage 2 and over apical prolapsus there is a great endopelvic fascia defect that cause prolapsus not only in apical compartment but also in the anterior and posterior compartments. If there is Stage 2 and over cystocele or rectocele according to the POP-Q we add anterior or posterior colporraphies to the operation.

The laparoscopy group underwent pectocolpopexy procedure. After entering into the abdominal cavity with 10mm (EndoEthicon) and 5 mm trocars, sufficient intraabdominal pressure (14 mm Hg) was achieved. First, the peritoneal layer above and lateral to the bladder was opened parallel to the round ligament toward the pelvic side wall on the right side. Then, with the guidance of the obliterated umbilical artery, lateral to the obliterated umbilical artery and medial to the external iliac vein, the iliopectineal ligament was located. At this point, a segment of approximately 3-4 cm2 was formed, exposing the iliopectineal (Cooper's) ligament. In this area, behind the obliterated umbilical artery, the obturator nerve could be seen. The same area on the left side was prepared using the same steps. Then, the anterior part of the vaginal cuff was prepared for the mesh fixation. Bilaterally, the ends of a polypropylene monofilament mesh (1.5 x 15 cm) were fixed to the iliopectineal ligament with nonabsorbable polypropylene or polyester sutures (Prolene & Ethibond Excel; Ethicon, Somerville, NJ, USA) (Figure-2). The vaginal cuff was elevated to the POP-Q level 0-1 with the help of a vaginal sponge, and the mesh was fixed anteriorly to the vaginal cuff with nonabsorbable polypropylene sutures. Finally, the mesh was embedded under the peritoneum with a continuous monofilament absorbable suture (Vicryl; Ethicon, Johnson & Johnson, Bridgewater, NJ, USA).

In L / S pectopexy surgery, after apical suspension we evaluate anterior and posterior vaginal walls. If there is Stage 2 and over cystocele or rectocele according to the POP-Q, we add anterior or posterior colporraphies to the operation.

Figure 2 - The mesh is fixated to the bilateral ileopectineal ligaments and anterior part of the vaginal cuff.



Statistics

The sample size calculation was done with the G Power 3.1 software. For the comparison of the categorical variables between the groups, with the significance level of **α**:0.05, the effect size 0.5 and the statistical power of 0.80, the required total sample size was 52.

The IBM SPSS Statistics for Windows version 21.0. (IBM Corp., Armonk, NY, USA) was used for the statistical analysis. The continuous variables were expressed as the mean±standard deviation, and the categorical variables were expressed as the number and percentage. The Mann-Whitney U test and independent sample t-tests were used to compare the quantitative variables, while the chi-squared test and Fischer's exact test were used to compare the categorical variables. The statistical significance was defined as $p < 0.05$.

RESULTS

Forty-three patients underwent VSSFs, and 36 patients underwent LPPs. Forty patients in the vaginal surgery group and 18 patients in the laparoscopic surgery group underwent hysterectomies (vaginal hysterectomy or total laparoscopic hysterectomy). Of the 79 patients, 21 patients had vaginal vault prolapses (three in the vaginal surgery group and eighteen in the laparoscopy group) (Table-1). Thirteen patients in the vaginal surgery group and three patients in the laparoscopy group underwent concomitant anti-incontinence surgery.

The mean ages of the patients were 58.95±8.33 and 60.03±9.05 in the vaginal surgery and laparoscopy group respectively, which was not statistically significant. The number of births of the patients in the surgery groups was not different. There were no significant differences in the duration of the follow-up period between the

Table 1 - Surgical procedures performed in the vaginal and laparoscopic surgery groups.

Procedure	Vaginal Sacrospinous Fixation group (n: 43)	Laparoscopic Pectopexy Group (n: 36)
Vaginal Hysterectomy	40 (93%)	-
Sacrospinous fixation	43 (100%)	-
Anterior colporrhaphy	28 (65.1%)	2 (5.6%)
Posterior colporrhaphy	23 (53.5%)	2 (5.6%)
Salphenjectomy	8 (18.6%)	-
Perineoplasty	17 (39.5%)	2 (5.6%)
Transobturator tape	11 (25.6%)	3 (8.3%)
Tension free vaginal tape	2 (4.7%)	-
Paravaginal repair	10 (23.3)	5 (13.9%)
Laparoscopic hysterectomy	-	18 (50%)
Laparoscopic pectocolpopexy	-	36 (100%)
Bilateral salphingoopherectomy	-	21 (58.3%)

Table 2 - Patient characteristics and previous pelvic surgery histories in the vaginal and laparoscopic surgery groups.

Parameter	Vaginal Sacrospinous fixation group (n: 43)	Laparoscopic Pectopexy group (n: 36)	p
Age (years)	58.95±8.33	60.03±9.05	0.585a
Mean body mass index (kg / m^2)	28.98±3.48	29.51±3.29	0.492a
Parity	4.02±2.01	3.72±1.75	0.613b
Follow-up (month)	17.09±10.29	13.06±6.35	0.165b
History of pelvic surgery, n (%)	5 (11.6%)	21 (58.3%)	0.000c
Abdominal hysterectomy + bilateral salpingoophorectomy	2	11	
Abdominal hysterectomy	1	1	
Vaginal Hysterectomy	-	6	
Tubal ligation	1	1	
Tubal ligation + unilateral salpingoophorectomy	1	-	
Cesarean section	-	2	
Sacrocolpopexy	-	2	
Sacrospinous fixation	-	1	

[a] Independent samples t-test; [b] Mann-Whitney U test; [c] Pearson Chi-squared test

Table 3 - Complications related to surgery groups.

	Vaginal sacrospinous fixation group (n: 43)	Laparoscopic pectopexy group (n: 36)
Ureteral kinging (n)	1	
Bladder injury (n)		1
Mesh erosion (n)		1

surgery groups. The vaginal surgery patients were examined 7 to 43 (min-max) months after the surgery with a mean of 17.09±10.29 months, and the laparoscopic surgery patients were examined 7 to 30 (min-max) months after surgery with a mean of 13.06±6.35. Three patients in the laparoscopy group had repeat surgery because of a recurrent prolapse; two of them had SCP and 1 had previously had VSSF (Table-2).

The patient's hospital records were evaluated for perioperative and postoperative complications. One patient had ureteral kinking in the vaginal surgery group. She underwent ureteral catheterizations four times postoperatively, and she is now in urology follow-up. One patient in the laparoscopy group had a bladder injury. Another patient in the laparoscopy group had mesh erosion at the vaginal apex, which was surgically extracted. The woman with mesh erosion was 50 years old, she had had a vaginal hysterectomy two years earlier and a laparoscopic sacrocolpopexy one year earlier (Table-3).

There were six apical descensus relapse cases in the vaginal surgery group and four cases in the laparoscopy group, although these numbers were of no statistical significance. The number of

women with de novo central or lateral defect cystoceles in the vaginal surgery group was significantly higher than the number of women in the laparoscopy group (Table-4). Those women who underwent VSSFs had more de novo cystoceles than the women who underwent the LPPs. Moreover, there was no significant difference in the de novo rectocele numbers between the vaginal and laparoscopic surgery groups. The treatment satisfaction rates were high in both groups (93% in the vaginal surgery and 91.7% in the laparoscopy groups), but this was not statistically significant.

There was no difference between the postoperative de novo urge and stress urinary incontinence rates in the VSSF and LPP groups (Table-4).

The postoperative PISQ-12 scores of the women in the laparoscopy group were significantly higher than those of the women in the vaginal surgery group (Table-5), which means that the postoperative sexual function scores were better in the laparoscopy group. The general scores of the P-QOL questionnaire were not significantly different between the surgery groups. However, there were differences in the general health perceptions, role limitations, and emotion domains between the groups (Table-5).

DISCUSSION

We found that the LPPs and VSSFs were equally effective in the treatment of the uterovaginal / vaginal vault prolapses. The apical descensus relapse rates and patient satisfaction rates were not different.

When the abdominal sacrocolpopexy(ASC) and vaginal surgery results for the apical vaginal prolapsus were compared, the ASC showed better objective anatomic outcomes (8, 9). In addition, the ASC provided better recurrent prolapse rates for 1-2 years and also better objective failure and repeat surgery rates for 2-4 years when compared to the vaginal surgery (10). Although good anato-

Table 4 - Follow-up results.

	Vaginal sacrospinous fixation group	Laparoscopic pectopexy group	p
Number of patients	43	36	
Apical descensus relapse (number of patients / all patients)	6 (14%)	4 (11.1%)	0.748[d]
De novo central or lateral defect cystocele (number of patients / all patients)	11 (25.6%)	3 (8.3%)	0.046[c]
De novo rectocele (number of patients / all patients)	2 (4.7%)	2 (5.6%)	1.000[d]
De novo stress urinary incontinence	1 (2.3%)	2 (5.6%)	0.589[d]
De novo urge incontinence	3 (7%)	0 (0%)	0.246[d]
Satisfied with surgery (number of patients / all patients)	40 (93%)	33 (91.7)	1.000[d]

[c]Pearson Chi-squared test; [d]Fisher's exact test

Table 5 - Comparison of the Pelvic Organ Prolapse / Urinary Incontinence Sexual Questionnaire (PISQ-12) and Prolapse Quality of Life (P-QOL) scores between the surgery groups.

	Vaginal sacrospinous fixation group	Laparoscopic pectopexy group	p
PISQ-12 score (mean±SD)	36.86±3.15	38.21±5.69	0.029[b]
GHP (mean±SD)	3.93±4.58	4.83±3.33	0.048[b]
PI (mean±SD)	3.09±2.56	2.33±1.76	0.304[b]
RL (mean±SD)	0.09±0.61	0.28±0.70	0.031[b]
PL (mean±SD)	0.14±0.64	0.28±0.61	0.059[b]
SL (mean±SD)	0.14±0.64	0.14±0.54	0.830[b]
PR (mean±SD)	0.51±1.05	0.83±1.27	0.183[b]
EM (mean±SD)	0.14±0.91	0.58±1.02	0.000[b]
SE (mean±SD)	0.09±0.48	0.11±0.32	0.303[b]
SM (mean±SD)	0.12±0.62	0.11±0.39	0.521[b]
GS (mean±SD)	8.49±10.82	9.61±6.99	0.088[b]

GHP = general health perceptions; **PI** = prolapse impact; **RL** = role limitations; **PL** = physical limitations; **SL** = social limitations; **PR** = personal relationships; **EM** = emotions; **SE** = sleep / energy; **SM** = severity measures; **GS** = general score

[b] Mann-Whitney U test

mical and functional results related to sacrocolpexy procedures were reported, new surgical modalities are being applied to more patients every day. Technical difficulties related to the sacrocolpopexy can be seen as due to the difficult surgical field at the ventral site of the sacrum, with a related injury risk to the presacral veins, sacral arteries, and hypogastric nerves. The mesh is usually fixated to the sacral longitudinal ligament, and problems related to the mesh exposure, such as erosion, ileus, and osteomyelitis can be seen (11). Additionally, the decrease in the pelvic space can cause de novo constipation (12).

Pectopexy is an alternative technique to sacropexy, which has certain advantages such as the technique is easy to learn, has a shorter operative time, and has similar positive results (4). Noe et al. compared the postoperative and intermediate-term follow-up results between 44 pectopexy and 41 sacropexy patients. They reported that the apical descensus relapse rates and de novo rectocele rates were no different. In addition, the laparoscopic pectopexy group had lower lateral defect cystocele and de novo constipation rates (12). Moreover, they noticed that the pectopexy had a protective effect on the anterior lateral compartments (12). Although during L / S pectopexy operations vaginal apex is suspended upwards and anteriorly, and the body's center of gravity deviated to the posterior, postoperative de novo rectocele rates were no different between pectopexy and sacropexy patients. Similarly in the present study, although the vaginal surgery group had more posterior colporrhaphy operations, de novo rectocele rates were no different between VSSF and LPP groups. Moreover in the present study although the vaginal surgery group had more anterior colporrhaphy operations, we found higher de novo cystocele rates in the vaginal sacrospinous fixation group when compared to the pectopexy group. This could be explained by the fact that the vaginal axis in the VSSF group deviated to the right and posterior, and the body's center of gravity deviated to the anterior, resulting in more weight on the anterior compartment.

It is reported that using mesh in the surgical treatment of cystocele and POP might worsen the sexual function of women in the dyspareunia

and behaviour domains (13, 14). Besides, in the study by Vitale et. al., women with severe cystocele were treated with biocompatible porcine dermis graft and they reported a significant improvement in the QoL and sexual function scores 12 months after surgery (14). In our study, the postoperative sexual function of the women in the laparoscopy group was better than that in the vaginal surgery group. This could be related to the scar formation in the vagina after vaginal surgery, vaginal axis deviation to the right and posterior because of unilateral SSF and related sexual problems. Hereof Vitale et. al. evaluated the efficacy and effect of bilateral sacrospinous fixation on QoL and sexuality of women with recurrent vaginal vault prolapse, and they showed a significant improvement in the QoL and sexuality scores (15).

In the current study, we found high patient satisfaction rates (93% in the vaginal surgery group and 91.7% in the laparoscopy group). The general quality of life scores were no different, and they could be accepted as good in both groups, which is compatible with the high patient satisfaction rates. Similarly, Tahaó lu et al. showed significant improvement in the P-QOL scores pre and postoperatively in the laparoscopic pectopexy group (16). Kale et al. evaluated the short-term outcomes of initial experience with pectopexy surgeries, they reported successful intraoperative and 6. month postoperative results (17). In the present study, although fewer patients in the pectopexy group underwent anti-incontinence surgery, the de novo urge and stress urinary incontinence rates were not different between the groups. This could be attributed to the protective effect of the pectopexy on the anterior lateral compartments (12).

The small number of cases, short term follow-up period and the retrospective design are the major limitations of this study. Since pectopexy procedure has been widely performed in the world since 2010, studies reporting long term results of pectopexy operation with more patients will be published in the following years. Besides VSSF group had more hysterectomies and colporrhaphy operations than LPP group. This could be explained as our hospital is a center that patients are referred from different cities. Most of the women who have completed her fertility and over 45 years want to have hysterectomy because of follow-up difficulties. Another limitation is that, unlike Noe et al. using polyvinylidenfluoride (PVDF) in their original work, we used meshes made of polypropylene, which is widely used in sacrocolpopexy operations (12). The major strengths of this study were that all of the patients (except one, who had relapses and was interviewed over the phone) were gynecologically re-examined postoperatively.

We believe that a pectopexy can be a good choice for prolapse surgery. Surgeons experienced in the laparoscopy and familiar with the pelvic anatomy can easily perform a laparoscopic pectopexy. However, the vaginal sacrospinous fixation maintains its value in prolapse surgery with the increasing importance of native tissue repair. Overall, a pectopexy presents a new, promising, safe method for prolapse surgery. Long-term multicenter follow-up results will help both surgeons and patients to better understand their role in prolapse surgery.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Food and Drug Administration, HHS. Obstetrical and Gynecological Devices; Reclassification of Surgical Mesh for Transvaginal Pelvic Organ Prolapse Repair; Final order. Fed Regist. 2016;81:353-61.
2. Gadonneix P, Ercoli A, Salet-Lizée D, Cotelle O, Bolner B, Van Den Akker M, et al. Laparoscopic sacrocolpopexy with two separate meshes along the anterior and posterior vaginal walls for multicompartment pelvic organ prolapse. J Am Assoc Gynecol Laparosc. 2004;11:29-35.
3. Joshi VM. A new technique of uterine suspension to pectineal ligaments in the management of uterovaginal prolapse. Obstet Gynecol. 1993;81(5 (Pt1)):790-3.
4. Banerjee C, Noé KG. Laparoscopic pectopexy: a new technique of prolapse surgery for obese patients. Arch Gynecol Obstet. 2011;284:631-5.

5. Haylen BT, de Ridder D, Freeman RM, Swift SE, Berghmans B, Lee J, et al. An International Urogynecological Association (IUGA)/International Continence Society (ICS) joint report on the terminology for female pelvic floor dysfunction. Int Urogynecol J. 2010;21:5-26.
6. Cam C, Sancak P, Karahan N, Sancak A, Celik C, Karateke A. Validation of the short form of the Pelvic Organ Prolapse/ Urinary Incontinence Sexual Questionnaire (PISQ-12) in a Turkish population. Eur J Obstet Gynecol Reprod Biol. 2009;146:104-7.
7. Cam C, Sakalli M, Ay P, Aran T, Cam M, Karateke A. Validation of the prolapse quality of life questionnaire (P-QOL) in a Turkish population. Eur J Obstet Gynecol Reprod Biol. 2007;135:132-5.
8. Maher C, Feiner B, Baessler K, Adams EJ, Hagen S, Glazener CM. Surgical management of pelvic organ prolapse in women. Cochrane Database Syst Rev. 2010;(4):CD004014.
9. Siddiqui NY, Grimes CL, Casiano ER, Abed HT, Jeppson PC, Olivera CK, et al. Mesh sacrocolpopexy compared with native tissue vaginal repair: a systematic review and meta-analysis. Obstet Gynecol. 2015;125:44-55.
10. Maher C, Feiner B, Baessler K, Christmann-Schmid C, Haya N, Brown J. Surgery for women with apical vaginal prolapse. Cochrane Database Syst Rev. 2016;10:CD012376.
11. Nygaard IE, McCreery R, Brubaker L, Connolly A, Cundiff G, Weber AM, et al. Abdominal sacrocolpopexy: a comprehensive review. Obstet Gynecol. 2004;104:805-23.
12. Noé KG, Schiermeier S, Alkatout I, Anapolski M. Laparoscopic pectopexy: a prospective, randomized, comparative clinical trial of standard laparoscopic sacral colpocervicopexy with the new laparoscopic pectopexy-postoperative results and intermediate-term follow-up in a pilot study. J Endourol. 2015;29:210-5.
13. Liang CC, Lin YH, Chang YL, Chang SD. Urodynamic and clinical effects of transvaginal mesh repair for severe cystocele with and without urinary incontinence. Int J Gynaecol Obstet. 2011;112:182-6.
14. Vitale SG, Caruso S, Rapisarda AMC, Valenti G, Rossetti D, Cianci S, et al. Biocompatible porcine dermis graft to treat severe cystocele: impact on quality of life and sexuality. Arch Gynecol Obstet. 2016;293:125-131.
15. Vitale SG, Laganà AS, Noventa M, Giampaolino P, Zizolfi B, Butticè S, et al. Transvaginal Bilateral Sacrospinous Fixation after Second Recurrence of Vaginal Vault Prolapse: Efficacy and Impact on Quality of Life and Sexuality. Biomed Res Int. 2018;2018:5727165.
16. Tahaoglu AE, Bakir MS, Peker N, Bagli İ, Tayyar AT. Modified laparoscopic pectopexy: short-term follow-up and its effects on sexual function and quality of life. Int Urogynecol J. 2018;29:1155-1160.
17. Kale A, Biler A, Terzi H, Usta T, Kale E. Laparoscopic pectopexy: initial experience of single center with a new technique for apical prolapse surgery. Int Braz J Urol. 2017;43:903-9.

Correspondence address:
Bahar Sariibrahim Astepe, MD
Cumhuriyet Mah. Lale sok.Cemre sitesi No: 6
Plajyolu, İzmit, Turkey
Telephone: +050 58 942-924
E-mail: baharsariibrahim@hotmail.com

ORIGINAL ARTICLE

Vol. 45 (5): 1008-1012, September - October, 2019
doi: 10.1590/S1677-5538.IBJU.2019.0132

Human Chorionic Gonadotropin monotherapy for the treatment of hypogonadal symptoms in men with total testosterone > 300 ng/dL

Vinayak Madhusoodanan [1], Premal Patel [2], Thiago Fernandes Negris Lima [2], Jabez Gondokusumo [3], Eric Lo [3], Nannan Thirumavalavan [3], Larry I. Lipshultz [3], Ranjith Ramasamy [2]

[1] University of Miami Miller School of Medicine, Miami, FL, USA; [2] Department of Urology, University of Miami Miller School of Medicine, Miami, FL, USA; [3] Scott Department of Urology, Baylor College of Medicine, Houston, TX, USA

ABSTRACT

Purpose: The 2018 American Urological Association guidelines on the Evaluation and Management of Testosterone Deficiency recommended that 300 ng/dL be used as the threshold for prescribing testosterone replacement therapy (TRT). However, it is not uncommon for men to present with signs and symptoms of testosterone deficiency, despite having testosterone levels greater than 300 ng/dL. There exists scant literature regarding the use of hCG monotherapy for the treatment of hypogonadism in men not interested in fertility. We sought to evaluate serum testosterone response and duration of therapy of hCG monotherapy for men with symptoms of hypogonadism, but total testosterone levels > 300 ng/dL.

Materials and Methods: We performed a multi-institutional retrospective case series of men receiving hCG monotherapy for symptomatic hypogonadism. We evaluated patient age, treatment indication, hCG dosage, past medical history, physical exam findings and serum testosterone and gonadotropins before and after therapy. Descriptive analysis was performed and Mann Whitney U Test was utilized for statistical analysis.

Results: Of the 20 men included in the study, treatment indications included low libido (45%), lack of energy (50%), and erectile dysfunction (45%). Mean testosterone improved by 49.9% from a baseline of 362 ng/dL (SD 158) to 519.8 ng/dL (SD 265.6), (p=0.006). Median duration of therapy was 8 months (SD 5 months). Fifty percent of patients reported symptom improvement.

Conclusions: Treatment of hypogonadal symptoms with hCG for men who have a baseline testosterone level > 300 ng/dL appears to be safe and efficacious with no adverse events.

ARTICLE INFO

Vinayak Madhusoodanan
http://orcid.org/0000-0002-5499-7876

Keywords:
Testosterone; Chorionic Gonadotropin; Hypogonadism

Int Braz J Urol. 2019; 45: 1008-12

Submitted for publication:
February 23, 2019

Accepted after revision:
June 16, 2019

Published as Ahead of Print:
July 22, 2019

INTRODUCTION

Hypogonadism is prevalent, currently affecting 38% of men over the age of 45, and 7% of men under the age of 40 (1, 2). Adult-Onset Hypogonadism is defined as having low testosterone in conjunction with clinical signs or symptoms such as low energy, low libido, decreased lean body mass, erectile dysfunction, fatigue, depression, anemia and infertility (3-6). Although it is primarily a clinical syndrome of the aging male (3), prevalence in younger generations has incre-

ased exponentially over the past decades, a trend that correlates with an increase in the prevalence of anabolic androgenic steroid use (1). It can be associated with various acute and chronic illnesses, and should be recognized and treated (7).

Despite the prevalence of hypogonadism, there are limited treatment options. Most often, testosterone replacement therapy (TRT) is offered to these men (3). It can be effective in improving sexual function, bone mineral density, muscle strength, energy, cognition and vitality (4, 7). However, per the recent American Urological Association (AUA) guidelines, it should be reserved for patients with testosterone deficiency, as defined as less than 300 ng/dL (8). This recommendation was determined based on a compromise between the inclusion criteria testosterone (less than 350 ng/dL) and the median testosterone levels (250 ng/dL) of most large testosterone therapy trials over the past decade, in part to minimize overtreatment of patients (8).

Unfortunately, this means that the scope of treatment with TRT can be limited, precluding treatment of patients with subclinical hypogonadism (SH), who may present with the clinical syndrome, although baseline testosterone levels remain above 300 ng/dL (9). Per the European Male Ageing Study, the prevalence of SH is close to 10%, increasing up to 21% in the 8th decade of life (9). Moreover, although TRT can be efficacious, it can be undesirable due to associated side effects and complications, including acne, gynecomastia, testicular atrophy, male infertility and increased hematocrit (4, 7).

Human chorionic gonadotropin (hCG) can be a promising alternative for these patients. This pharmacotherapy can be self-administered subcutaneously on a scheduled basis. It acts as an analogue of luteinizing hormone (LH), with the added benefit of a longer half-life (1, 4). As LH would do, hCG acts on Leydig cells, stimulating them to produce and release intratesticular testosterone (1).

Despite its promise, scant literature exists on use of hCG as a monotherapy for patients with suspicion of late-onset hypogonadism, whose primary indication for therapy is not fertility. The purpose of this study was to assess the efficacy and safety of its use in such a population. We sought to evaluate the response of serum testosterone to hCG monotherapy as evidence of its efficacy at various doses and therapeutic durations, as well as its safety. We hypothesize that hCG monotherapy will be effective at increasing serum testosterone levels and improving symptomatology.

MATERIALS AND METHODS

This was an IRB approved retrospective case series of 44 men (age 26-77) with symptoms of hypogonadism treated by two Andrologists at the University of Miami Miller School of Medicine and the Baylor College of Medicine from February 2015 to May 2018. Of the 44, 9 men were excluded from the study due to concurrent treatment with TRT or Clomiphene Citrate (CC). The men who met inclusion criteria had symptoms of hypogonadism and were ineligible for treatment with TRT with initial T >300 ng/dL, as this implied they did not meet the definition of Testosterone Deficiency. They were prescribed an average of 2000 IU hCG weekly, which is based on the bi/tri-weekly regimen of 1500 IU hCG generally prescribed to men with hypogonadotropic hypogonadism (HH) and infertility, educated on administering it subcutaneously and followed up consistently with proper documentation of follow-up testosterone (T) levels (1). Patients were asked to schedule follow-up clinic visits every 3 months, with labs taken at these times.

On first encounter, patients were evaluated for symptoms associated with low libido, low energy and erectile dysfunction. Patients were asked about sex drive, exercise tolerance, insomnia and difficulty sleeping, and weight gain. Past medical history and patient co-morbidities, as well as management for these were considered. Patients were then sent for lab testing, which included, testosterone (T), luteinizing hormone (LH), follicular stimulating hormone (FSH), estradiol, hematocrit and prostate specific antigen (PSA). Repeat T was taken, and the mean of two samples established baseline and informed diagnosis. The study collected and evaluated all mentioned parameters, but only follow-up T was mandated for inclusion in study. The study also evaluated baseline characteristics such as age, treatment indications, hCG

dosage, past medical history and physical exam findings. Dates of each patient's treatment initiation and the latest follow-up visits were recorded to evaluate the average duration of treatment, and patient reports of side effects, complications and symptom improvement were recorded.

The major parameters of concern for analysis were T improvement from initial to follow-up as well as the degree of T changes as they correlated to dosage and other baseline characteristics such as LH, FSH and therapy duration. The Mann Whitney U test was utilized to compare initial to follow-up T, and a Multiple Linear Regression was used to describe correlation and the significance of correlation between dose, LH, FSH and duration of therapy with percent change in T. All statistical analysis was performed using Microsoft Excel.

RESULTS

The study included 20 men. Average age was 50.3 (SD 15.6) years and ranged from 26 to 77. On past medical history, 2 of the patients had a history of anabolic steroid use, and two had a history of prostate cancer, one of whom was post--radical prostatectomy. These patients had an average testicular volume of 14.2cc (SD 4.3), and 3 presented with varicoceles, 2 of which were grade II and 1 of which was grade I (Table-1). Indications for treatment were largely attributed to persistent complaints of one or multiple of either low libido, low energy or erectile dysfunction, but also included infertility and insomnia. Patients received an average dose of 2000 IU weekly.

These men presented with an average initial T of 361.8 ng/dL (SD 158.2), and improved to an average follow-up T of 519.8 ng/dL (SD 265.6). Duration of therapy for these men averaged 6 months, with an average weekly hCG dose of 2000 IU. Over this period, they experienced an average change in T of 60%. One-tail Mann Whitney U test demonstrated this improvement was significant, as the sample of T at baseline was significantly less ($p<0.005$) than that of follow-up T. This corresponded with 50% of men subjectively reporting symptom improvement. Of the 10 men who reported symptom improvement, only 2 had negative changes in testosterone levels, both by less than 15%.

Table 1. Summary of baseline characteristics of men included in study.

Sample	n=20
Age in years	50.3 (15.6)
Testes Volume	14.2 (4.3)
Testosterone (ng/dL)	361.8 (158.2)
No Testicular Abnormality	n=17
Varicoceles	n=3
Grade I	n=1
Grade II	n=2
Grade III	n=0
Past Medical History	
None	n=6
Hyperlipidemia	n=4
Anabolic Steroid Use	n=2
Prostate Cancer	n=2

Mean and (Standard Deviation)

After performing a multivariable adjusted analysis, the two variables that were statistically associated and positively correlated with percent T changes were hCG dosage ($p=0.0005$) and duration of therapy ($p=0.03$). Considering both variables resulted in an R-square value of 0.62, but this still is not a comprehensive explanation of the variance. Patient age and pre-treatment testicular size were not significantly associated with percent T changes.

DISCUSSION

In our study, we retrospectively evaluated 20 men who were treated for symptoms of hypogonadism with human chorionic gonadotropin (hCG) monotherapy. We presented these patients in terms of their baseline characteristics, and compared pre-treatment and post-treatment Testosterone levels, evaluating the relationship of treatment period testosterone changes with both hCG dosage and duration of therapy. We found that hCG monotherapy, over an average therapy duration of 6 months, significantly improved testosterone levels in this cohort of men. This corresponded with reports of symptom improvement in 50% of patients, with no reports of side effects or

complications. We also found a strong relationship between both hCG dosage and duration of therapy with percent testosterone changes. Administration of hCG is effective in improving intratesticular testosterone, while preventing many of the undesirable side effects associated with exogenous testosterone, including testicular atrophy and fertility preservation (4, 10).

HCG's ability to preserve spermatogenesis, and even improve semen parameters in patients who had been using exogenous testosterone, have been established (10-13). Vicari et al. looked at long term hCG treatment in 17 men with isolated hypogonadotropic hypogonadism, observing a significant increase in testicular volume, in a time-dependent manner, and testosterone, at 15 and 24 months of treatment (13). Similarly, Habous et al. studied a cohort of 282 men with hypogonadism, separating them into 3 arms - CC alone, hCG alone and a combination of hCG and CC. 94 patients from this study received hCG monotherapy with 5000 IU hCG twice weekly and experienced statistically significant increases in T levels at 1 and 3 months (14). However, most of the above-mentioned studies deal with patients who desire fertility, or desire to maintain fertility, as an outcome of treatment with hCG alone.

Our data suggests that hCG can be a safe and efficacious treatment option for patients with symptoms of hypogonadism who do not desire fertility. We observed significant increases in testosterone over an average therapy duration of 6 months. We also observed that this response to treatment was primarily positively correlated with hCG dosage and duration of therapy and lacked association with initial testicular size and patient age. No side effects or complications were reported by the subjects. It should be noted that 6 men who started hCG therapy failed to follow up after the initial 3-month follow-up visit, and therefore fell out of the study.

Further study is needed to investigate the viability of hCG as a monotherapy for symptoms of hypogonadism. Limitations of this study include non-randomized and retrospective design, small sample size, lack of control group, variability in physical exam and dosing regimens between the two Andrologists, variability in hormonal changes between patients, lack of clinically important information to characterize variability in the study population (such as FSH, estradiol and LH) and analysis of follow-up labs with potentially significant implications for safety, such as hematocrit. If repeated, studies can consider use of a standardized patient questionnaire, such as a qADAM questionnaire. The utility of such questionnaires may lie as an adjunct, in establishing a quantifiable baseline clinical presentation that can be followed thereafter. However, it should be recognized that a thorough history and measurement of serum testosterone are equally important in diagnosis and follow-up, due to the lack of specificity of available questionnaires (15). Future studies should observe patients prospectively, use a standardized measure to survey patient improvement and more consistently monitor hematocrit changes in patients on hCG monotherapy, as this could impact treatment safety and patient eligibility. Effect of baseline LH levels on efficacy of therapy may also be important to establish. Of course, although difficult, randomized studies using control groups for comparison are necessary to confirm the clinical and physiological significance of this treatment regimen. Despite these limitations, the current manuscript provides valuable data in proposing the efficacy and safety of hCG monotherapy for men not meeting criteria for testosterone therapy.

CONCLUSION

Our study indicated that hCG monotherapy is a safe and efficacious treatment option for patients with symptoms of hypogonadism who do not desire fertility or may not have initial testosterone levels greater than 300 ng/dL, significantly improving testosterone levels with no associated reports of complications or side effects. The role of hCG monotherapy in treating these men is promising. Future studies should evaluate changes in hematocrit levels in these patients, as well as the effect that baseline luteinizing hormone may play on response to hCG monotherapy.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Lee JA, Ramasamy R. Indications for the use of human chorionic gonadotropic hormone for the management of infertility in hypogonadal men. Transl Androl Urol. 2018;7(Suppl 3):S348-S52.
2. Rohrmann S, Platz EA, Selvin E, Shiels MS, Joshu CE, Menke A, et al. The prevalence of low sex steroid hormone concentrations in men in the Third National Health and Nutrition Examination Survey (NHANES III). Clin Endocrinol (Oxf). 2011;75:232-9.
3. Surampudi PN, Wang C, Swerdloff R. Hypogonadism in the aging male diagnosis, potential benefits, and risks of testosterone replacement therapy. Int J Endocrinol. 2012;2012:625434
4. Lo EM, Rodriguez KM, Pastuszak AW, Khera M. Alternatives to Testosterone Therapy: A Review. Sex Med Rev. 2018;6:106-13.
5. Mulligan T, Frick MF, Zuraw QC, Stemhagen A, McWhirter C. Prevalence of hypogonadism in males aged at least 45 years: the HIM study. Int J Clin Pract. 2006;60:762-9.
6. Khera M, Broderick GA, Carson CC 3rd, Dobs AS, Faraday MM, Goldstein I, et al. Adult-Onset Hypogonadism. Mayo Clin Proc. 2016;91:908-2.
7. Surampudi P, Swerdloff RS, Wang C. An update on male hypogonadism therapy. Expert Opin Pharmacother. 2014;15:1247-64.
8. Mulhall JP, Trost LW, Brannigan RE, Kurtz EG, Redmon JB, Chiles KA, et al. Evaluation and Management of Testosterone Deficiency: AUA Guideline. J Urol. 2018;200:423-32.
9. Giannetta E, Gianfrilli D, Barbagallo F, Isidori AM, Lenzi A. Subclinical male hypogonadism. Best Pract Res Clin Endocrinol Metab. 2012;26:539-50.
10. Ramasamy R, Armstrong JM, Lipshultz LI. Preserving fertility in the hypogonadal patient: an update. Asian J Androl. 2015;17:197-200.
11. Kohn TP, Louis MR, Pickett SM, Lindgren MC, Kohn JR, Pastuszak AW, et al. Age and duration of testosterone therapy predict time to return of sperm count after human chorionic gonadotropin therapy. Fertil Steril. 2017;107:351-7.e1.
12. Hsieh TC, Pastuszak AW, Hwang K, Lipshultz LI. Concomitant intramuscular human chorionic gonadotropin preserves spermatogenesis in men undergoing testosterone replacement therapy. J Urol. 2013;189:647-50.
13. Vicari E, Mongioì A, Calogero AE, Moncada ML, Sidoti G, Polosa P, et al. Therapy with human chorionic gonadotrophin alone induces spermatogenesis in men with isolated hypogonadotrophic hypogonadism--long-term follow-up. Int J Androl. 1992;15:320-9.
14. Habous M, Giona S, Tealab A, Aziz M, Williamson B, Nassar M, et al. Clomiphene citrate and human chorionic gonadotropin are both effective in restoring testosterone in hypogonadism: a short-course randomized study. BJU Int. 2018;122:889-97.
15. Bernie AM, Scovell JM, Ramasamy R. Comparison of questionnaires used for screening and symptom identification in hypogonadal men. Aging Male. 2014;17:195-8.

Correspondence address:
Vinayak Madhusoodanan, BS
Department of Urology
University of Miami Miller School of Medicine
1120 NW 14th Street, Room 1560
Miami, FL, USA 33136
Telephone: +1 305 243-6090
E-mail: vmkodoth@med.miami.edu

ORIGINAL ARTICLE

Vol. 45 (5): 1013-1019, September - October, 2019
doi: 10.1590/S1677-5538.IBJU.2019.0092

Portable model for vasectomy reversal training

Luis Otávio Amaral Duarte Pinto [1], Charles Alberto Villacorta de Barros [1], Anderson Bentes de Lima [1], Deivid Ramos dos Santos [1], Herick Pampolha Huet de Bacelar [1]

[1] *Programa de Mestrado Profissional em Cirurgia e Pesquisa Experimental, Universidade do Estado do Pará - Uepa, Belém, PA, Brasil*

ABSTRACT

Objectives: To validate an experimental non-animal model for training of vasectomy reversal.
Materials and Methods: The model consisted of two artificial vas deferens, made with silicon tubes, covered by a white resin, measuring 10 cm (length) and internal and external diameters of 0.5 and 1.5 mm, respectively. The holder of the ducts is made by a small box developed with polylactic acid, using a 3D print. The objective of the invention is to simulate the surgical field of vasovasostomy, when the vas deferens are isolated from other cord structures. For validation, it was verified the acquisition of microsurgical skills during its use, in a capacitation course with 5 urology residents from a Hospital of the region. Along the training sessions, it was analyzed the time (speed) of microsurgical sutures, and quantification of the performance using a checklist. Collected data were analyzed using de BioEstat®5.4 software.
Results: Medium time for the completion of microsurgical sutures improved considerably during the course, and reached a plateau after the third day of training (p=0.0365). In relation to the checklist, it was verified that during capacitation, there was significant improvement of the scores of each participant, that reached a plateau after the fourth day of training with the model (p=0.0035).
Conclusion: The developed model was able to allow the students that attended the course to gain skills in microsurgery, being considered appropriate for training vasectomy reversal.

ARTICLE INFO

Luis Otávio Amaral Duarte Pinto
https://orcid.org/0000-0003-3065-7516

Keywords:
Vasovasostomy; Vas Deferens; Fertility

Int Braz J Urol. 2019; 45: 1013-9

Submitted for publication:
February 08, 2019

Accepted after revision:
March 23, 2019

Published as Ahead of Print:
May 28, 2019

INTRODUCTION

Vasectomy is a safe and efficient contraceptive method. Worldwide, it is estimated that nearly 60 million men had been submitted to this procedure (1). According to DATASUS database, only in November 2018, 3,127 surgeries were performed in public health services in Brazil (2).

Although widely accepted, men submitted to vasectomy may seek reversal of fertility due to death of children, divorces, new relationships, among other life circumstances (3). It is estimated that 6% of vasectomized men look for fertility reversal sometime in their lives (4).

Among possible options, vasectomy reversal (vavovasostomy) is considered the gold

standard procedure, with patency and pregnancy rates of up to 89.4% and 73.0% respectively (5).

In order to perform vasovasostomy, urologists must train skills in microsurgery, mastering the use of optical microscope and very delicate surgical instruments, made with cutting-edge technology (6). Unfortunately, most Brazilian public services lack those equipment, and an expressive quantity of urologists finish their residences without learning microsurgical skills, and have to spend money and time in capacitation courses.

Some authors advocate the use of experimental models for training and gaining microsurgical skills. Grober et al. (7) and Shurey et al. (8) developed models of vasovasostomy training using laboratory mice, with good results of capacitation. However, pressure of society to lower the use of experimental animals along with restrictions for their use by only some selected research centers are stimulating the development of artificial models, that reliably simulate *in vivo* surgical procedures (9).

The first artificial vasovasostomy model was described by Li et al. in 1992 (10). It consisted of the use of microsurgical sutures in silicon tubes. In that time, the author used tubes with 1.5 mm of internal diameter, higher than the human vas deferens lumen, that varies from 0.4 to 0.7 mm, limiting its importance (11).

Therefore, there is a need to produce training models for vasovasostomy that are affordable, that spare the use of experimental models and that mimic efficiently human vas deferens. Such models could be introduced to training urologists to capacitate them in microsurgery, filling that formation gap.

The objective of the present study is to validate an experimental non-animal model, developed for the training of vasectomy reversal.

MATERIALS AND METHODS

Ethical aspects

The study was developed according to Helsinki statements and Nuremberg Code, respecting the rules of researches with human beings.

Model development

The training model for vasectomy reversal was developed at the Experimental Surgical Laboratory of University of the State of Pará (LCE-UEPA). Artificial vas deferens ducts were made of translucid silicon tubes, measuring 10 cm width, with internal and external diameters of 0.5 and 1.5 mm respectively. They were covered externally with a white PVA resin film (vinil poliacetate), allowing the simulation of all vas deferens layers, such as lumen mucosa, and muscular and adventitia layers.

The holder of the artificial ducts was made by a small box (with base and cover) developed with polilatic acid using a 3D printer Makerbot®. The base has a non-slip cover, assuring adhesion and firmness of the device during training sessions. The cover has a rectangular 4.5x3.5 cm opening, made with latex, containing two small orifices through which the ducts are exteriorized, with stability during realization of the sutures. Figure-1 shows the components of the experimental model and the steps for mounting. Figure-2 shows a vasovasostomy performed using the device.

Validation of the model

In order to validate the model, it was analyzed the gain of skills in microsurgery during training. It was proposed a study with 5 urology residents from a reference public hospital of the region, and none had previous experience with microsurgery.

The participants were submitted to a capacitation workshop in microsurgery, using the previously described model. The course consisted of a first day of first impressions (D0), followed by 5 training sessions, with weekly intervals (D7, D14, D21, D28 and D35).

At D0, the residents watched a theorical 30 minutes video demonstrating basic aspects of microscope use, positioning and operatory technique, followed by practice with microsurgical sutures using training plates, with 1 hour duration.

The other sessions (D7 to D35) involved the performance of vasovasostomies using the experimental model. In the beginning and in the end of each session, the residents performed two microsurgical sutures in the training plate (each with a double semi-knot and two simple semi-knots), that were named pre-training, post-training and vasovasostomy.

Figure 1 - Training model for vasectomy reversal (A and B: model components; C and D: assembling of the components).

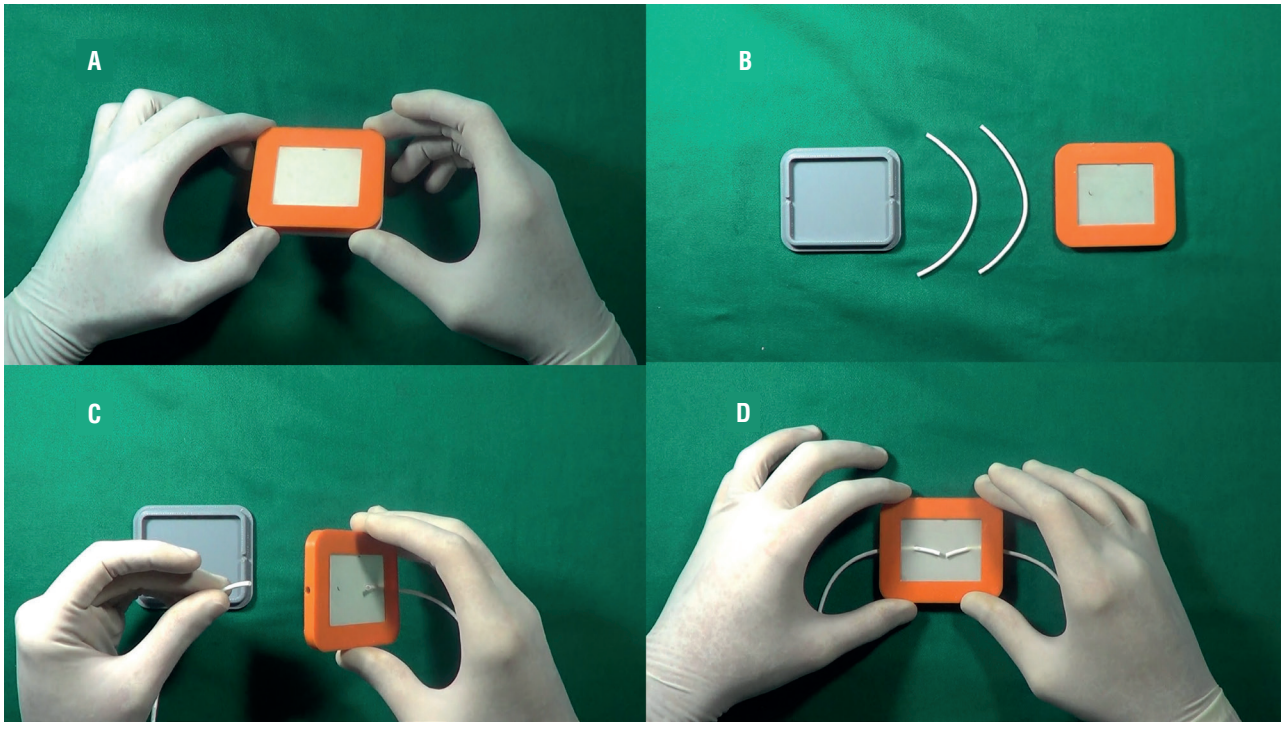
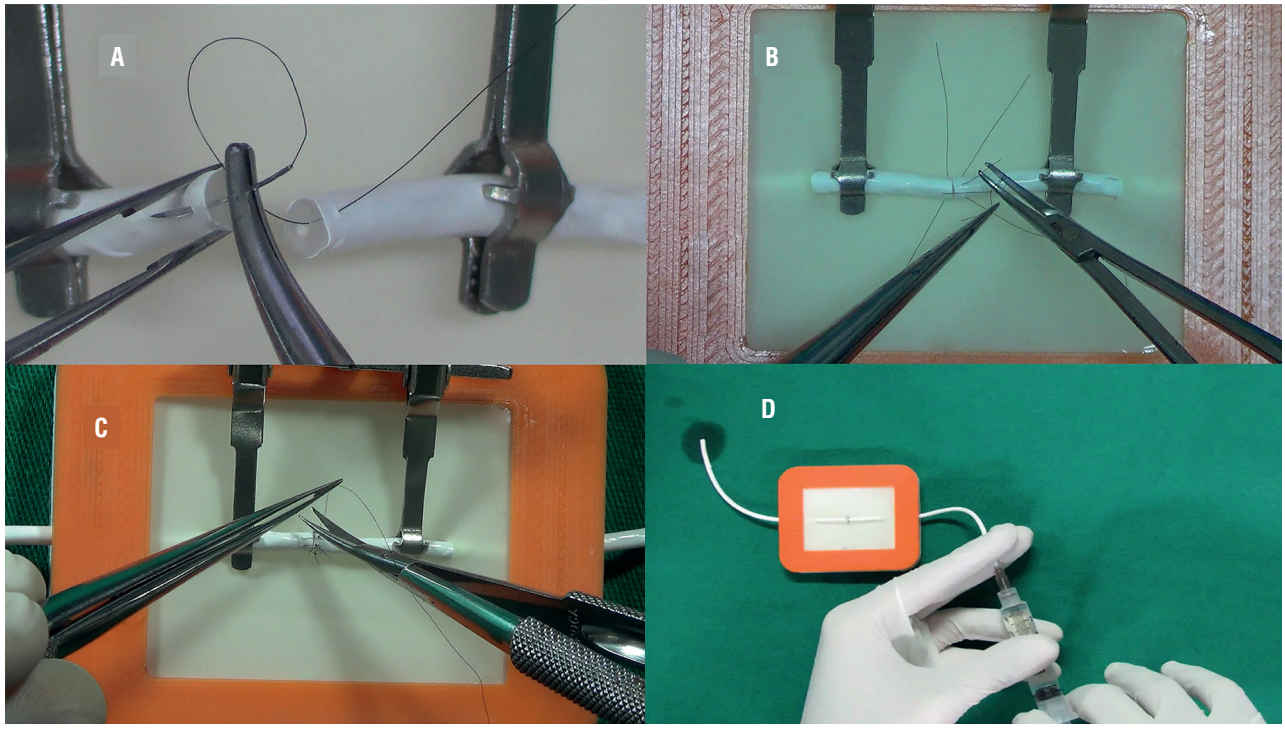


Figure 2 - Vasovasostomy in a training model (A: stitches applied through all duct layers; B and C: microsurgical sutures; D: proof of a patent anastomosis).



Also, during the training sessions, the participants were also evaluated with a checklist (Appendix), that assigned a score according to the performance during surgery.

Materials and recommended technique

For the microsurgical suture in the model it was used: microsurgical needle holder Castroviejo 10 cm width without rack; dissection clamp watchmaker straight, 10 cm width; curved Castroviejo scissors, 10 cm width, and microspike clamp. It was also used Nylon 8-0 suture, with two spatulated1/4 0.65 cm needles. Anastomosis was made using stereoscopic magnification by a conventional optical microscope D.F. Vasconcelos®.

Recommended vasovasostomy technique is characterized by 4 simple equidistant sutures, englobing all layers of the model, at 3, 6, 9 and 12 hours; interleaved with 4 sutures that spared the lumen, allowing complete coaptation of all circumference, according to the recommended technique described by Benlloch et al. (12).

Statistical analysis

Analytic parameters were processed in the software Microsoft Excel® and Word® 2013 creating tables and graphics that posteriorly were submitted to statistical analysis using BioEstat® 5.4 software. Data were initially submitted to Shapiro Wilk normality test. For normal distributed data it was used ANOVA parametric test. For those with abnormal distribution it was used the Kruskal Wallis non parametric test. And finally, for comparison, it was used the paired t-Student. Statistical significance was set at p≤0.05.

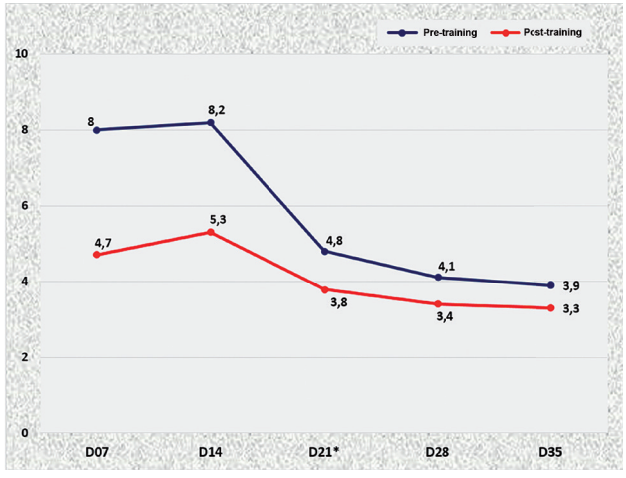
RESULTS

In Figure-3 shows a graphic comparison between time of suture media of all participants, between pre-training and post-training, in all training sessions. It is observed that the medium time for complete suture improved considerably during the 5 days of the course, as in the end of each session.

When D7 was analyzed singly (first capacitation session, using the model) it is observed an important improvement between the post-training time and respective pre-training time. This transitory gain in skills became more consistent along the other sessions, which is demonstrated by the graphic showing approximation of the curves pre- and post-training. After the third day of training (D21), the participants reached a plateau, consolidating the gain of skills in microsurgery (p=0.0365).

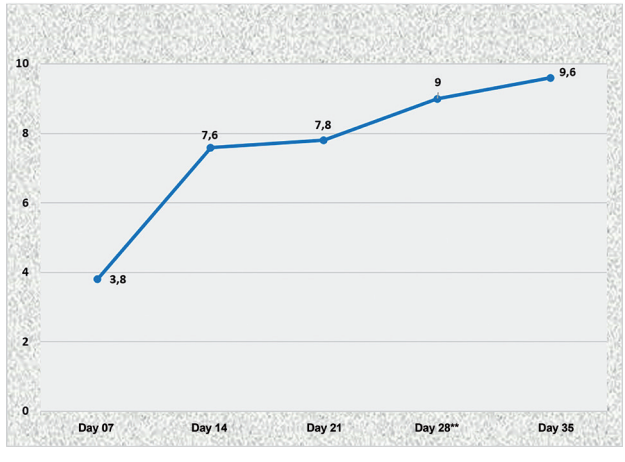
In Figure-4 shows the progression of skills of residents in microsurgery, using the media results obtained with the checklist throughout capacitation. The analysis of the graphic shows an important

Figure 3 - Medium time, in minutes, of sutures during pre and post-training throughout all sessions.



* Paired t-Student test (p=0.0365)

Figure 4 - Medium cheklist score throughout training sessions.



** ANOVA test one criteria (p=0.0035)

increase of score right after session two (D14), that remains progressive until reach a plateau on the fourth session of training (D28), with a medium score of 9 points (p=0.0035).

DISCUSSSION

The objective of this study was to validate a non-animal experimental model to develop skills in microsurgery, particularly vasectomy reversal. The model simulates the surgical field, when the ducts are isolated from other elements of the spermatic cord.

The model in the shape of a small box is easy to handle and storage, and can be reused several times; the used segment is severed and the stubs are approached again. It is estimated the model allows for 35 vasovasostomies.

The coat with a layer of PVA simulates the different layers of the vas deferens, allowing for different sutures (total or partial layers). Benlloch technique was chosen since it is considered the easiest available for urologists in initial training.

The course, with 5 training weekly sessions, is similar to most microsurgery courses with international relevance (13). Evidences show that acquired skills is higher when there is an interval between session, in comparison to consecutive day training (14).

Literature demonstrates that direct evaluation of training in experimental models (artificial or animal) are highly reliable for microsurgery training (15, 16). Temple et al. (17) and Grober et al. (18) state that this kind of evaluation may be performed using timely parameters, as well as the use of checklists or scales.

In the present study, model validation was checked with improvement of time spent for microsurgical sutures and the progressive increase of score of the checklist during capacitation. We believe that the use these two criteria allowed for more concise result interpretation than the analysis of only one parameter.

Results show a plateau of skill acquisition at the third session of training (D21), when only time was considered, and at the fourth session (D28), when checklist score was considered. These aspects reinforce the idea that evaluation with detailed and specific criteria (in a checklist or scale) represent more reliably the acquisition of skill throughout training, rather than only the analysis of time.

Analysis of time spent in this study was a complementary evaluation of acquired skills in microsurgery. We timed the suture time spent at pre and post-training instead that of vasovasostomy *per se*, based on the publication of Starkes et al. (19). According to these authors, time is secondary in the analysis of a good microsurgical anastomosis; other criteria are more important, such as correct handling of tissue, stitches applied equidistantly, good edge cooptation, among others. A "fast" vasectomy reversal is not always the "best".

Therefore, time analysis only at the moment of microsurgical knots (at pre and post--training) allowed for an objective interpretation: better skills are observed with faster stitches.

The artificial model has some disadvantages: it simulates only the surgical field and does not allow for training the other steps of vasovasostomy, such as identification of buds, fibrosis section, and calibration of deferens lumen. Another unfavorable aspect is that silicon obviously does not present the same physical proprieties than human vas deferens; therefore, for a good coaptation and patency analysis, it was necessary to stabilize and align the stumps, with the aid of the microspike clamp, and the use of a double semi-knot at first, not necessary in real surgeries.

Our original idea when we proposed the current study was to broaden the teaching of microsurgery for urology residents, particularly those that work at public services with low budget. We hope that this study encourage the development of more realistic models for vasectomy reversal or other microsurgical procedures in Urology, such as varicocele correction, neophaloplasties, penile reimplantation, among others.

Literature reinforces the use of high definition video systems, that produces image magnification similar to surgical microscope, with the advantage of being much less expensive, needing only a camera and a TV set (20). We believe that this kind of technology may be used along with similar models , and that, in the future, urologists that intend to improve their microsurgical skills

can do so at home, with reliable simulators, without the use of laboratory animals or the need to go to a hospital or facility that has a microscope.

CONCLUSIONS

The developed experimental model was efficient to train vasectomy reversal, allowing for skills improvement in microsurgery.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Eisenberg ML, Lipshultz LI. Estimating the number of vasectomies performed annually in the United States: data from the National Survey of Family Growth. J Urol. 2010;184:2068-72.
2. DATASUS - Tecnologia da Informação a Serviço do SUS: Procedimentos hospitalares do SUS – por local de internação [citado 22 de agosto de 2017]. available at. <http://tabnet.datasus.gov.br/cgi/tabcgi.exe?sih/cnv/qiuf.def>.
3. Li PS, Ramasamy R, Goldstein M. Male infertility microsurgical training. In: Sandlow JI, editor. Microsurgery for Fertility Specialists. New York: Springer; 2012.
4. Potts JM, Pasqualotto FF, Nelson D, Thomas AJ Jr, Agarwal A. Patient characteristics associated with vasectomy reversal. J Urol. 1999;161:1835-9.
5. Crain DS, Roberts JL, Amling CL. Practice patterns in vasectomy reversal surgery: results of a questionnaire study among practicing urologists. J Urol. 2004;171:311-5.
6. Parekattil SJ, Gudeloglu A, Brahmbhatt J, Wharton J, Priola KB. Robotic assisted versus pure microsurgical vasectomy reversal: technique and prospective database control trial. J Reconstr Microsurg. 2012;28:435-44.
7. Grober ED, Hamstra SJ, Wanzel KR, Reznick RK, Matsumoto ED, Sidhu RS, et al. Laboratory based training in urological microsurgery with bench model simulators: a randomized controlled trial evaluating the durability of technical skill. J Urol. 2004;172:378-81.
8. Shurey S, Akelina Y, Legagneux J, Malzone G, Jiga L, Ghanem AM. The rat model in microsurgery education: classical exercises and new horizons. Arch Plast Surg. 2014;41:201-8.
9. Weber D, Moser N, Rösslein R. A synthetic model for microsurgical training: a surgical contribution to reduce the number of animal experiments. Eur J Pediatr Surg. 1997;7:204-6.
10. Li PS, Schlegel PN, Goldstein M. Use of silicone medical grade tubing for microsurgical vasovasostomy training. Urology. 1992;39:556-7.
11. Middleton WD, Dahiya N, Naughton CK, Teefey SA, Siegel CA. High-resolution sonography of the normal extrapelvic vas deferens. J Ultrasound Med. 2009;28:839-46.
12. Ramada Benlloch FJ, de la Torre Abril L, Tramoyeres Galvañ A, Cánovas Ivorra JA, Sánchez Ballester F, Ordoño Domínguez F, et al. [Our experience with simplified vasovasostomy. Review of our results during the last 5 years]. Arch Esp Urol. 2004;57:59-63.
13. Leung CC, Ghanem AM, Tos P, Ionac M, Froschauer S, Myers SR. Towards a global understanding and standardisation of education and training in microsurgery. Arch Plast Surg. 2013;40:304-11.
14. Moulton CA, Dubrowski A, Macrae H, Graham B, Grober E, Reznick R. Teaching surgical skills: what kind of practice makes perfect?: a randomized, controlled trial. Ann Surg. 2006;244:400-9.
15. Kalu PU, Atkins J, Baker D, Green CJ, Butler PE. How do we assess microsurgical skill? Microsurgery. 2005;25:25-9.
16. Ramachandran S, Ghanem AM, Myers SR. Assessment of microsurgerycompetency-where are we now? Microsurgery. 2013;33:406-15.
17. Temple CL, Ross DC. A new, validated instrument to evaluate competency in microsurgery: the University of Western Ontario Microsurgical Skills Acquisition/Assessment instrument [outcomes article]. Plast Reconstr Surg. 2011;127:215-22.
18. Grober ED, Hamstra SJ, Wanzel KR, Reznick RK, Matsumoto ED, Sidhu RS, et al. Validation of novel and objective measures of microsurgical skill: Hand-motion analysis and stereoscopic visual acuity. Microsurgery. 2003;23:317-22.
19. Starkes JL, Payk I, Hodges NJ. Developing a standardized test for the assessment of suturing skill in novice microsurgeons. Microsurgery. 1998;18:19-22.
20. Sergio R, de Barros M, Brito MV, Leal RA, Teixeira RK, Sabbá MF, et al. A Low-Cost High-Definition Video System for Microsurgical Hindlimb Replantation in Rats. J Reconstr Microsurg. 2017;33:158-62.

Correspondence address:
Luis Otávio Amaral Duarte Pinto, MD
Universidade do Estado do Pará - Uepa, Belém, PA, Brasil
Avenida Gentil Bittencourt 2086 / 1203
São Brás, Belém, PA, 66063-018, Brasil
Telephofone: + 55 91 3222-7817
E-mail: luis_otavio_pinto@yahoo.com.br

Appendix - Checklist used for validation of experimental model.

Checklist - Experimental model for training vasectomy reversal				
1. Did the resident remained in a comfortable position with forearms and wrists support?				
[ ]D7	[ ]D14	[ ]D217	[ ]D28	[ ]D35
2. Did the resident cut the suture in half for better use?				
[ ]D7	[ ]D14	[ ]D217	[ ]D28	[ ]D35
3. Did the microspike clamp was used for approaching the stubs?				
[ ]D7	[ ]D14	[ ]D217	[ ]D28	[ ]D35
4. Did the resident handled correctly the microsurgical thread? (not using the needle)				
[ ]D7	[ ]D14	[ ]D21	[ ]D28	[ ]D35
5. Did the resident make some sutures with backhand?				
[ ]D7	[ ]D14	[ ]D21	[ ]D28	[ ]D35
6. Did the resident perform the 3 semi-knots?				
[ ]D7	[ ]D14	[ ]D21	[ ]D28	[ ]D35
7. Did the resident perform 8 suture stitches? (4 total layer and 4 partial layer)				
[ ]D7	[ ]D14	[ ]D21	[ ]D28	[ ]D35
8. Were the stitches equidistant?				
[ ]D7	[ ]D14	[ ]D21	[ ]D28	[ ]D35
9. Were the suture stubs satisfactory? (not too short, not too long)				
[ ]D7	[ ]D14	[ ]D21	[ ]D28	[ ]D35
10. Did the needle was kept at the sponge in order to avoid its loss?				
[ ]D7	[ ]D14	[ ]D21	[ ]D28	[ ]D35
Onus/bonus:				

11. Did the resident lost the needle during training? (yes = -1 point)	[ ]D7	[ ]D14	[ ]D21	[ ]D28	[ ]D35
12. Did the needle was damaged during procedure? (yes = -1 point)	[ ]D7	[ ]D14	[ ]D21	[ ]D28	[ ]D35
13. Did the resident use only half the suture thread? (yes = +1 point)	[ ]D7	[ ]D14	[ ]D21	[ ]D28	[ ]D35
14. Did the vasovasostomy was patent? (yes= +1 point)	[ ]D7	[ ]D14	[ ]D21	[ ]D28	[ ]D35

Total score:	

[ ]D7	[ ]D14	[ ]D21	[ ]D28	[ ]D35

EDITORIAL COMMENT

Vol. 45 (5): 1020-1021, September - October, 2019
doi: 10.1590/S1677-5538.IBJU.2019.0092.1

Editorial Comment: Portable model for vasectomy reversal training

Rodrigo R. Vieiralves [1]

[1] *Serviço de Urologia, Hospital Federal da Lagoa, Rio de Janeiro, RJ, Brasil*

We know that microsurgery training is still far from becoming a reality during the process of training residents in Brazil. In fact, there is a gap around the world and therefore, just the fact that we have an article dealing with this topic is already of great relevance. In this very interesting article conducted at Para State University, Brazil (1), a portable training model for vasectomy reversal was adopted. We know that with the current juncture of society and the number of vasectomies performed worldwide, there is an increasing demand for vasectomy reversal mainly associated with a new post-vasectomy relationship. The paper in question becomes even more relevant because of it´s bimodal certification for microsurgical training based on a time performance and on a check list not only measuring the execution time of vaso-vasostomy but also with a checklist questionnaire assessing the most diverse specific items of an perfect anastomosis (eg, if the resident is placed in a comfortable position, equidistance between knots, number of knots, continuous needle vision, etc). We understand that this bimodal evaluation increases the accuracy in the training, since the time does not necessarily correlate with the quality of the anastomosis.

However, some relevant aspects need to be highlighted. Vasectomy reversal surgery involves a complex number of factors for its true success. The preparation of the surgical field itself, the section of adhesions and previous fibrosis, the calibration and approximation of the deferens ends without tension, the stabilization of the anastomosis, the patency test, the observation of the four deferent layers in the intraoperative period -mucosa, two layers of muscle and the adventitia- (failure to observe two muscle layers may indicate residual vasectomy scarring). Blood supply evaluation by thin mucosal bleeding is also important (2). None of these fundamental points for surgical success is reproduced through the present model.

Moreover, we know that the technique used in the model, proposed by Benlloch (3) with only 4 sutures in a single layer is not a reference in the literature. Today, we found no statistical difference in patency or pregnancy results for two- and one-layer vasovasostomy but a minimum number of 6 sutures presumably offers a high quality anastomosis by preventing sperm leakage and the associated risk of granuloma (4, 5).

Some considerations regarding the materials used should be made. A technical point is the fact that using an 8-0 suture could facilitate training without simulating real anastomosis situations. We understand that further in vivo studies with thicker sutures in a single anastomotic layer are needed to validate this model (6, 7). Also, as much as the 3D printed model addresses important aspects such as the presence of two layers allowing the training of two types of anastomoses, single layer or double layer, the consistency of the material hardly simulates the vas deferens real physical proprieties, since the external PVA coating probably offers a much higher resistance than the real one. Other point is that there is no exact description of how the model is fixed on a training table, since any mobilization makes all training difficult and inaccurate. The magnification used while using the microscope has not been described also.

Regarding the validation model, the low number of residents who perform the training draws attention. We understand that results based on training of only 5 residents can present an important bias, due to individual aspects besides the fact that they are part of the same Hospital (similar previous training), making the external validation of this model difficult. Another point about this model is the aspect of 5 different days of training. Although undeniably a quality model, we understand that a final assessment 8-12 weeks after the last training session would be important for permanent/long term skill acquisition assessment. This is of great relevance as we know that vasectomy reversal and microsurgery will not be a routine urologist procedure.

To conclude, we see in this important paper the presentation of a vasectomy reversal training model that is reproducible, storable, transportable, low cost and durable. It allows a single or double layer training, and despite the need for improvement in the validation model, we undoubtedly have a non-animal 3D model that sets a benchmark for future improvements, serving as a basis for more realistic ones. It will be possibly used as the first stage of basic training in microsurgery and vasectomy reversal.

REFERENCES

1. Pinto LOAD, de Barros CAV, de Lima AB, Dos Santos DR, de Bacelar HPH. Portable model for vasectomy reversal training. Int Braz J Urol. 2019 May 28;45:1013-9.
2. Hayden RP, Li PS, Goldstein M. Microsurgical vasectomy reversal: contemporary techniques, intraoperative decision making, and surgical training for the next generation. Fertil Steril. 2019;111:444-53.
3. Ramada Benlloch FJ, de la Torre Abril L, Tramoyeres Galvañ A, Cánovas Ivorra JA, Sánchez Ballester F, Ordoño Domínguez F, Navalón Verdejo P, Zaragoza Orts J. Our experience with simplified vasovasostomy. Review of our results during the last 5 years. Arch Esp Urol. 2004;57:59-63.
4. Chan PT. The evolution and refinement of vasoepididymostomy techniques. Asian J Androl. 2013;15:49-55.
5. Patel AP, Smith RP. Vasectomy reversal: a clinical update. Asian J Androl. 2016;18:365-71.
6. Belker AM, Thomas AJ Jr, Fuchs EF, Konnak JW, Sharlip ID. Results of 1,469 microsurgical vasectomy reversals by the Vasovasostomy Study Group. J Urol. 1991;145:505-11.
7. Sheynkin YR, Li PS, Magid ML, Carlson D, Chen EC, Goldstein M. Comparison of absorbable and nonabsorbable sutures for microsurgical vasovasostomy in rats. Urology. 1999;53:1235-8.

ARTICLE INFO

Rodrigo Vieiralves
http://orcid.org/0000-0003-4745-0319

Int Braz J Urol. 2019; 45: 1020-1

Correspondence address:
Rodrigo R. Vieiralves, MD
Serviço de Urologia,
Hospital Federal da Lagoa, Rio de Janeiro, RJ
R. Jardim Botânico, 501 - Jardim Botânico
Rio de Janeiro - RJ, 22470-050, Brasil
E-mail: rrvieiralves@gmail.com

ORIGINAL ARTICLE

Vol. 45 (5): 1022-1032, September - October, 2019
doi: 10.1590/S1677-5538.IBJU.2018.0366

An augmented patient-specific approach to administration of contrast agent for CT renal angiography

Charbel Saade [1], Nadine Hamieh [1], Ibrahim Al-Sheikh Deeb [1], Maurice Haddad [1], Alain S. Abi-Ghanem [1], Diamond Ghieh [1], Fadi El-Merhi [1]

[1] *Department of Radiology, American University of Beirut, Beirut, Lebanon*

ABSTRACT

Purpose: This hybrid retrospective and prospective study performed on 200 consecutive patients undergoing renal CTA, investigates the opacification of renal vasculature, radiation dose, and reader confidence.

Materials and Methods: 100 patients were assigned retrospectively to protocol A and the other 100 were allocated prospectively to protocol B. Both protocols implemented a contrast material and saline flow rate of 4.5 mL/sec. Protocol A utilized a 100 mL of low-osmolar nonionic IV contrast material (Ioversol 350 mg I/mL) while protocol B employed a patient-tailored contrast media formula using iso-osmolar non-ionic (Iodixanol 320 mg I/mL).

Results: Arterial opacification in the abdominal aorta and in the bilateral main proximal renal arteries demonstrated no statistical significance ($p>0.05$). Only the main distal renal artery of the left kidney in protocol B was statistically significant ($p<0.046$). In the venous circulation, the IVC demonstrated a significant reduction in opacification in protocol B (59.39 HU ± 19.39) compared to A (87.74 HU ± 34.06) ($p<0.001$). Mean CNR for protocol A (22.68 HU ± 13.72) was significantly higher than that of protocol B (14.75 HU ± 5.76 $p< 0.0001$). Effective dose was significantly reduced in protocol B (2.46 ± 0.74 mSv) compared to A (3.07 ± 0.68 mSv) ($p<0.001$). Mean contrast media volume was reduced in protocol B (44.56 ± 14.32 mL) with lower iodine concentration. ROC analysis demonstrated significantly higher area under the ROC curve for protocol B ($p< 0.0001$), with inter-reader agreement increasing from moderate to excellent in renal arterial visualization.

Conclusion: Employing a patient-tailored contrast media injection protocol shows a significant refinement in the visualization of renal vasculature and reader confidence during renal CTA.

ARTICLE INFO

Fadi El-Merhi
https://orcid.org/0000-0002-7346-2308

Keywords:
Computed Tomography Angiography; Kidney; Radiation Dosage

Int Braz J Urol. 2019; 45: 1022-32

Submitted for publication:
August 06, 2018

Accepted after revision:
December 14, 2018

Published as Ahead of Print:
March 22, 2019

INTRODUCTION

CT Angiography (CTA) is established as one of the noninvasive imaging modalities for the evaluation of vascular diseases. Since its development, Renal CTA (rCTA) has emerged as a reliable tool for the diagnosis of renal artery stenosis. The sensitivity and specificity of rCTA for the diagnosis of greater than 50% renal artery stenosis range from 67%-100% and 77%-98%, respectively (1). On the other hand, renal magnetic resonance angiography (MRA) has sensitivity and specificity of 88%–100%

and 70%–100% with low interobserver variability, especially for severe stenosis greater than 70% (2, 3). However, the sensitivity and specificity are dependent upon the opacification levels in the renal vasculature. Over the years, improvements in CTA to evaluate renal artery stenosis have resulted from optimization of acquisition (4, 5), image presentation with various rendering algorithms, as well as contrast media administration protocols. Recent studies have reported attenuation values of the renal arteries being as high as 435±48 HU, while those of the renal veins have reached 277±29 HU (6), whilst employing large contrast media volumes (60-125 mL) (5-8).

There are three main approaches in determining contrast media volume. The first approach is body weight range and fixed contrast volume-based protocols; 80 mL for <61 Kg, 90 mL for 61-91 Kg and 120 mL for > 91 kg (9). The second is linear body weight and contrast volume 1-1.5 mL/kg (10) and finally; fixed contrast volumes ranging from 60 to 125 mL (11). Furthermore, previous studies have reported that weight-based protocols are not considered to be a determining factor during CTA (3, 12-14). The aim of our study is to investigate the opacification of renal vasculature, radiation dose, and reader confidence by a patient-tailored contrast administration protocol during rCTA.

MATERIALS AND METHODS

Patient Selection

This hybrid retrospective and prospective study was approved by the institutional review board. Written informed consents were only waived for protocol A, whilst, informed consents were mandatory and obtained for protocol B. Two hundred rCTA were evaluated from July 2012 to September 2015. Between July 2012 and June 2014, one hundred patients with suspected renovascular disease went through the conventional CTA contrast protocol (protocol A). Between July 2014 and September 2015, the other one hundred underwent the patient-tailored contrast material injection protocol (protocol B) (Table-1). Patients were distributed normally. Patients with serum creatinine >1.2 mg/dL or eGFR <60 mL/min/1.73 m^2, and pregnant patients were excluded from the final patient cohort (n=8).

Renal CT Angiography Acquisition

All examinations were done using a 256-slice MDCT scanner (Brilliance iCT); patients were placed in supine position. Before the scan acquisition, anterior-posterior scout scan was performed, with a scan range from the diaphragm to the iliac crest. CT scan parameters employed in both protocols were: detector width of 256×0.625 mm, pitch of 0.881:1 ratio, and rotation time of 0.27 sec, 120 kVp, effective 180 mAs, with x,y and z-axis modulation (DoseRight), and hybrid iterative reconstruction iDose4, level 5.

Bolus triggering technique

The two protocols used distinct bolus-tracking techniques. Protocol A harnessed a dynamic bolus tracking: the region of interest (ROI) is marked in the lumen of the suprarenal segment of the

Table 1 - Demographics.

	Protocol A	Protocol B
Gender		
Females	30	33
Males	70	68
Age	58.49±19.09	52.09±16.05
Height	1.70±0.90	1.71±0.09
Weight	78.11±15.37	78.91±14.78
BMI	26.96±4.75	27±4.67

abdominal aorta with a constant contrast volume of 100 mL. 100 HU was chosen as a trigger attenuation value threshold above the baseline with a delay of 5s (upon reaching the peak threshold to the beginning of the CTA acquisition). Each bolus employed free breathing and scanner parameters; rotation time of 0.5 sec, 100 kVp, effective 50 mAs and interscan delay 1 sec. The volume of contrast was based on current departmental work practice and in line with current literature (3, 12, 16, 17), it was not adjusted to the patient's body mass index (15). Protocol B harnessed the test bolus technique: the ROI is marked within the abdominal aorta (suprarenal segment) using a small amount of contrast (5 mL), which is not part of the total contrast volume (CV) measured using the formula. It is administered at the same rate, as we measured the time to peak (TTP) and the main bolus. Both protocols employed a 100 mL saline chaser injected at 4.5 mL/s.

Contrast Medium Administration

An automated dual barrel power injector (Optivantage®) was used to inject warmed contrast material (37º) through a 20 gauge venous catheter in the right arm. Patients were examined by two contrast media protocols. Protocol A, conventional protocol consisting of a 100 mL of contrast (Ioversol 350 mg I/mL) injected intravenously at a flow rate of 4.5 mL/s. Protocol B utilized a patient-specific contrast media formula: CV = (ST + TTP - OVWP) x FR (Iodixanol 320 mg I/mL). ST: scan time; TTP: time to peak of the contrast at the level of the renal arteries; OVWP: optimal venous washout phase (12 seconds) (3, 12, 16); FR: flow rate. Both protocols employed 100 mL saline at 4.5 mL/s. Two separate iodinated contrast media agents were chosen to reduce the iodine concentration administered to patients with the patient--specific contrast media formula.

Radiation Dose Measurement

The dose-length products (DLP [mGy × cm]) were recorded from the patient protocol, then individual effective dose (E[mSv]) was calculated from the DLP for each of the CT scans (17). In order to calculate the E, a normalized conversion factor (k[mSv / mGy × cm]) for the abdomen –0.015 mSv/mGy × cm– was used (18): E=DLP x k.

Image assessment

With a smooth convolution kernel (field of view 380 × 380 mm, image matrix, 512 × 512), we reconstructed trans-axial images with 1.5 mm slice thickness (1 mm increment). Our department's CT experts (CS, 15 years) determined the technical inclusion criteria, to ensure a correct scan range, as well as an anatomical inclusion of the pathway, origin and termination of the renal vasculature for each of the prospective and retrospective cases. Using a primary reporting workstation (IMPAX 6.3.1, AGFA) with a GSDF-calibrated 3-megapixel monitor, quantitative measurements of all images were performed.

Vascular Opacification Analysis

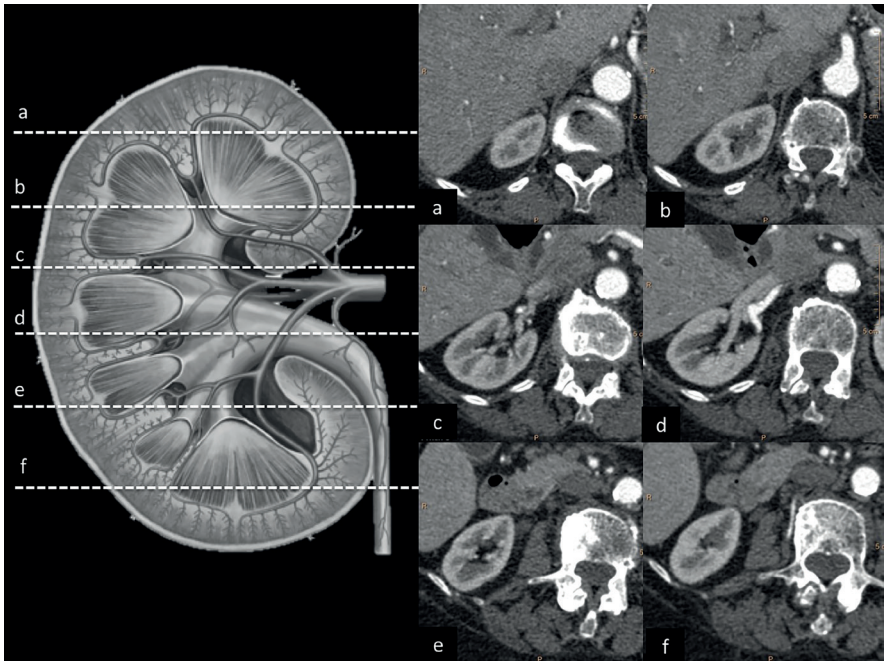
A circular ROI diameter was fitted within the lumen of the vessel, and opacification was measured in the axial plane within it in Hounsfield units (HU). Then, within the ROI of each vessel, the mean and standard deviation (SD) were recorded. In the pre-contrast and arterial phase, both arterial and venous structures were measured. Arterial measurements of the abdominal aorta, the main segments (proximal and distal) of bilateral renal arteries, and the interlobular segments (superior and inferior) of bilateral renal arteries were determined. Venous measurements included the inferior vena cava (IVC), right and left renal veins in both the proximal and distal segments (Figure-1).

Contrast-to-Noise Ratio Measurement

Using a 1.5 mm thick trans-axial image, we calculated the contrast to noise ratio (CNR).

Image quality depends on several factors among which are noise, resolution, and mechanical along with electrical stability of the instrument in use. Noise is not an independent factor as it always depends on the clarity of the available information, therefore it is to be correlated with the contrast in the image under study. The CNR serves as a quantitative assessment tool for noise

Figure 1 - Anatomical location of measurements of the renal vasculature. The segmental lines are as follows: (a and b) upper pole of the kidney that demonstrates the renal cortex, medulla and renal pyramids as well as the minor calyx and interlobular arteries; (c and d) renal cortex, medulla, renal pyramids, interlobular and main segmental renal arteries; and (e and f) inferior pole of the kidney that shows the renal cortex, medulla and renal pyramids as well as the minor calyx and interlobular arteries.



relative to the signal between high and low density structures.

The ROI was drawn at the same size of the vessel lumen diameter, avoiding soft and/or calcified plaques of the vessel wall. When calculating the CNR, we measured the attenuation of the right psoas muscle (ROI_{PSM}) at the level of the 1st Lumbar vertebrae, followed by the second measurement of noise as the standard deviation. The mean opacification of each patient was measured at the origin of the renal arteries (ROI_{RA}) in order to compare the overall degree of vascular opacification within the renal vasculature. Finally, the CNR was calculated based on the measured parameters described above with an empirically derived formula;

$$CNR = (ROI_{RA} - ROI_{PSM})/Noise$$

Diagnostic Efficacy

Thirty two (n=32) cases were chosen with an equal number of normal (n=16) and abnormal cases (n=16), for each contrast protocol (total=64). The normal cases showed normal renal vasculature, while the abnormal cases showed varying degrees of atherosclerotic changes, as defined by the radiologists' reports. Images were selected by one of our department's experts in CT imaging, and not involved in the image reading protocol. Readers viewed images in a blinded manner and in a single sitting. All pathology was visible on the trans-axial images. Vascular pathology prevalence in the image bank was not disclosed to the readers.

Three radiologists with a mean of *17 years' experience (F.M 14 years, M.H 35 years and A.A 4 years)* certified by the American Board of Radiology and *The Royal College of Radiologists,* were the base of the multi-reader analysis. Manipulating the level of the images and the window was permitted to the readers. Each reader indicated the locations of suspicious findings with a confidence level noted from 1-5 where 5 indicated a definite presence of vascular pathology whereas 1 indicated pathology was definitely not present.

Visual Grading Assessment

In order to illustrate viewer preference of one technique over another (based on the visibility of the renal vasculature), the visual grading characteristic (VGC) method (19) was used. VGC is widely used to assess for clinical image quality in radiography, where the observer rates his confidence with the image quality depending on whether or not it has met the image quality criteria. For this study, confidence level ranged from 1-5 where 5 indicated excellent renal artery visualization and 1 represented poor renal artery visualization.

Statistical analysis

Data entry and statistical analyses were performed using SPSS, V.23, 2009. Descriptive analyses for age distribution between the two patient groups was carried out by reporting frequencies and percentages. Continuous variables were presented: means and standard deviations were calculated using independent-samples t-test to compare: age, anteroposterior and transverse diameter, abdominal circumference, contrast media volume, dose length product, radiation dose, and measured opacity between the two patient groups.

To account for the potential confounding effect of the abdominal circumference on the decrease in radiation dose, multivariate logistic regression analyses were carried out. This was also controlled by stratified analyses adjusting our population into four subgroups according to patients' abdominal circumference range, and the usage of independent t-tests to compare the variables.

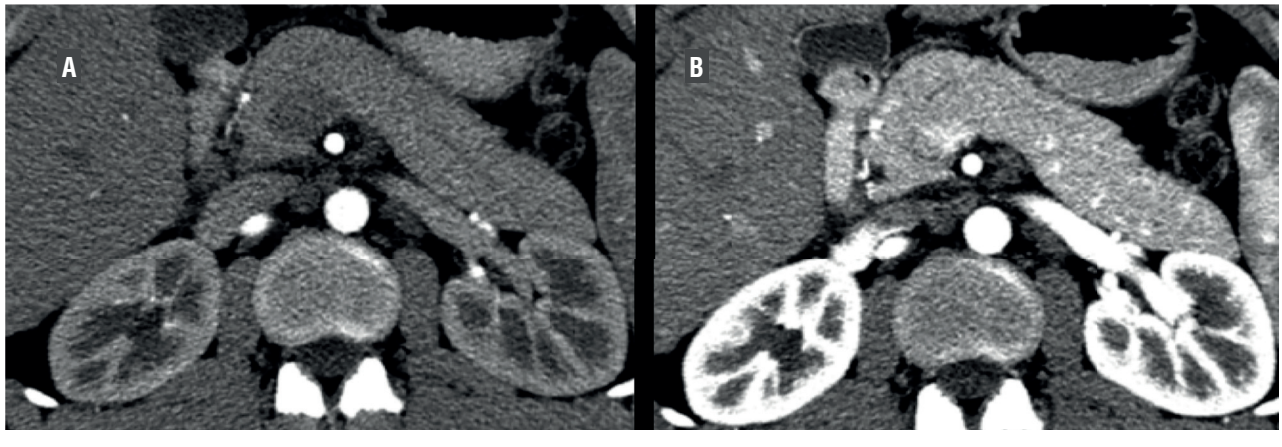
The Dorfman-Berbaum-Metz approach was employed in order to do the ROC analyses. This approach uses cases as fixed and readers as random. Cases were treated as fixed on the basis that the limited image sample size was not taken as a representative of all images. Cohen's kappa analysis was used to calculate. Inter-observer agreements were calculated using k values of 0.60-1, 0.41-0.60, 0.21-0.40, and <0.20 that defined excellent, moderate, fair, and poor agreement respectively. Results were considered statistically significant if $p \leq 0.05$.

RESULTS

Vascular Measurements and CNR

Arterial measurements in the abdominal aorta demonstrated no statistical significance: protocol A=290.35 ± 105.82 vs Protocol B = 269.47 ± 58.74 (p=0.086) (Figure-2). In both protocols, the right and left main proximal renal arteries demonstrated no statistical significance (p>0.05). As for the distal segments of the renal arteries, only that of the left kidney in protocol

Figure 2 - Demonstrates contrast media timing technique for protocol A (b) and protocol B (a). Image a clearly displays only arterial opacification of the renal arteries as well as interlobular renal arteries, whereas image b demonstrates venous contamination within the renal collecting system as well as renal parenchyma.



B was statistically significant (p<0.046). The arterial opacification of the right and left interlobular arteries showed a clear difference in the two protocols (Table-2). In the venous circulation, the IVC demonstrated a significant reduction of opacification in protocol B (59.39 ± 19.39) compared to A (87.74 ± 34.06) (p<0.001). Also, protocol B demonstrated a significant reduction in venous opacification of the proximal and distal segments of bilateral renal veins comparing to protocol A (p<0.001) (Table-2). Mean CNR for protocol A (22.68 HU ± 13.72) was significantly higher than that for protocol B; (14.75 HU ± 5.76 P<0.0001).

Renal parenchymal measurements

Renal parenchymal segmental measurements in the non-contrast phase demonstrated no significant differences in the upper, middle, and lower segments of the cortex and medulla, except for the upper, middle, and lower medulla of the left kidney. In the arterial phase, the upper middle cortex, the lower cortex, and the medulla demonstrated significant differences between the two protocols (p<0.001), with Protocol B being lower than protocol A (Table-3).

Contrast media volume

Contrast media volume was significantly reduced in protocol B (44.56 ± 14.32 mL) compared to A (100 ± 1.0 mL) (p<0.001), with the total CM calculation does not include the 5 mL contrast media test-bolus.

Radiation Dose

Radiation dose was significantly decreased in protocol B (2.46 ± 0.74 mSv) compared to protocol A (3.07 ± 0.68 mSv) (p<0.001). To account for the potential confounding effect of the abdominal circumference on the decrease in radiation dose, stratified analyses were carried out.

Table 2 - Opacification measurements of the renal vasculature.

Vascular Measurements	Right Kidney			Left Kidney		
	Protocol A	Protocol B	*P*	Protocol A	Protocol B	*P*
Arterial						
Main Renal artery						
Proximal	261.45 ± 99.30	255.76 ± 52.49	0.613	267.74±101.17	250.38 ± 56.44	0.136
Distal	254.47 ± 91.95	238.83 ± 55.23	0.146	253.45 ± 88.77	232.34 ± 56.19	0.046
Interlobular						
Superior	196.31 ± 42.16	200.67 ± 54.60	0.527	197.27 ± 42.73	204.63 ± 54.91	0.291
Inferior	184.99 ± 43.99	206.34 ± 60.55	0.005	182.10 ± 38.34	210.27 ± 64.53	0.001
Venous						
Renal Vein						
Proximal	123.03 ± 61.11	78.49 ± 38.43	0.001	116.84 ± 53.24	77.83 ± 39.59	0.001
Distal	121.77 ± 55.60	76.99 ± 37.44	0.001	120.99 ± 49.48	73.04 ± 34.75	0.001

Data are mean ± standard deviation

Table 3 - Opacification measurements of the renal parenchyma.

Renal Parenchyma	Right Kidney			Left Kidney		
	Protocol A	Protocol B	*P*	Protocol A	Protocol B	*P*
Pre Contrast Phase						
Renal Pelvis	14.01 ± 6.51	11.09 ± 4.56	0.001	12.81 ± 6.82	10.36 ± 4.88	0.004
Cortex						
Upper	32.51 ± 11.20	33.77 ± 4.58	0.301	32.67 ± 11.54	33.63 ± 4.65	0.442
Middle	34.71 ± 12.07	32.60 ± 4.07	0.100	33.26 ± 9.68	33.17 ± 4.66	0.936
Lower	31.39 ± 9.51	32.58 ± 4.34	0.285	33.65 ± 12.12	32.22 ± 4.13	0.266
Medulla						
Upper	30.60 ± 8.40	34.27 ± 5.52	0.001	31.16 ± 10.90	34.00 ± 4.57	0.017
Middle	32.00 ± 9.56	33.30 ± 5.23	0.223	31.12 ± 10.68	33.77 ± 4.69	0.025
Lower	31.39 ± 9.51	32.75 ± 4.67	0.200	30.68 ± 9.27	34.97 ± 4.93	0.001
Arterial Phase						
Renal Pelvis	26.29 ± 17.03	38.34 ±60.50	0.056	23.03 ± 17.48	36.81 ± 52.05	0.013
Cortex						
Upper	151.53 ± 48.92	100.28 ± 33.80	0.001	149.88 ± 38.82	100.18 ± 33.55	0.001
Middle	159.10 ± 48.04	105.30 ± 36.12	0.001	158.45 ± 41.92	102.94 ± 35.31	0.001
Lower	163.07 ± 50.08	108.28 ± 35.43	0.001	160.22 ± 45.22	103.04 ± 36.19	0.001
Medulla						
Upper	106.50 ± 55.92	48.76 ± 20.60	0.001	105.58 ± 58.23	48.84 ± 19.15	0.001
Middle	107.26 ± 58.98	51.47± 21.47	0.001	106.20 ± 60.28	50.00 ± 18.61	0.001
Lower	110.56 ± 58.24	51.86 ± 20.48	0.001	108.62 ± 58.44	50.64 ± 19.73	0.001

Data are mean ± standard deviation

Our population was adjusted into three subgroups according to the patients' abdominal circumference range, and radiation dose. Independent t-tests were used for comparison. Abdominal circumference was calculated after measurements of the anterior-posterior length and the transverse length, using this formula (20):

$$\frac{44}{7}\sqrt{(Transverse\ Length/2)\text{^}2+\frac{(Anteriorposterior\ Length/2)\text{^}2)}{2}}$$

In Table-4 summarizes the results of the stratified analyses and shows that radiation dose was still decreased in each of the subgroups. Furthermore, on multivariate analysis, radiation dose was also decreased in protocol B after adjustment for abdominal circumference (r=-0.634, p-value < 0.001).

Image Evaluation

Receiver operating characteristic - the five-point scale revealed a significant difference (p<0.005) between the two protocols with mean ROC values demonstrating increased reader confidence in protocol B compared to A with the area under the curve reaching 0.935

Table 4 - Stratified analyses for the radiation dose.

Abdominal Circumference (mm)	Number of Cases		Radiation Dose		P
	A	B	A	B	
<849	27	43	3.05 ± 0.61	2.49 ± 0.55	<0.001
>850-<940	29	37	3.04 ± 0.79	2.49 ± 0.91	0.012
>941	44	21	3.10 ± 0.65	2.33 ± 0.74	<0.001

Radiation dose: (mSv)

with reader confidence interval between 0.719 and 0.993 (Figure-3a).

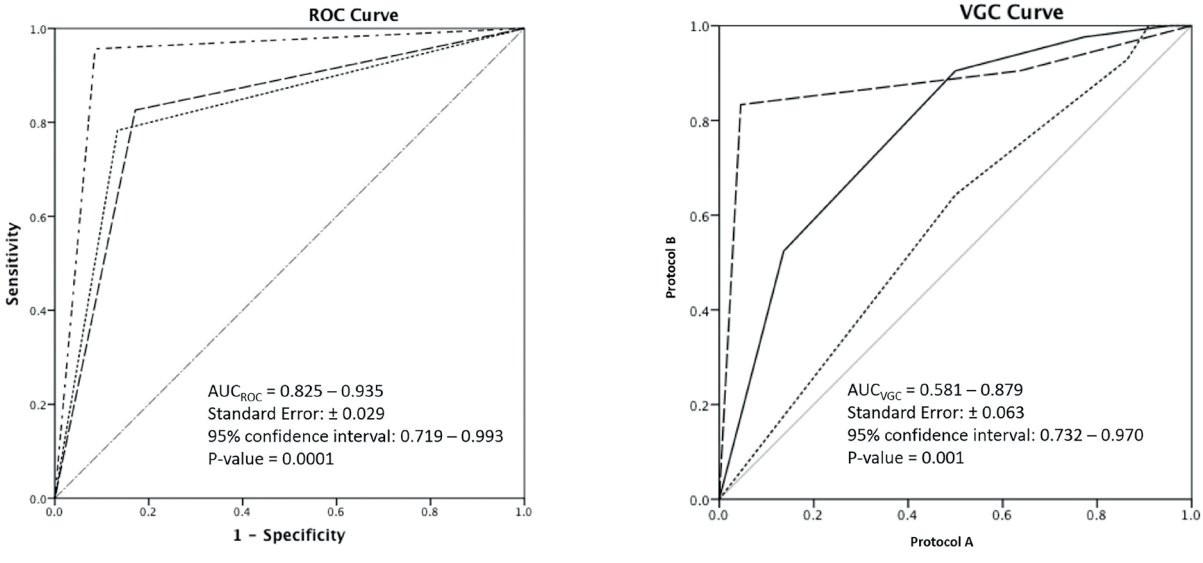
Visual grading characteristic - the five--point scores were individually graded by the three readers for each protocol. The results were represented as a graph shown in Figure-3. When a preference is shown towards one protocol the curve is convex to that protocol's axis. The graphs clearly demonstrate that when the renal arteries were assessed for opacification, the preference is for protocol B over A (Figure-3b).

Kappa analysis - rCTA yielded moderate interobserver agreement with protocol A (k=0.51) and B (k=0.73). There was a strong positive relationship between mean renal arterial opacification, good image quality, and reader confidence in protocol B compared to A (r=0.51, p<0.001).

DISCUSSION

In the current study, we examined a patient-tailored contrast media protocol compared to the conventional contrast media injection protocol. We employed a multi-parametric model to perform the comparison, in which we considered opacification levels within blood vessels, CNR, and ROC analysis with the overall aim of investigating the effect of the protocol on the diagnosis of renovascular diseases. The results were consistent: The patient-tailored approach clearly reduced the opacification of the veins without compromising the arterial vasculature opacification, thus potentially reducing vascular artifact, however, there was increased noise in protocol B that resulted in a lower CNR, but, without affecting the subjective VGC.

Figure 3 - a) ROC curve and b) VGC curve. Each curve demonstrates the individual readers (lines) area under the curve at 95% confidence intervals. In both graphs there is statistical significance in area under the curve in protocol B compared to A.



Interestingly, a reduction in iodine concentration in protocol A (350 mg/mL) compared to B (320 mg/mL) revealed that iodine concentration has no effect on vascular opacification since the emphasis is based on cardiovascular timing and contrast media volume control. Additionally, when observers are blinded in reading arterial studies, it was noticeable that the effect of venous contamination reduces the relative arterial opacification in Protocol A, however, when compared to protocol B, lower arterial opacification with significantly reduced venous contamination in the background gave rise to the observers' perception that higher arterial opacification is best judged relative to low venous contamination. Hence, arterial opacification is determined based on the level of surrounding venous contamination which may distract observers when grading studies for their quality and was evident by greater reader confidence with narrower confidence intervals at 95% CI at lower iodine concentrations and vascular opacification of the renal vasculature. Expert radiologists demonstrated higher AUC values in protocol B compared to protocol A. The consistency of the improvement with the patient-tailored approach, regardless of the metric used, clearly accentuates the positive impact of our proposed technique.

Previous studies have shown a cost to achieving optimal image quality with rCTA examinations, in particular in regards to radiation dose (7, 21). Exceptionally, in our work, radiation dose was actually reduced with reduced contrast media volume, however, further work is required to validate this claim. This was due to the flying focal spot detecting changes in tissue attenuation throughout mA modulation by reduced contrast media within the parenchyma when administering patient-specific contrast media. This dose saving offers significant benefits to the examination since radiation levels at adjacent radiosensitive anatomical structures such as the adrenal glands and liver are reduced. The interplay between the radiation dose and contrast media protocols have often been overlooked, with the chief focus being on peri-venous artifact reduction via patient-specific contrast material formulas during CT angiography (3, 16), reduced x-ray tube voltage (22), and contrast media with low iodine concentration, while attempting to maintain image quality (23). The current study highlights the value of patient--tailored contrast media administration technique that can reduce radiation dose to patients during rCTA (irrespective of body habitus as proved after accounting for the potential confounding effect of the abdominal circumference). This decrease in CV and radiation comes at no cost since it is associated with increased image quality and reader confidence. Currently, it is somewhat difficult to draw an accurate comparison with the literature, and to our knowledge, we are the first to compare the patient-tailored to conventional contrast media approach for renal artery disease during rCTA.

There are limitations in this study; the use of conventional angiography could further clarify the diagnostic accuracy and patient outcome on the basis of our patient-tailored contrast media protocol. We did not test the same patients under both protocols. We did not compare renal arterial cross sections and luminal diameters with those of filtered back projection, hybrid, and model-based iterative reconstruction algorithms. Therefore, predicting accurate clinical outcomes in renal vasculature with our patient-tailored contrast media technique would ideally be confirmed with the use of conventional angiography and effects on clinical outcomes. Finally, we did not entertain the observer performance of image quality compared to the CNR, since observer performance employs noise texture (noise power spectrum) (24) when reducing radiation dose during iterative reconstruction.

In summary, we present a patient-tailored contrast media injection protocol that demonstrates significant improvements in the visualization of renal vasculature reader confidence during rCTA.

Key points:

1. Iodixanol improved visualization at reduced radiation dose during renal CT Angiography.
2. Iodixanol reduced the opacification of the veins without compromising the arterial vasculature.
3. When administering Iodixanol, radiation dose was reduced with reduced contrast media volume.

ABBREVIATIONS

rCTA = Renal Computed Tomography Angiography
IV = Intravenous
eGFR = Estimated glomerular filtration rate
CNR = Contrast-to-noise ratio
IVC = Inferior Vena Cava
ROC = Receiver operating characteristic
MRA = Magnetic resonance Angiography
HU = Hounsfield Unit
MDCT = Multidetector Computed tomography
ROI = Region of Interest
ROI_{PSM} = region of interest in the psoas muscle
ROI_{RA} = region of interest in the renal arteries
CV = Contrast Volume
TTP = Time To Peak
ST = Scan Time
OVWP = Optimal Venous Washout Phase
FR = Flow Rate
DLP = Dose-Length Products
E = Effective dose
SD = Standard Deviation
AVCR = Artery and Vein Contrast Ratio
CI = Confidence Interval

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Pellerin O, Sapoval M, Trinquart L, Redheuil A, Azarine A, Chatellier G, et al. Accuracy of multi-detector computed tomographic angiography assisted by post-processing software for diagnosis atheromatous renal artery stenosis. Diagn Interv Imaging. 2013;94:1123-31.
2. Gilfeather M, Yoon HC, Siegelman ES, Axel L, Stolpen AH, Shlansky-Goldberg RD, et al. Renal artery stenosis: evaluation with conventional angiography versus gadolinium-enhanced MR angiography. Radiology. 1999;210:367-72.
3. Saade C, Deeb IA, Mohamad M, Al-Mohiy H, El-Merhi F. Contrast medium administration and image acquisition parameters in renal CT angiography: what radiologists need to know. Diagn Interv Radiol. 2016;22:116-24.
4. Lell MM, Jost G, Korporaal JG, Mahnken AH, Flohr TG, Uder M, et al. Optimizing contrast media injection protocols in state-of-the art computed tomographic angiography. Invest Radiol. 2015;50:161-7.
5. Pang L, Zhao Y, Dong H, Shi H, Yang W, Zhang H, et al. High-Pitch Dual-Source Computed Tomography Renal Angiography Comparison With Conventional Low-Pitch Computed Tomography Angiography: Image Quality, Contrast Medium Volume, and Radiation Dose. J Comput Assist Tomogr. 2015;39:737-40.
6. Helck A, Schönermarck U, Habicht A, Notohamiprodjo M, Stangl M, Klotz E, et al. Determination of split renal function using dynamic CT-angiography: preliminary results. PLoS One. 2014;9:e91774.
7. Duddalwar VA. Multislice CT angiography: a practical guide to CT angiography in vascular imaging and intervention. Br J Radiol. 2004;77 Spec No 1:S27-38.
8. Carrascosa P, Capunay C, Rodriguez-Granillo GA, Deviggiano A, Vallejos J, Leipsic JA. Substantial iodine volume load reduction in CT angiography with dual-energy imaging: insights from a pilot randomized study. Int J Cardiovasc Imaging. 2014;30:1613-20.
9. Andrabi Y, Kambadakone A, Sahani DV. Experiences with the use of iteratively reconstructed dose-modified MDCT angiography examinations of living renal donors. J Comput Assist Tomogr. 2014;38:535-43.
10. Meng X, Mi Q, Fang S, Zhong W. Preoperative evaluation of renal artery anatomy using computed tomography angiography to guide the superselective clamping of renal arterial branches during a laparoscopic partial nephrectomy. Exp Ther Med. 2015;10:139-44.
11. Cho ES, Yu JS, Ahn JH, Kim JH, Chung JJ, Lee HK, et al. CT angiography of the renal arteries: comparison of lower-tube-voltage CTA with moderate-concentration iodinated contrast material and conventional CTA. AJR Am J Roentgenol. 2012;199:96-102.
12. Saade C, Mayat A, El-Merhi F. Exponentially Decelerated Contrast Media Injection Rate Combined With a Novel Patient-Specific Contrast Formula Reduces Contrast Volume Administration and Radiation Dose During Computed Tomography Pulmonary Angiography. J Comput Assist Tomogr. 2016;40:370-4.
13. Saade C, Jamal O, Kobrossi S, Araji A, Haddad M, El-Rassi I. Synchronization of contrast media administration with retrograde blood flow in patients with hypoplastic ascending aorta. Diagn Interv Imaging. 2016;97:265-8.
14. Saade C, El-Merhi F, Mayat A, Brennan PC, Yousem D. Comparison of Standard and Quadruple-Phase Contrast Material Injection for Artifacts, Image Quality, and Radiation Dose in the Evaluation of Head and Neck Cancer Metastases. Radiology. 2016;279:571-7.

15. Nakaura T, Awai K, Yauaga Y, Nakayama Y, Oda S, Hatemura M, et al. Contrast injection protocols for coronary computed tomography angiography using a 64-detector scanner: comparison between patient weight-adjusted- and fixed iodine-dose protocols. Invest Radiol. 2008;43:512-9.
16. Saade C, Bourne R, Wilkinson M, Evanoff M, Brennan PC. Caudocranial scan direction and patient-specific injection protocols optimize ECG-gated and non-gated thoracic CTA. J Comput Assist Tomogr. 2013;37:725-31.
17. Boone JM. Reply to "Comment on the 'Report of AAPM TG 204: Size-specific dose estimates (SSDE) in pediatric and adult body CT examinations'" [AAPM Report 204, 2011]. Med Phys. 2012;39(7Part2):4615-6.
18. Huda W, Ogden KM, Khorasani MR. Converting dose-length product to effective dose at CT. Radiology. 2008;248:995-1003.
19. Båth M, Månsson LG. Visual grading characteristics (VGC) analysis: a non-parametric rank-invariant statistical method for image quality evaluation. Br J Radiol. 2007;80:169-76.
20. Sykora S. Approximations of ellipse perimeters and of the complete elliptic integral E (x). Review of known formulae Stan's Library. 2005.
21. Saade C, Deeb IA, Mohamad M, Al-Mohiy H, El-Merhi F. Contrast medium administration and image acquisition parameters in renal CT angiography: what radiologists need to know. Diagn Interv Radiol. 2016;22:116-24.
22. Cho ES, Yu JS, Ahn JH, Kim JH, Chung JJ, Lee HK, et al. CT angiography of the renal arteries: comparison of lower-tube-voltage CTA with moderate-concentration iodinated contrast material and conventional CTA. AJR Am J Roentgenol. 2012;199:96-102.
23. Iyama Y, Nakaura T, Yokoyama K, Kidoh M, Harada K, Tokuyasu S, et al. Impact of Knowledge-Based Iterative Model Reconstruction in Abdominal Dynamic CT With Low Tube Voltage and Low Contrast Dose. AJR Am J Roentgenol. 2016;206:687-93.
24. Christianson O, Chen JJ, Yang Z, Saiprasad G, Dima A, Filliben JJ, et al. An Improved Index of Image Quality for Task-based Performance of CT Iterative Reconstruction across Three Commercial Implementations. Radiology. 2015;275:725-34.

Correspondence address:
Fadi El-Merhi, MD
Diagnostic Radiology Department
American University of Beirut Medical Center
PO Box: 11-0236
Riad El Solh, Beirut 1107 2020
Beirut, Lebanon
Telephone: + 96 113 50000
E-mail: fe19@aub.edu.lb

ORIGINAL ARTICLE

Vol. 45 (5): 1033-1042, September - October, 2019
doi: 10.1590/S1677-5538.IBJU.2019.0016

Evaluation of relaxant responses properties of cinnamon essential oil and its major component, cinnamaldehyde on human and rat corpus cavernosum

Alev Onder [1], Didem Yilmaz-Oral [2, 3], Igor Jerkovic [4], Alp Ozgur Akdemir [5], Serap Gur [2]

[1] Department of Pharmacognosy, Faculty of Pharmacy, Ankara University, Ankara, Turkey; [2] Department of Pharmacology, Faculty of Pharmacy, Ankara University, Ankara, Turkey; [3] Department of Pharmacology, Faculty of Pharmacy, Cukurova University, Adana, Turkey; [4] Department of Organic Chemistry, Faculty of Chemistry and Technology, University of Split, Split, Croatia; [5] Department of Urology, Ankara Numune Education and Research Hospital, Ankara, Turkey

ABSTRACT

Cinnamomum cassia (Cinnamon) is a well-known traditional medicine with therapeutic benefits for centuries. We evaluated the effects of cinnamon essential oil (CEO) and its main component cinnamaldehyde (CA) on human corpus cavernosum (HCC) and rat CC. The essential oil of cinnamon was analyzed for the confirmation of the oil profile. HCC specimens from patients undergoing penile prosthesis surgery (age 48-69 years) were utilized for functional studies. In addition, erectile responses in anesthetized control and diabetic rats were evaluated in vivo after intracavernosal injection of CEO and CA, and rat CC strips were placed in organ baths. After precontraction with phenylephrine (10μM), relaxant responses to CEO and CA were investigated. CA (96.9%) was found as the major component. The maximum relaxation responses to CEO and CA were 96.4±3.5% and 96.0±5.0% in HCC and 97.5±5.5% and 96.8±4.8% in rat CC, respectively. There was no difference between control and diabetic rats in relaxation responses to CEO and CA. The relaxant responses obtained with essential oil and CA were not attenuated in the presence of nitric oxide synthase (NOS) inhibitor, and soluble guanylate cyclase inhibitor (sGS) in CC. In vivo, erectile responses in diabetic rats were lower than in control rats, which was restored after intracavernosal injection of CEO and CA. CEO and CA improved erectile function and relaxation of isolated strips of rat CC and HCC by a NO/cGMP-independent mechanism. Further investigations are warranted to fully elucidate the restorative effects of CEO and CA on diabetic erectile dysfunction.

ARTICLE INFO

Serap GUR
https://orcid.org/0000-0002-1730-7282

Keywords:
cinnamic aldehyde [Supplementary Concept]; Penile Induration; Humans

Int Braz J Urol. 2019; 45: 1033-42

Submitted for publication:
January 07, 2019

Accepted after revision:
April 20, 2019

Published as Ahead of Print:
June 20, 2019

INTRODUCTION

Erectile dysfunction (ED) is one of the most common health problems for men. In the current treatment for ED, phosphodiesterase-5 inhibitors (PDE-5i) are considered the first-line therapy (1). Plant-derived products have been used for the management of ED for a long time, and over 15% of men use natural-based therapies (2). Yohimbine, Korean ginseng, and ginkgo are popular exam-

ples used as male sexual performance enhancers in traditional medicine (3). Previous clinical trials with Yohimbine demonstrated that the combination with L-arginine was effective in improving erectile function in patients with mild to moderate ED (4). In addition, oral gavage with Ginsenoside Rg3 (100mg/kg) normalized in vivo erectile responses in a diabetic rat model (5). Furthermore, a clinical trial showed the positive effects of ginseng on IIEF-5 scores compared with placebo. The treatment with ginseng was effective in four of the five IIEF-15 domains (2). Another study indicated that four weeks of daily treatment with high-dose Ginkgo biloba extract significantly increased erectile function in comparison with the vehicle--only treatment (6). Recently, we found that the treatment with pomegranate juice improved ED in a diabetic rat model (7).

The genus *Cinnamomum* (Cinnamon) Schaeff (Lauraceae) comprises over 250 species, which are aromatic evergreen shrubs and trees growing in tropical rain-forests in a majority of Asia (8). Cinnamon is one of the oldest spices, which has been used in many cultures for centuries. *Cinnamomum cassia* or Chinese cinnamon is one of the most important and popular species in this genus. The bark and stem of the plant are an excellent natural source of aromatic spices (8). *Cinnamomum cassia* includes several secondary metabolites such as coumarins, diterpenoids, polyphenols, and essential oils. The essential oil of the plant bark mainly contains cinnamaldehyde (CA) besides cinnamic acid (9). Moreover, the plant is well-known for its medicinal properties for the treatment of some diseases with many critical pharmacological effects such as platelet anti--aggregation, antidiabetic, anti-inflammatory and antioxidant (9-12).

In traditional oriental herbal medicine, *Cinnamomum cassia* extract (8) has been suggested to improve sexual performance (13). Moreover, the methanolic extract of *Cinnamomum cassia* improved the sexual function in aged rats by increase in smooth muscle/collagen ratio and decrease in oxidative stress in rat penile smooth muscle (10). Furthermore, the methanol extract of the plant barks has been potentially inhibited arginase activity on isolated rat corpus cavernosum (CC) smooth muscles (14).

In the present study, the cinnamon essential oil (CEO, from *Cinnamomum cassia* Blume) was evaluated for the potential effect on i*n vitro* on isolated human CC (HCC), diabetic and control rat CC, as well as *in vivo* erectile function in diabetic rats. In addition, the essential oil of *Cinnamomum cassia* Blume was analyzed by GC (Gas Chromatography) and spectroscopy (GC/MS) for the identification and confirmation of the major components. To understand the effect of related compounds in diabetic conditions, its major compound CA-induced relaxation responses were evaluated on both *in vitro* and *in vivo* in diabetic rats compared with control rats.

MATERIALS AND METHODS

Plant material

The essential oil of *Cinnamomum cassia* Blume has been supplied from Heal with Essential Oil Company (Florida, USA).

GC/Flame Ionization Detector (FID) and GC/MS analysis procedure for the identification of essential oil components.

Analyses of CEO (diluted 1:100 v/v with n-pentane: diethyl ether 1:2 v/v) were performed using GC/FID and GC/MS in triplicate. GC analyses were carried out on a gas chromatograph (Agilent Technologies, CA, USA) equipped with FID. Chromatographic separations were performed on a 30m x 0.25mm HP-5MS capillary column [(5%-phenyl)-methylpolysiloxane, Agilent J and W GC column] with a coating thickness of 0.25µm. The oven was temperature-programmed isothermal at 70°C for 2 min and then increased to 200°C at a rate of 3°C/min and held isothermal for 15 min. Helium at 1mL/min was used as a carrier gas. The injector temperature was 250°C, and the detector temperature was 300°C. The injected volume was 1µL, and the split ratio was 1:50. The mass detector operated in the electron impact ionization mode at 70 eV; the mass range was m/z 30-300, and the ion source temperature was 280°C. The volatile compound separation was obtained

using the same column and oven temperature program as previously described. The individual peaks were identified by the comparison of their retention indices (relative to C9-C25n-alkanes for HP-5MS column), as well as by comparing their mass spectra with the Wiley 275MS library (Wiley, New York, USA) and NIST98 (Gaithersburg, Germany) mass spectral database. The percentage composition of the samples was computed from the GC peak areas using the normalization method (without correction factors).

HCC tissue strips

A total of 16 men with ED and/or Peyronie's disease were enrolled in the present study with consent under Institutional Review Board guidelines. CC samples were obtained from the patients (age: 48-69) who had undergone penile prosthesis surgery. HCC tissue strips were placed in cold Krebs isotonic solution [consisting in (mM): NaCl, 118; $NaHCO_3$, 25; glucose, 5.6; KCl, 4.7; KH_2PO_4, 1.2; $MgSO_4$ 7 (H_2O), 1.17; and $CaCl_2$ $2H_2O$, 2.5] and immediately transported (between 15-30 min) to the laboratory for in vitro experiments.

Animal experiments

Ten adult male Sprague-Dawley rats (300-350g) were randomly divided into control and diabetic groups. Diabetic rats received a single intraperitoneal injection of streptozotocin (40mg/kg), which was dissolved in a citrate buffer (pH=5.5). Measurement of blood glucose levels was performed using an Accu-Chek glucometer (Roche Diagnostics, Indianapolis, IN) after the induction of diabetes. Rats were housed in separate cages and were provided with food and water ad libitum in a temperature-controlled room (22±1°C) that was artificially lit from 7:00 AM to 7:00 PM daily. The present study was approved by the Institutional Animal Care and Use Committee of Ankara University (2014-9-66).

***In vivo* studies**

Eight weeks after the induction of diabetes, rats were anesthetized with sodium pentobarbital (50mg/kg, i.p.) to measure intracavernosal pressure (ICP). The trachea was cannulated using polyethylene-240 tubing to maintain the constant airway. The carotid artery was also cannulated (polyethylene-50 tubing) to measure mean arterial pressure (MAP) using a transducer (Statham, Oxnard, CA) attached to a data acquisition system (Biopac MP 100 System, Santa Barbara, CA). A 25-gauge needle filled with 250U/mL of heparin and connected to the polyethylene tubing was inserted into the right crura of the penis and connected to the pressure transducer to measure ICP continuously. The right major pelvic ganglion and cavernous nerve (CN) were identified. A stainless steel bipolar hook electrode was placed around the CN posterolateral to the prostate for the stimulation. The CN was stimulated (2.5, 5 and 7.5 V, 15 Hz, 30-second pulse width) with a square pulse stimulator (Grass Instruments, Quincy, MA), and MAP and ICP were continuously measured. The measurements were repeated after the intracavernosal administration of CEO and CA (1µM) in the diabetic group.

Measurement of isometric tension in CC strips

CC was placed in a petri dish containing Krebs-bicarbonate and was oxygenated with a mixture of 95% O_2, and 5% CO_2. On average 4 strips of HCC tissue (1 x 1 x 8mm) were prepared from each cavernosal sample. Strips were suspended in 20mL organ bath chambers (Radnoti Glass Technology Inc, Monrovia, California) with one end fixed to a tissue holder and the other secured to a force transducer (FT03 Grass Instruments, Quincy, Massachusetts). Organ chamber temperature was maintained at 37°C via a circulating water bath. After the placement of tissue strips in the organ bath chamber, the preparations were allowed to equilibrate for about 90 min, and the bath solution was replaced every 15 min. The CC strips were pre-contracted with phenylephrine (PE, 10µM) and allowed to relax in response to the administration of CEO (26, 52 and 104mg) and CA (26, 52 and 104mg). The relaxant responses to CEO and CA were recorded before and after the administration of the nonspecific NO synthase (NOS) inhibitor, L-NAME (NG-nitro-L-arginine methyl ester, 100µM) and soluble guanylyl cyclase (sGC) inhibitor, ODQ (1H-[1,2,4] oxadiazole [4,3-a] quinoxaline-1-one, 30µM). CC strips were incubated

with inhibitors for 20 min before obtaining the relaxation curves.

Sodium nitroprusside-(SNP, 10nM) and sildenafil (10nM)-induced relaxation responses were evoked after precontraction of CC strips with PE (10^{-5}M) in the presence or the absence of CEO (26mg) in HCC.

Drugs and chemicals

All drugs, as well as CA, were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA).

Data analysis

All values are expressed as mean±SEM. Statistical differences were determined by one--way analysis of variance (ANOVA) with repeated measures followed by Bonferroni post-test performed using Prism 4 statistical analysis packages for Windows (GraphPad Software, La Jolla, CA, USA). A ***p***-value <0.05 was considered significant.

RESULTS

Identification of the essential oil components

In essential oil analysis, components of CEO (99.3%) were identified. CA (96.9%) has been determined as a major component in the oil of cinnamon as seen in Table-1.

CEO and its major compound CA-induced relaxation of HCC strips and involvement of NO/sGC pathway.

Representative relaxation traces of CEO (Figure-1A) and its major compound CA (Figure-1B) in isolated HCC are shown in Figure-1. The maximum relaxation induced by CEO was 96.4±3.5%, was not inhibited in the presence of either L-NAME (98±4%) or ODQ (90±3%) (Figures 1C and D). Also, CA-induced maximal relaxation response was 96±5%. Similarly, the maximal relaxation response to CA did not change in the presence of L-NAME (86.0±4.5%) and ODQ (89±4%) (Figures 1E and F).

Effect of CEO on SNP-and sildenafil-mediated relaxation of HCC

SNP-induced relaxation response at 10nM dose was increased in the presence of CEO (26mg), which was not statistically significant (Figure-1G). There was no difference in sildenafil-induced relaxation response at 10nM between the absence and presence of CEO (26mg, Figure-1H).

***In vitro* responses of rat CC strips**

The maximal relaxation induced by CEO was 97.5±5.5%, which was not altered in diabetic rats (87.3±1.0, Figure-2A). The relaxant responses to CEO were not inhibited by the presence of either L-NAME (95±6%) or ODQ (90±2%) (Figure 2B and C). Moreover, CA-induced maximal relaxation response in control and diabetic rats were 96.8±4.8% and 86.5±13.6%, respectively (Figure-2F). The maximal relaxation response to CA did not change in the presence of L-NAME or ODQ (Figures 2G and H).

In vivo erectile responses in both groups

Total ICP and ICP/MAP values for erectile responses in diabetic rats were lower than those in control rats (P <01; Figure-2). After the intracavernosal administration of CEO (Figures 2 D and E) and CA (Figures 2I and J), the total ICP

Table 1 - The essential oil composition of *Cinnamomum cassia* Blume.

Peak number	Compounds	RRI	Area %
1	Benzaldehyde	965	0.3
2	Phenylacetaldehyde	1048	0.2
3	*trans*-Cinnamic aldehyde	1277	96.9
4	trans-Cinnamic acid	1457	1.9

Figure 1 - Representative traces are showing the relaxation effect of cinnamon essential oil (CEO, A) and cinnamaldehyde (CA, B) on phenylephrine (PE) pre-contracted human corpus cavernosum (HCC) strips. Relaxation-response curves for CEO and CA in HCC strips after pre-contraction with PE (10µM) in the presence of L-NAME (100µM, n=8), ODQ (30µM, n=7). Relaxation-response curves for sodium nitroprusside (SNP, G) and sildenafil (H) in HCC strips after pre-contraction with PE (10µM) in the presence of CEO (26mg, n=8). Data represent the mean SEM of 6-8 observations.

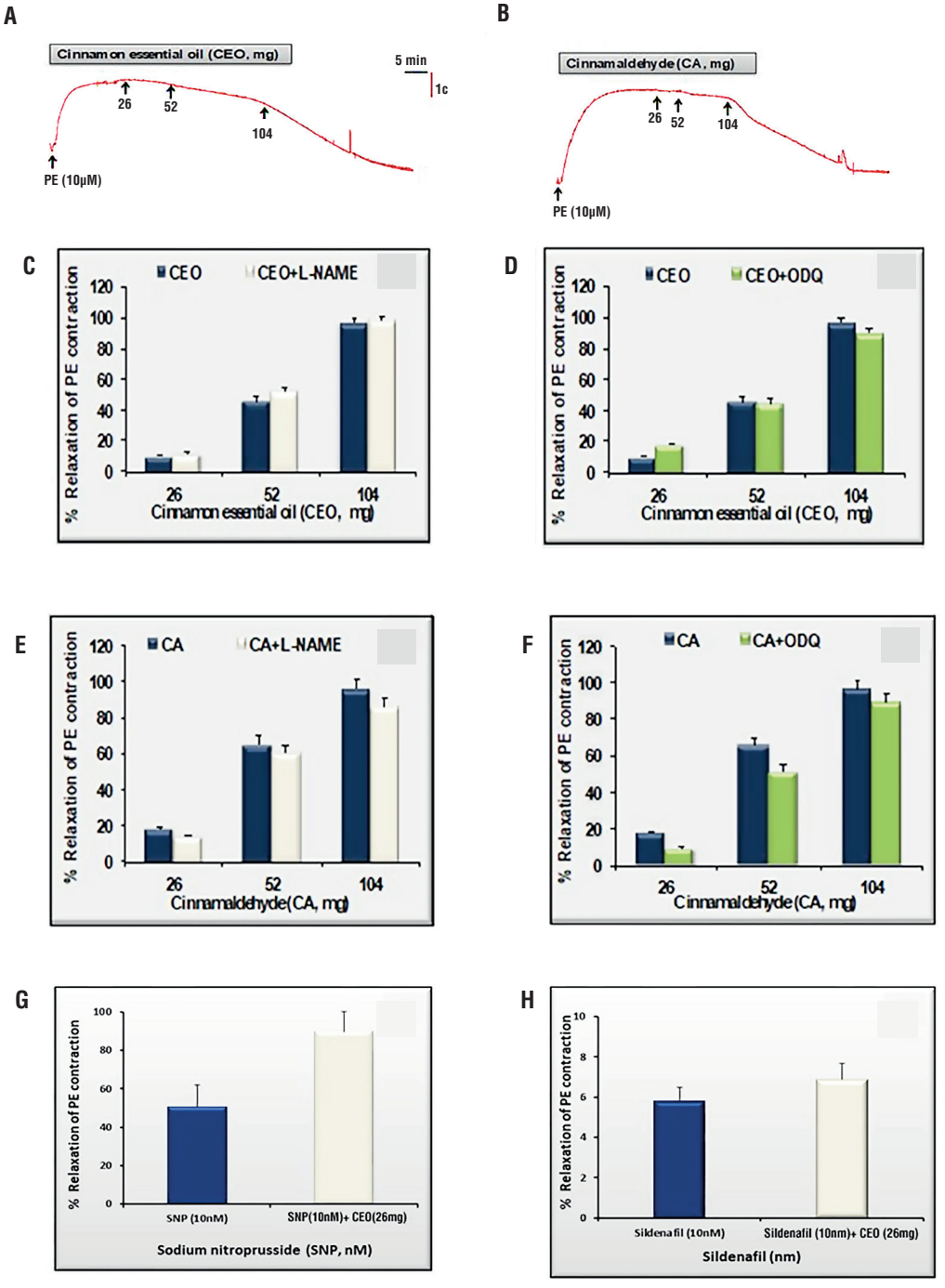
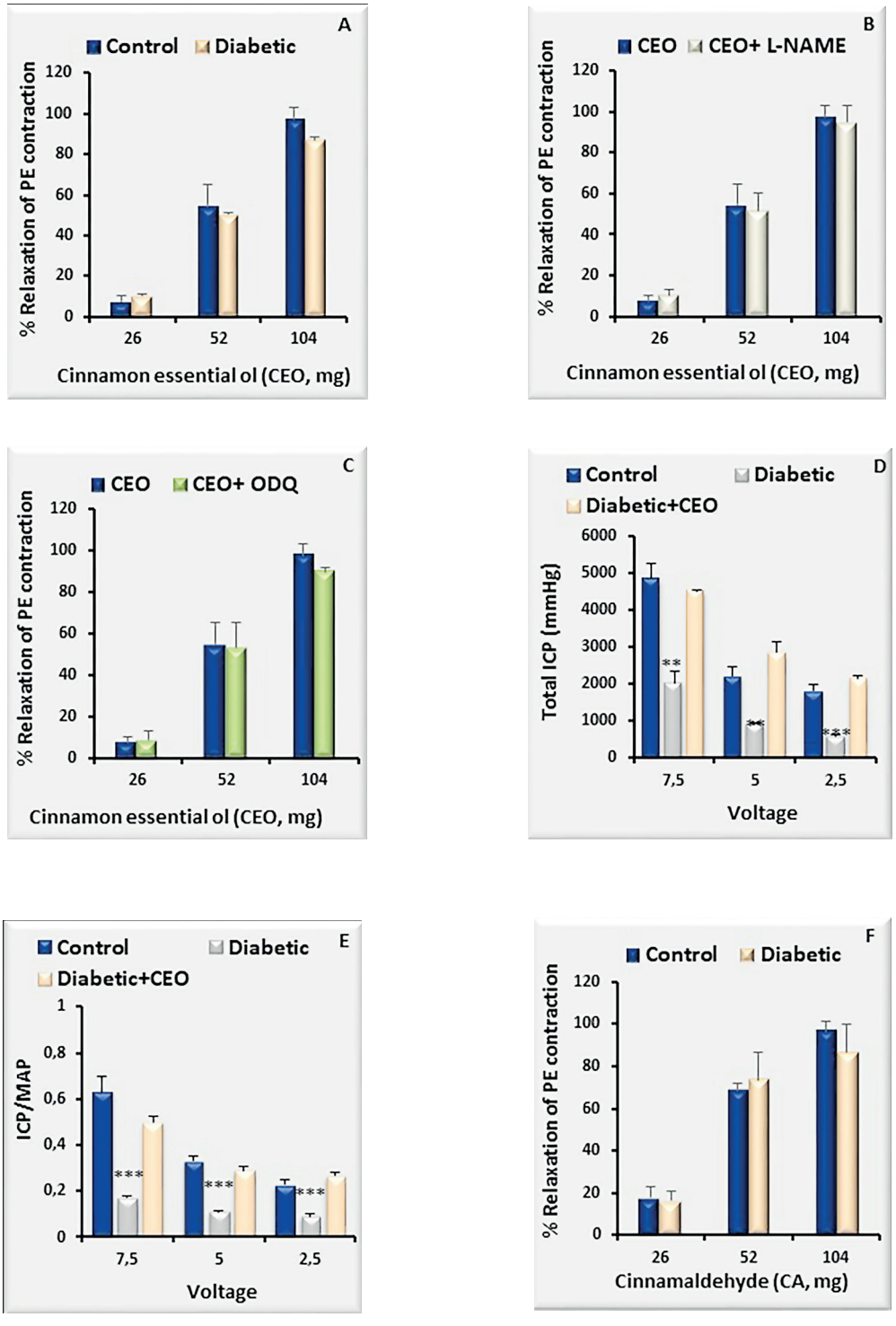
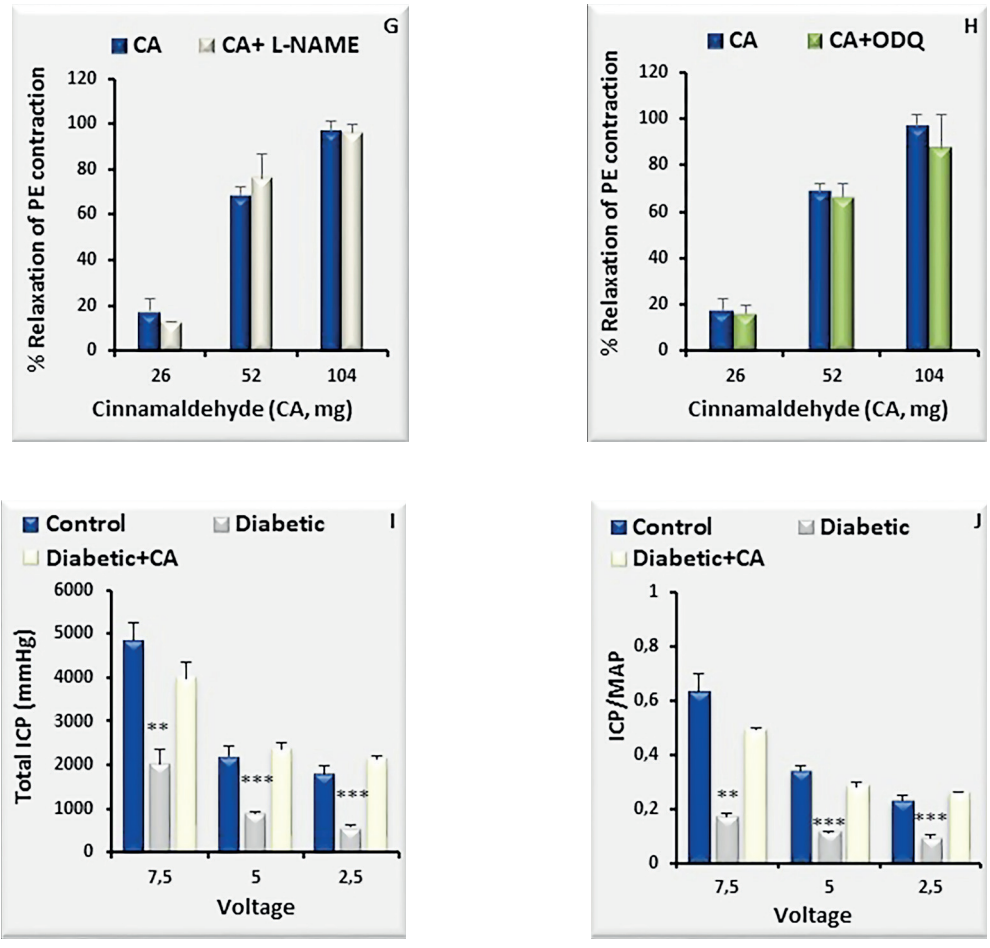


Figure 2 - Relaxation-response curves for cinnamon essential oil (CEO) and cinnamaldehyde (CA) in rat corpus cavernosum (CC) strips after pre-contraction with phenylephrine (PE, 10μM) in the presence of L-NAME (100μM, n=8), ODQ (30μM, n=7). In vivo intracavernosal effect of CEO and CA on the penile erection of diabetic and control rats. Data represent the mean±standard error of the mean of 6-8 observations.





**P <01, ***P <001 vs. control value.

and the ICP/MAP values were restored in the diabetic rats at all voltage levels (Figure 2).

DISCUSSION

This is the first report that provides basic mechanistic information concerning CEO and CA-induced dose-dependent relaxation in human and rat CC. The major findings of the present study show that (1) CEO and CA relax human and rat CC in a concentration-dependent manner; (2) NO-cyclic guanosine monophosphate (cGMP) pathway is not involved in the relaxation response to CEO and CA; and (3) CEO and CA improve in vivo erectile function in diabetic rats.

In the current study, CA (96.9%) has been determined as a major component in the oil of cinnamon by GC and GC/MS analyzes. In previous studies, CA has also been found as a major component of 90.22% in *Cinnamomum cassia* barks (15); 90% in Vietnamese Cinnamomum cassia barks (16).

The present study showed that CA and CEO remarkably induced relaxation of HCC and rat CC strips. There are no previous reports on the effects of CEO and CA on human and rat CC. However, Alotaibi (17) recently demonstrated that cinnamon extract decreased the force of uterine contraction, even when the uterus was stimulated by agonists. The maximum relaxation induced by CEO was not inhibited in the presence of either L-NAME or ODQ. Similarly, the maximal relaxation response to CA did not change in the presence of L-NAME and ODQ. These results suggest that the relaxation effect

of CEO and CA is not mediated through the NO/cGMP pathway. There are few studies including these compounds and interactions with the NO/cGMP system. Davaatseren et al. (18) recently reported that trans-cinnamaldehyde, an active compound of cinnamon did not affect the production of NO. In addition, the relaxation response to CA in rat aorta and porcine coronary artery did not alter in the presence of NOS and sGC inhibitors (19, 20). Furthermore, the methanol extract of *Cinnamomum zeylanicum* stem bark displayed antihypertensive and organ protective effects in L-NAME-induced hypertensive rats (21).

In the present study, the SNP-induced relaxation response was enhanced after incubation with CEO, which was not statistically significant. In addition, there was no difference in sildenafil-induced relaxation response between the absence and presence of CEO. The previous study showed that endothelium-independent relaxation in mice aorta did not change after the incubation with CA (22). To our knowledge, this is the first data regarding the effects of CEO on cavernosal smooth muscle.

We found that ICP/MAP and total ICP values for erectile responses in diabetic rats were lower than those in control rats. After the intracavernosal administration of CEO and CA, the ICP/MAP and the total ICP values were restored in diabetic rats at all voltage levels. Furthermore, CA and CEO remarkably relaxed both control and diabetic rat CC. We suggest that these compounds may have a restorative effect involving in hyperglycemia-induced reactive oxygen species (ROS) production. In a study by Wang et al. (22), CA is a crucial flavor compound in CEO that enhanced the antioxidant defense against ROS by activating the transcription factor Nuclear factor (erythroid--derived 2)-like 2 (NF-E2-related factor 2) indicating a cardiovascular protective effect. Based on these findings, CA preserved endothelial function under high glucose conditions, but the underlying mechanism is unknown. In addition, Raffai et al. (20) showed that CA-loaded and poly-CA micelles include vasodilator properties, and thus may be used both to relieve coronary vasospasm and for therapeutic drug delivery. Lee et al. suggested that the hypoglycemic activity and pancreas-protective effects of cinnamon in diabetic rats induced by streptozotocin were observed (11). Li et al. (12) demonstrated that cinnamon polyphenols could exert the hypoglycemic and hypolipidemic effects through improving its anti-oxidative capacity, as well as attenuating cytotoxicity via inhibition of inducible NOS, nuclear factor kappa B activation in diabetic mice. We suggest that these compounds could have restorative effects involving in hyperglycemia-induced ROS production under diabetic conditions.

Cinnamon is a dietary component that has been demonstrated to include biologically active substances that regulate blood glucose by insulin-mimetic properties (23). Several clinical trials exhibited that Cinnamon and its extracts achieved a therapeutic effect on diabetic patients (24, 25). In clinical trials, cinnamon displayed positive effects on glycemic control lipid markers in type 2 diabetes populations (24, 26). In light of the previous data supporting cinnamon dietary supplement improves glycemic parameters. Thus, a cinnamon dietary supplement may be beneficial in deterring the incidence of diabetes-induced ED by preventing glycemic parameters. It remains unknown that the benefits of different cinnamon extract supplementations on the prevention and treatment of diabetes-induced ED. Furthermore, these results may be supported further clinical and preclinical studies using combinations of cinnamon dietary supplement and PDE5i for the treatment of diabetic ED, especially in patients who do not respond to PDE5i therapy.

Overall, our results suggest that cinnamon and its major compound, CA may be effective, although a number of some limitations that should be discussed to help shape future research. For instance, limited trial numbers, total sample sizes, methodological differences, imprecise standardization of herbal extracts used, and unclear risk of bias may decrease interest for possible utility in clinical practice. Several compounds have shown promise in vitro and animal studies, but do not provide any

clinical benefit. Previous data obtained from in vitro cell cultures and in vivo animal experiments must be translated into human activities. In addition, more rigorous clinical trials in the field are required before the consumption of herbal dietary supplements can be definitively suggested for the treatment of ED.

In conclusion, the present study demonstrates that CEO and CA induce relaxation in HCC and rat CC in a NO/cGMP-independent manner. Our investigation revealed novel biological functions of CEO and CA for erectile function. Further research is required to address the underlying molecular mechanisms of CEO and CA responsible for cavernosal smooth muscle relaxation.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Hong JH, Kwon YS, Kim IY. Pharmacodynamics, pharmacokinetics and clinical efficacy of phosphodiesterase-5 inhibitors. Expert Opin Drug Metab Toxicol. 2017;13:183-92.
2. Borrelli F, Colalto C, Delfino DV, Iriti M, Izzo AA. Herbal Dietary Supplements for Erectile Dysfunction: A Systematic Review and Meta-Analysis. Drugs. 2018;78:643-73.
3. Cui T, Kovell RC, Brooks DC, Terlecki RP. A Urologist's Guide to Ingredients Found in Top-Selling Nutraceuticals for Men's Sexual Health. J Sex Med. 2015;12:2105-17.
4. Akhondzadeh S, Amiri A, Bagheri AH. Efficacy and Safety of Oral Combination of Yohimbine and L-arginine (SX) for the Treatment of Erectile Dysfunction: a multicenter, randomized, double blind, placebo-controlled clinical trial. Iran J Psychiatry. 2010;5:1-3.
5. Liu T, Peng YF, Jia C, Yang BH, Tao X, Li J, et al. Ginsenoside Rg3 improves erectile function in streptozotocin-induced diabetic rats. J Sex Med. 2015;12:611-20.
6. Wu YN, Liao CH, Chen KC, Liu SP, Chiang HS. Effect of Ginkgo biloba Extract (EGb-761) on Recovery of Erectile Dysfunction in Bilateral Cavernous Nerve Injury Rat Model. Urology. 2015;85:1214.e7-1214.e15.
7. Onal E, Yilmaz D, Kaya E, Bastaskın T, Bayatlı N, Gur S. Pomegranate juice causes a partial improvement through lowering oxidative stress for erectile dysfunction in streptozotocin-diabetic rat. Int J Impot Res. 2016;28:234-40.
8. Bandara T, Uluwaduge I, Jansz ER. Bioactivity of cinnamon with special emphasis on diabetes mellitus: a review. Int J Food Sci Nutr. 2012;63:380-6.
9. Kim SY, Koo YK, Koo JY, Ngoc TM, Kang SS, Bae K, et al. Platelet anti-aggregation activities of compounds from Cinnamomum cassia. J Med Food. 2010;13:1069-74.
10. Goswami SK, Inamdar MN, Jamwal R, Dethe S. Efficacy of Cinnamomum cassia Blume. in age induced sexual dysfunction of rats. J Young Pharm. 2013;5:148-53.
11. Lee SC, Xu WX, Lin LY, Yang JJ, Liu CT. Chemical composition and hypoglycemic and pancreas-protective effect of leaf essential oil from indigenous cinnamon (Cinnamomum osmophloeum Kanehira). J Agric Food Chem. 2013;61:4905-13.
12. Li R, Liang T, Xu L, Li Y, Zhang S, Duan X. Protective effect of cinnamon polyphenols against STZ-diabetic mice fed high-sugar, high-fat diet and its underlying mechanism. Food Chem Toxicol. 2013;51:419-25.
13. Dell'Agli M, Galli GV, Dal Cero E, Belluti F, Matera R, Zironi E, et al. Potent inhibition of human phosphodiesterase-5 by icariin derivatives. J Nat Prod. 2008;71:1513-7.
14. Goswami SK, Inamdar MN, Jamwal R, Dethe S. Effect of Cinnamomum cassia methanol extract and sildenafil on arginase and sexual function of young male Wistar rats. J Sex Med. 2014;11:1475-83.
15. Firmino DF, Cavalcante TTA, Gomes GA, Firmino NCS, Rosa LD, de Carvalho MG, et al. Antibacterial and Antibiofilm Activities of Cinnamomum Sp. Essential Oil and Cinnamaldehyde: Antimicrobial Activities. ScientificWorldJournal. 2018;2018:7405736.
16. Trinh NT, Dumas E, Thanh ML, Degraeve P, Ben Amara C, Gharsallaoui A, et al. Effect of a Vietnamese Cinnamomum cassia essential oil and its major component trans-cinnamaldehyde on the cell viability, membrane integrity, membrane fluidity, and proton motive force of Listeria innocua. Can J Microbiol. 2015;61:263-71.
17. Alotaibi M. The effect of cinnamon extract on isolated rat uterine strips. Reprod Biol. 2016;16:27-33.
18. Davaatseren M, Jo YJ, Hong GP, Hur HJ, Park S, Choi MJ. Studies on the Anti-Oxidative Function of trans-Cinnamaldehyde-Included β-Cyclodextrin Complex. Molecules. 2017;22(12).
19. Xue YL, Shi HX, Murad F, Bian K. Vasodilatory effects of cinnamaldehyde and its mechanism of action in the rat aorta. Vasc Health Risk Manag. 2011;7:273-80.
20. Raffai G, Kim B, Park S, Khang G, Lee D, Vanhoutte PM. Cinnamaldehyde and cinnamaldehyde-containing micelles induce relaxation of isolated porcine coronary arteries:

role of nitric oxide and calcium. Int J Nanomedicine. 2014;9:2557-66.
21. Nyadjeu P, Nguelefack-Mbuyo EP, Atsamo AD, Nguelefack TB, Dongmo AB, Kamanyi A. Acute and chronic antihypertensive effects of Cinnamomum zeylanicum stem bark methanol extract in L-NAME-induced hypertensive rats. BMC Complement Altern Med. 2013;13:27.
22. Wang F, Pu C, Zhou P, Wang P, Liang D, Wang Q, et al. Cinnamaldehyde prevents endothelial dysfunction induced by high glucose by activating Nrf2. Cell Physiol Biochem. 2015;36:315-24.
23. Ranasinghe P, Jayawardana R, Galappaththy P, Constantine GR, de Vas Gunawardana N, Katulanda P. Efficacy and safety of 'true' cinnamon (Cinnamomum zeylanicum) as a pharmaceutical agent in diabetes: a systematic review and meta-analysis. Diabet Med. 2012;29:1480-92.
24. Davis PA, Yokoyama W. Cinnamon intake lowers fasting blood glucose: meta-analysis. J Med Food. 2011;14:884-9.
25. Hasanzade F, Toliat M, Emami SA, Emamimoghaadam Z. The Effect of Cinnamon on Glucose of Type II Diabetes Patients. J Tradit Complement Med. 2013;3:171-4.
26. Mang B, Wolters M, Schmitt B, Kelb K, Lichtinghagen R, Stichtenoth DO, et al. Effects of a cinnamon extract on plasma glucose, HbA, and serum lipids in diabetes mellitus type 2. Eur J Clin Invest. 2006;36:340-4.

Correspondence address:
Serap Gur, MD, PhD
Department of Pharmacology,
Faculty of Pharmacy, Ankara University
06100, Tandogan, Ankara, Turkey
Fax: + 90 312 213-1081
E-mail: serapgur@ankara.edu.tr

ORIGINAL ARTICLE

Vol. 45 (5): 1043-1054, September - October, 2019
doi: 10.1590/S1677-5538.IBJU.2018.0843

Improvement of fertility parameters with Tribulus Terrestris and Anacyclus Pyrethrum treatment in male rats

Dariush Haghmorad [1,2], Mohammad Bagher Mahmoudi [3], Pardis Haghighi [4], Paria Alidadiani [4], Ensieh Shahvazian [4], Parsova Tavasolian [4], Mahmoud Hosseini [5], Mahmoud Mahmoudi [4]

[1] Department of Pathology and Laboratory Medicine, School of Medicine, Semnan University of Medical Sciences, Semnan, Iran; [2] Department of Immunology, School of Medicine, Semnan University of Medical Sciences, Semnan, Iran; [3] Department of Genetics, Shahid Sadoughi University of Medical Sciences, Yazd, Iran; [4] Immunology Research Center, BuAli Research Institute, Department of Immunology and Allergy, School of Medicine, Mashhad University of Medical Sciences; [5] Neuroscience Research Center, Department of Physiology, School of Medicine, Mashhad University of Medical Sciences

ABSTRACT

Objective: Anacyclus Pyrethrum (AP) and Tribulus Terrestris (TT) have been reported as male infertility treatment in several studies; however, in Iranian traditional medicine these two plants are prescribed simultaneously. In this study, we aimed to determine the effects of AP and TT extracts both separately and simultaneously on the male Wistar rat fertility parameters.

Materials and Methods: 32 male Wistar rats were divided into 4 groups: Control, TT, AP, and AT treated groups. Treatment continued for 25 days and rats were weighed daily. Their testes were dissected for histological studies. Sperm analysis including sperm count, viability and motility were performed. Serum was obtained to evaluate testosterone, LH and FSH levels. Histological studies were conducted to study Leydig, and Sertoli cells, spermatogonia and spermatid cell numbers, and to measure seminiferous diameter and epithelium thickness.

Results: Sperm count increased in all the treatment groups. Sperm viability and motility in AT and AP groups were elevated. TT and AT groups showed significantly increased testosterone level compared to control group (P=004, P=0.000, respectively) and TT, AP and AT treatment groups showed increased LH level (P=0.002, P=0.03 and P=0.000, respectively) compared to control, while only AT group showed increased FSH (p=0.006) compared to control. Histological studies showed significant increase of spermatogonia, Leydig and Sertoli cell numbers and epithelial thickness in AT group compared to other groups. All the treatment groups had higher number of Leydig, spermatogonia and spermatid cells.

Conclusion: TT and AP improved sexual parameters; however, their simultaneous administration had higher improving effects on studied parameters.

ARTICLE INFO

Dariush Haghmorad
http://orcid.org/0000-0002-9876-4943

Keywords:
Tribulus; Testosterone; Receptors, FSH; Receptors, LH

Int Braz J Urol. 2019; 45: 1043-54

Submitted for publication:
December 10, 2018

Accepted after revision:
May 06, 2019

Published as Ahead of Print:
July 15, 2019

INTRODUCTION

Infertility refers to inability to achieve pregnancy after twelve months of regular and unprotected intercourse (1, 2). About 40-50% of infertilities are due to male sexual dysfunction, as one out of twenty men suffer from this issue, worldwide (3-6). The majority of infertile and sub fertile men have deficiency in the semen quality which is determined by low sperm numbers, sperm morphology and insufficient sperm motility. Other cases may appear by hormonal imbalances, anatomical problems and genetic defects (1, 2). Male fertility is the direct consequence of spermatogenesis, a multistep process in seminiferous tubules of testis, which is highly regulated by sophisticated hormonal signaling pathways (6, 7). Testosterone is the major androgen in the process of spermatogenesis promoting the maintenance of blood-testis barrier, Sertoli-spermatid adhesion and mature sperm release (8). Gonadotropin releasing hormone (GnRH) has a central role in controlling spermatogenesis. It performs its role by inducing follicle-stimulating hormone (FSH) and luteinizing hormone (LH) secretion from the anterior pituitary gland. LH stimulates adult Leydig cells to generate testosterone (7, 9), while FSH supports spermatogenesis by increasing Sertoli cell numbers and preventing apoptosis of spermatogonia and spermatocytes (9, 10).

Medicinal plants, as a traditional treatment, play a great role in remedies, due to their accessibility, availability and affordability, particularly in non-industrialized countries (11). Despite major advances in assisted reproductive techniques (ART), according to World Health Organization (WHO) estimation, almost 80% of world population trust on traditional health care (12). Plants empirical studies have shown their significant role in alternative therapy of sexual dysfunction (13).

Anacyclus Pyrethrum (AP) root, commonly known as pillitory, belongs to Asteraceae family. It is abundant in India and also found in Africa and Asia. AP has been known as male sexual stimulant. Ebn-e-Sina (14), great medieval Persian physician, prescribed it in Erectile Dysfunctions (ED) treatment. Other records indicate this herb constitute a major part of Polyherbal Ayurvedic Medicine (PAM), widely used for treating **male sexual dysfunction** (MSD) in the Indian subcontinent (15). In studies conducted by Sharma et al. on the effect of AP in rat sexual parameters, several observations were found, including enhanced sperm number, bodyweight, testis weight, seminal vesicle fructose and sexual hormones concentrations (16, 17).

Tribulus terrestris (TT) (Zygophyllaceae) is a Mediterranean plant, traditionally used against various ailments such as sexual inability, edemas, abdominal distention and cardiovascular diseases in India, China, Bulgaria and South Africa (18). A great number of studies have been performed on the effect of TT extract on sperm parameters in both human and animal models, leading to controversial results (13, 19-24).

There are several reports indicating TT effect on increasing body and sexual organs weight (13, 25). In a similar trend, Testosterone and LH elevation were observed (26). On the contrary, other studies reported no increase in testosterone and LH levels (21, 27). In 2014 Santos CA et al. observed that Tribulus terrestris was not more effective than placebo in improving symptoms of erectile dysfunction or serum total testosterone (19).

In several regions of Iran, including northern Khorasan, TT and AP are prescribed simultaneously as a traditional treatment for male sexual dysfunctions. Accordingly, this paper aims to evaluate serum levels of sexual hormones, sperm analysis and histological studies in male rats treated by separate extracts of Tribulus terrestris and Anacyclus Pyrethrum as well as mixture of both extracts as prescribed in traditional medicine.

MATERIALS AND METHODS

Plant material

Dried root of AP and flowers of TT were purchased from local market in Bojnourd (North Khorasan province), and it was recognized and authenticated in Botanical Systematic Laboratory, Department of Biology, faculty of science in Ferdowsi University of Mashhad.

Preparation of extracts

The root and flowers were crushed to powder and were macerated in ethanol (70% v/v) for 72h at room temperature to prepare ethanolic extract. The yield for TT was 6.8% and for AP 7.2%. The salve extract was removed under reduced pressure until the volume reached to 50mL, then it was left in room temperature in petri dish and the dried mass was stored at 4°C.

Animals

Thirty-two Healthy adult male Wistar rats (weight average 255±5g) were obtained from the Animal House of Mashhad University of Medical Sciences. They were kept in well-ventilated house conditions (temperature 28-31°C and humidity 50-55%); photoperiod: 12h natural light and 12h dark; with free access to rat chow and tap water. This project was approved by the ethics committee of Mashhad University of Medical Science on the use of animal's laboratory.

Treatment

All rats were completely randomized into 4 groups containing 8 rats in each group. The groups were force fed as follows: 1) Control group was given Phosphate Buffer Saline (PBS) 2) TT Group was treated by 10mg/kg of Tribulus terrestris extract 3) AP group was treated by 100mg/kg of Anacyclus pyrethrum extract 4) AT group was administered by 10mg/kg of Tribulus terrestris extract and 100mg/kg of Anacyclus pyrethrum extract, simultaneously. The daily oral administration was carried out by the use of metal oropharyngeal cannula for 25 days. Each rat was administered by as much as 0.5mL of the solution. The extracts were dissolved in PBS; therefore, the control group was administered by the same volume of PBS as treatment group. The dose of administration for AP was chosen from Sharma's study 100mg/kg of which had the best results and for TT 10mg/kg was chosen from Gauthaman study (17, 26).

Body and organ weight

The body weight of animals was recorded daily. After 25 days, the animals were sacrificed; their testis and prostate glands were carefully removed and weighed.

Sperm quality analysis

The cauda epididymis was directly isolated after cervical dislocation of the animals and placed in a Petri dish containing 1mL DMEM with 1% BSA. 1mL of the medium was placed in another petri dish, and a section of the cauda epididymis was isolated in this dish that remained in an incubator at 37°C to allow the spermatozoa to 'swim out' into the medium for approximately 10 second.

Sperm quality was analyzed by three parameters: sperm concentration, motility and viability. Sperm concentration was analyzed using hemocytometer method (15). One drop of cauda epididymal spermatozoa was diluted 1: 100 in DMEM supplemented with 1% BSA and placed in the center of the lower disc and further examined with a microscope. The diluted solution was put into the counting chamber and the sperm number was counted using hemocytometer under light microscope. Sperm motility was calculated by calculating the percentages of total and progressive motile spermatozoa using invert microscope and expressed as percentage of motility. Sperm viability was analyzed by Eosin-Nigrosin method under light microscope, where unstained spermatozoa counted as viable and stained spermatozoa counted as non-viable. The viability of spermatozoa was announced in percentage terms.

Serum hormone analysis

Representative animals were bled by cardiac puncture, and the blood was allowed to clot at 4°C overnight. The samples were centrifuged, and the sera were collected and stored at-80°C until hormone analysis was performed.

The sera obtained from all groups were analyzed for testosterone, LH and FSH by ELISA using commercial kits. Testosterone was measured using Free Testosterone ELISA kit from IBL Germany. LH and FSH level were analyzed using Rat LH and FSH Assay kits from BioVendor, Czech Republic. Absorbance was read at 450nm using microplate ELISA reader (Stat Fax 2100, Awarness, and Phoenix, Arizona, USA). The concentrations of hormones were estimated from a standard curve generated with each run.

Histology

After fixing the left testis in Bouin's fixative, the testis were dehydrated using a gradient of ethanol and then cleared in xylene. It was then embedded in paraffin and microtomed into 5µm sections and stained using Hematoxylin and Eosin. Epithelium thickness, seminiferous tubule's diameter, number of Leydig and Sertoli cells, number of spermatids and spermatogonia were assessed under a light microscope. Seminiferous tubule diameter and epithelium thickness were measured using ocular micrometer, measuring respectively 40 and 20 random seminiferous tubule diameters and thickness of their epithelium in each slide; afterwards the mean was calculated for each rat testis, the results are expressed as mean±SD for each group. Sertoli cells, spermatids and spermatogonia were counted in all seminiferous tubules of about 20µm in diameter, and then the mean was calculated for each rat; the results are expressed as mean±SD for each group. In order to calculate the Leydig cell number we counted 6 random areas of all slides, and then the mean was calculated for each rat. The results are expressed as mean±SD.

Statistical analysis

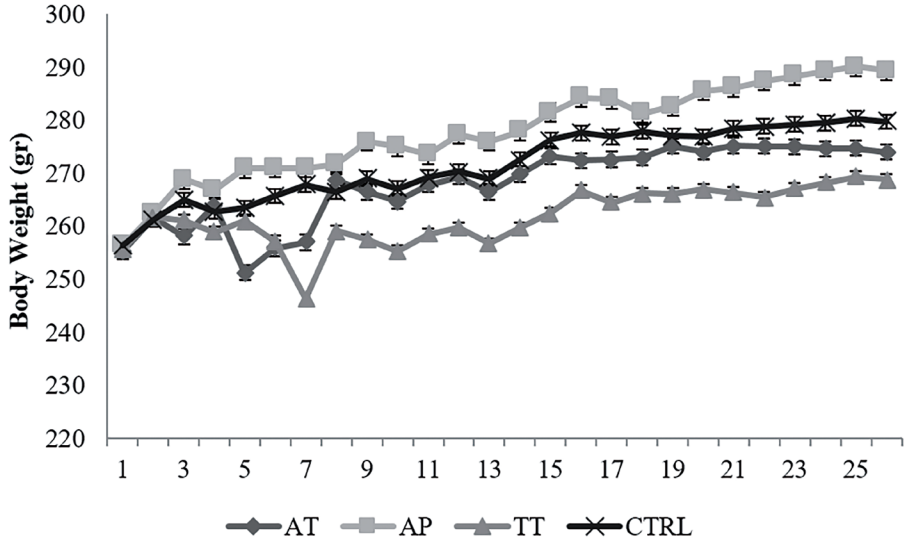
One-way ANOVA was used for statistical comparison of the results. Fischer`s LSD multiple comparison test was applied to see if the differences were statistically significant. Significance was defined at the 0.05 level. SPSS 19 were used to analyze the data. Results are expressed as the mean±SD.

RESULTS

Body and sexual organ weights

An increase was observed in body weight of AP group, while a decrease was obvious in TT group compared to control group, however, AT group showed nearly the same weights as control group; however, none of the alterations were significant (Figure-1). Prostate weight showed elevation in all treatment groups but it was not statistically significant. Testis weight showed no significant change (Table-1).

Figure 1- The body weight of animals was recorded daily.



An increase was observed in body weight of AP group, while a decrease was obvious in TT group compared to control group, however, AT group showed nearly the same weights as control group; however, none of the alterations were significant.

Ctrl=control group, **TT=***Tribulus terrestris*, **AP=***Anacyclus pyrethrum*, **AT=***Anacyclus pyrethrum* and *Tribulus terrestris*

Table 1 - Sexual organ weights and sperm parameters taken in this study (mean ± SD, n=8)

Parameters	Mean ± SD	Range	P Value
Sperm Count (No. of Sperm ×10^6 /MM)			
Control Group (n=8)	1.078 ± 0.621	0.457-1.699	
TT Group (n=8)	1.7225 ±0.482*	1.7225-2.2045	0.01
AP Group (n=8)	1.671 ± 0.404*	1.267-2.075	0.02
AT Group (n=8)	1.7245 ± 0.334*	1.3905-2.0585	0.01
Prostate Weight /mg			
Control Group (n=8)	63.7 ± 0.005	63.695-63.705	
TT Group (n=8)	75.3 ± 0.005	75.295-75.305	0.10
AP Group (n=8)	74.9 ± 0.005	74.895-74.905	0.11
AT Group (n=8)	70.9 ± 0.002	70.898-70.902	0.30
Testis Weight /g			
Control Group (n=8)	1.3412 ± 0.047	1.2942-1.3882	
TT Group (n=8)	1.275 ± 0.070	1.205-1.345	0.43
AP Group (n=8)	1.3588 ± 0.053	1.3058-1.4118	0.83
AT Group (n=8)	1.345 ± 0.060	1.2912-1.4012	0.96
Prostate / Body (×10^3)			
Control Group (n=8)	0.2335 ± 0.054	0.1795-0.2875	
TT Group (n=8)	0.2797 ± 0.046	0.2337-0.3257	0.07
AP Group (n=8)	0.2605 ± 0.057	0.2035-0.3175	0.29
AT Group (n=8)	0.2535 ± 0.043	0.2105-0.2965	0.43
Motility (%)			
Control Group (n=8)	70.375 ± 2.800	67.575-73.175	
TT Group (n=8)	72.75 ± 2.829	69.921-75.579	0.59
AP Group (n=8)	90 ± 2.619*	87.381-92.619	0.00
AT Group (n=8)	82.75 ± 3.845*	78.905-86.595	0.01
Viability (%)			
Control Group (n=8)	83.875 ± 6.0460	77.829-89.921	
TT Group (n=8)	86.5 ± 6.563	79.937-93.063	0.09
AP Group (n=8)	87 ± 10.797*	76.203-97.797	0.05
AT Group (n=8)	90.75 ± 10.403*	80.347-101.153	0.00

ANOVA followed by Fischer`s LSD multiple comparison test. Data are presented as mean ± SD
* Significant results
Ctrl= control group; **TT=***Tribulus terrestris;* **AP=***Anacyclus pyrethrum;* **AT=***Anacyclus pyrethrum* and *Tribulus terrestris*

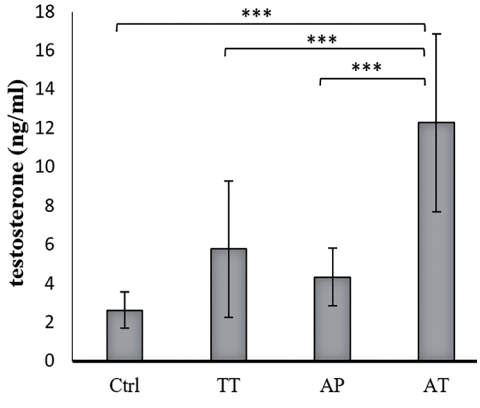
Sperm parameters

Sperm count showed a significant increase (55-57%, increase) in all treatment groups compared to control group; however, none of the treatment groups showed significant change compared to each other. AT and AP treated groups showed significant elevation in their sperm viability (P=0.000 and P=0.05, respectively), however the increase in AT treated group`s viability was significant compared to AP and TT treated groups (P=0.03 and P=0.01, respectively). Motility had significant increase in AT and AP treated groups (Table-1).

Hormonal levels

Testosterone level was increased significantly in AT treated group compared to AP, TT and control groups (P=0.000, P=0.000 and P=0.000, respectively). TT group showed significantly increased testosterone level compared to control group (P=0.004). Increased testosterone level was observed in AP treated group as well, but it was not significant (Figure-2). LH levels increased significantly in AT treated group compared to AP, TT and control groups (P=0.000, P=0.000 and P0.000, respectively); it was also significant, in lower scales, in TT and AP groups in comparison to control group (P=0.002 and P=0.03 respectively) (Figure-3). FSH level was significant only in AT group compared to control group and TT group (P=0.000, P=0.000, respectively) (Figure-4).

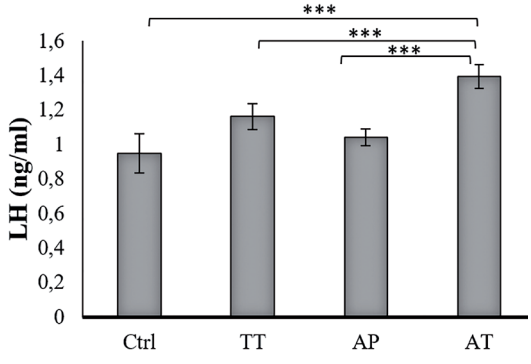
Figure 2- Effect of AP, TT and AT extracts on serum level of testosterone (mean ± SD).



The sera obtained from all groups were analyzed for testosterone, by ELISA using Free Testosterone ELISA kit from IBL Germany. Testosterone level was increased significantly in AT treated group compared to AP, TT and control groups (P=0.000, P=0.000 and P=0.000, respectively). TT group showed significantly increased testosterone level compared to control group (P=0.004). Increased testosterone level was observed in AP treated group as well, but it was not significant.

Ctrl=control group, **TT=***Tribulus terrestris*, **AP=***Anacyclus pyrethrum*, **AT=***Anacyclus pyrethrum* and *Tribulus terrestris*. *P <0.05, ** P < 0.01, ***p<0.001

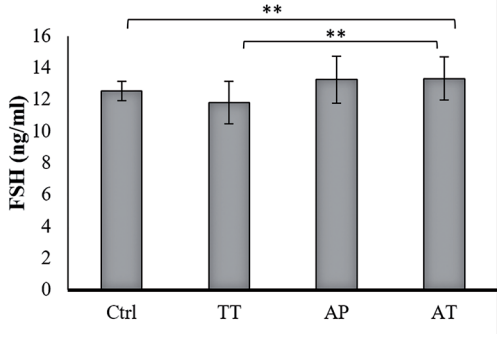
Figure 3- Effect of AP, TT and AT extract on serum level of LH (mean ± SD).



The sera obtained from all groups were analyzed for LH by ELISA using Rat LH Assay kit from BioVendor, Czech Republic. LH levels increased significantly in AT treated group compared to AP, TT and control groups (P=0.000, P=0.000 and P0.000, respectively); it was also significant, in lower scales, in TT and AP groups in comparison to control group (P=0.002 and P=0.03 respectively).

Ctrl=control group, **TT=***Tribulus terrestris*, **AP=***Anacyclus pyrethrum*, **AT=***Anacyclus pyrethrum* and *Tribulus terrestris*. *P <0.05, ** P < 0.01, ***p<0.001

Figure 4- Effect of AP, TT and AT extract on serum level of FSH (mean ± SD).



The sera obtained from all groups were analyzed for FSH by ELISA using Rat FSH Assay kit from BioVendor, Czech Republic. FSH level was significant only in AT group compared to control group and TT group (P=0.000, P=0.000, respectively).

Ctrl=control group, **TT=***Tribulus terrestris*, **AP=***Anacyclus pyrethrum*, **AT=***Anacyclus pyrethrum* and *Tribulus terrestris*. *P <0.05, ** P < 0.01, ***p<0.001

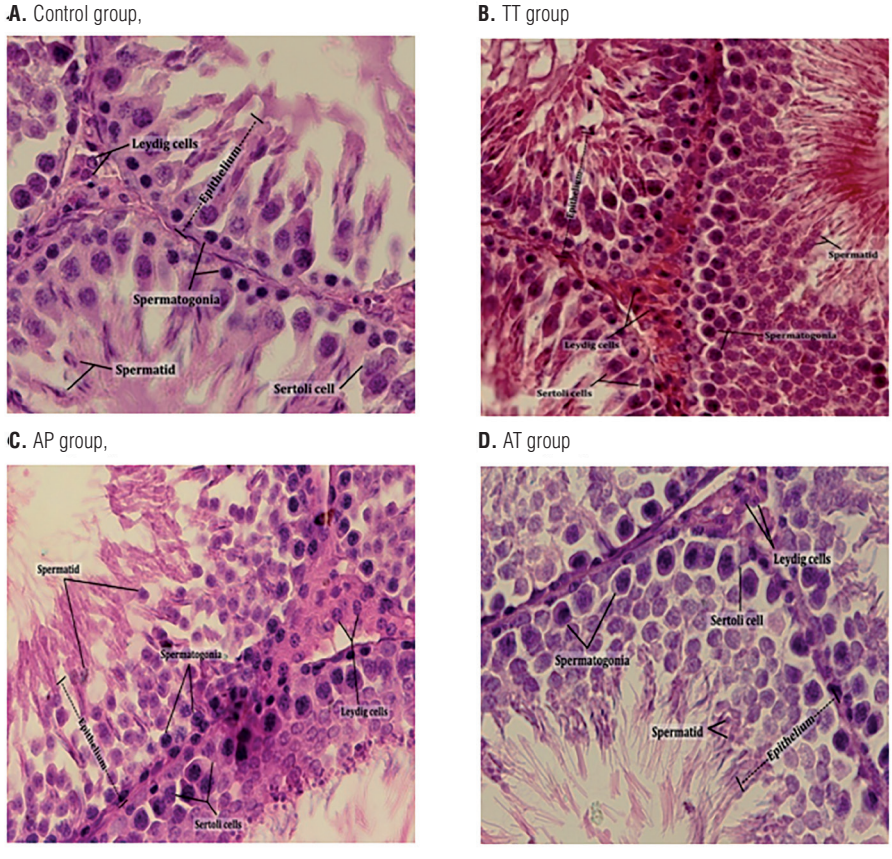
Histology

Seminiferous epithelium thickness significantly increased in AT group compared to control group (P=0.04). Leydig, Sertoli, spermatid and spermatogonia cell numbers elevated significantly in AT group compared to all other groups. Seminiferous diameter did not show any significant changes among all groups (Figure 5 and Table 2).

DISCUSSION

The present study demonstrates effects of TT and AP either separately or together, on reproductive parameters of male Wistar rats. Measured sperm parameters, sperm count, mobility and viability, in AT and AP treatment group compared to controls were significantly influenced. Also, TT treatment alone showed effects on sperm count, which is consistent with previous studies (18, 28). Cell numbers and properties related to sexual Wistar rat organs were analyzed. Seminiferous diameter, epithelium thickness, Sertoli, Leydig, spermatid and spermatogonia cell number were significantly increased in AT group compared to controls. However, treatment with either TT or AP just effected on Leydig and spermatogonia cell numbers significantly. Hormonal level of testosterone, LH and FSH showed considerable increase in AT group

Figure 5 - Comparative H&E staining of seminiferous epithelium demonstrated increased thickness and Leydig, Sertoli, spermatid and spermatogonia cell numbers in AT group.



H&E staining of seminiferous epithelium from all groups was performed. Left testis from each group, collected on day 25, fixed in Bouin's fixative and embedded in paraffin. Five µm sections from different regions of testis from each of the groups were stained with H&E. Epithelium thickness, seminiferous tubule's diameter, number of Leydig and Sertoli cells, number of spermatids and spermatogonia were assessed under a light microscope. Seminiferous epithelium thickness significantly increased in AT group compared to control group. Leydig, Sertoli, spermatid and spermatogonia cell numbers elevated significantly in AT group compared to all other groups. Seminiferous diameter did not show any significant changes among all groups (Additional data have been shown in Table 2).

Table 2 - Seminiferous diameter, epithelium thickness and Sertoli, Leydig, spermatid, spermatogonia cell numbers taken in this study (mean ± SD)

Parameters	Mean ± SD	Range	P Value
Seminiferous diameter (µm)			
Control Group (n=8)	24.27 ± 0.61	23.66-24.88	
TT Group (n=8)	24.35 ± 1.50	22.85-25.85	0.89
AP Group (n=8)	23.10 ± 0.94	22.16-24.04	0.06
AT Group (n=8)	23.00 ± 1.58*	21.42-24.58	0.04
Epithelium thickness (µm)			
Control Group (n=8)	0.67 ± 0.04	0.63-0.71	
TT Group (n=8)	0.73 ± 0.9	0.17-1.63	0.13
AP Group (n=8)	0.66 ± 0.3	0.36-0.96	0.67
AT Group (n=8)	0.75 ± 0.10*	0.65-0.85	0.04
Sertoli cell number			
Control Group (n=8)	19.65 ± 2.12	17.53-21.77	
TT Group (n=8)	20.63 ± 2.39	18.24-23.02	0.36
AP Group (n=8)	19.50 ± 2.20	17.3-21.7	0.87
AT Group (n=8)	33.88 ± 4.39*	29.49-38.27	0.00
Leydig cell number			
Control Group (n=8)	12.00 ± 0.53	11.47-12.53	
TT Group (n=8)	14.25 ± 1.49*	12.76-15.74	0.00
AP Group (n=8)	13.88 ± 0.83*	13.05-14.71	0.01
AT Group (n=8)	18.75 ± 1.98*	16.77-20.73	0.00
Spermatid cell number			
Control Group (n=8)	52.13 ± 7.49	44.64-59.62	
TT Group (n=8)	56.87 ± 6.51	50.36-63.38	0.30
AP Group (n=8)	62.50 ± 5.35*	57.15-67.85	0.03
AT Group (n=8)	70 ± 13.89*	56.11-83.89	0.00
Spermatogonia cell number			
Control Group (n=8)	113.00 ± 19.49	93.51-132.49	
TT Group (n=8)	134.50 ± 14.15*	120.35-148.65	0.01
AP Group (n=8)	135.87 ± 3.74*	132.13-139.61	0.01
AT Group (n=8)	220.00 ± 17.42*	202.58-237.42	0.00

ANOVA followed by Fischer`s LSD multiple comparison test. Data are presented as mean ± SD

* Significant results

Ctrl=control group, **TT=***Tribulus terrestris*, **AP=***Anacyclus pyrethrum*, **AT=***Anacyclus pyrethrum* and *Tribulus terrestris.*

compared to other groups except FSH level in AT group compared to AP group. The weight of prostate and testes were not significantly altered in none of the groups; nevertheless, prostate had a mild increase of weight in all treated groups. Our results for body weight were consistent with the study performed by Sujith et al. on the toxicity of AP in Wistar rats, and inconsistent with Sharma et al. study on the aphrodisiac effects of AP on male rats (28, 29).

A traditional Iranian, northern Khorasan, remedy for enhancing male sexual activity is consumption of a mixture of both TT flower and AP root. TT active components are furostanol saponins called protodioscin and protogracilin, which are responsible for the TT reported biological activities, they up-regulate testosterone and LH and increase libido and spermatogenesis (29-32). It is believed that protodioscin is capable of being converted to dehydroepiandrosterone (DHEA) (13). It is also postulated that TT might increase DHEA levels due to elevating the cAMP level in adrenals (26). The reported main active components of the root of AP are anacyclin, pellitorine, hydrocarolin, inulin and traces of volatile oil and seasamin. To our knowledge, no study has been conducted on its components effects on male fertility (28).

Sperm count, motile sperm count and normal sperm morphology have been reported as indices of male fertility (33, 34). Steroidogenesis and spermatogenesis are two major functions of testis, which are the results of coordination between various cell types, including Sertoli, Leydig and germ cells (35, 36). Hypothalamus-pituitary--gonadal axis increases Leydig cell numbers and stimulates their testosterone production through up-regulating LH; on the other hand, the axis is important for Sertoli cell function and its number (16, 36). Sertoli cells encompass different germ cells which are distributed in seminiferous epithelium, where multiple germ cells are in contact with a single Sertoli cell (36, 37). Testosterone, LH and FSH, are three hormones related to main role in sexual activity. LH stimulates Leydig cells to produce androgen; it also increases Leydig cell number in testis (38). Testosterone regulates the spermatogenesis through phosphorylation of cAMP response element-binding protein (CREB) and its increase has a pivotal role in sperm quantity and quality (8, 39). FSH also triggers the phosphorylation of CREB (40, 41). It has been shown that the presence of mutant CREB in testis of rat causes over 42±5.8% of the seminiferous tubules to have disrupted spermatogenesis, due to apoptosis, causing the loss of 75% of spermatids (10). This proves the important role of phosphorylation of CREB. Testosterone and FSH share similar final activities in different ways, and interestingly testosterone is twofold less efficient in phosphorylating CREB (42). Perhaps, that is why the presence of FSH is necessary for full fertility (43). FSH also causes higher glucose uptake (44, 45) and controls the synthesis of major fuel used by germ cells. FSH prevents apoptosis in spermatogonia and spermatocytes; thus, their viability increases (7, 10). Testosterone induces spermatogenesis and can potentially improve spermatogenesis, if injected exogenously. However, what is really needed for spermatogenesis is intratesticular testosterone; moreover, exogenous testosterone suppresses GnRH, leading to reduced LH, and consequently lower testosterone production by Leydig cells, i.e lower intratesticular testosterone (46, 47).

In our study, the number of Leydig cells increased significantly in all treated groups coordinately with the LH serum level. However, the number of Sertoli cells was elevated significantly only in AT group compared to all other groups, which might be a confirmation for simultaneous increase of serum LH, FSH and testosterone levels compared to other studied groups. Spermatid and spermatogonia numbers increased significantly in all three groups, which might be due to higher testosterone and LH levels; however, their number in AT group was significantly higher than other groups, which can be due to the increased number of Sertoli cells and higher sexual hormonal levels in this group.

In conclusion, TT and AP have positive effects on sexual parameters and sexual hormonal levels in male rats. However, the mixture of AP and TT, as prescribed in traditional medicine of northern Khorasan has considerably remarkable effects on sexual parameters of Wistar rats including enhanced sperm quality, sexual hor-

monal levels and histoarchitecture of male Wistar rats. The extracts seem to have combined effects. Thus, further studies should be performed on different doses and also to find active agents and their effective combinations. Both TT and AP have been reported to be non-toxic and have no side effects; accordingly, they are safe choices for drug purposes. These findings and future studies based on these results can lead to new drugs for male sexual dysfunction.

ACKNOWLEDGEMENT

The authors would like to thank the authorities in research council of Mashhad University of Medical Sciences (MUMS) for their financial support grant number (900141).

Sincere thanks go to Mr. Zahedi and Mr. Naseri for the generous contribution to histological data analysis.

Sincere thanks go to Dr. Ehsan Farashahi Yazd, assistant professor of Shahid Sadoughi University of medical sciences of Yazd, for the generous contribution to data analysis and article revision.

Dariush Haghmorad and Mohammad Bagher Mahmoudi contributed equally this work.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Olayemi FO. A review on some causes of male infertility. African Journal of Biotechnology. 2010;9:2834-42.
2. Hafeez M, Ahmed A, Usmanghani K, Mohiuddin E, Asif HM, Akram M, et al. Clinical Evaluation of Herbal Medicine for Oligospermia. Pakistan Journal of Nutrition 2011;10:238-40.
3. Nantia EA, Moundipa PF, Monsees TK, Carreau S. Medicinal plants as potential male anti-infertility agents:a review. Androl. 2009;19:148-58.
4. Shefi S, Turek PJ. Definition and current evaluation of subfertile men. Int Braz J Urol. 2006;32:385-97.
5. Dada R, Kumar M, Jesudasan R, Fernández JL, Gosálvez J, Agarwal A. Epigenetics and its role in male infertility. J Assist Reprod Genet. 2012;29:213-23.
6. Meri ZB, Irshid IB, Migdadi M, Irshid AB, Mhanna SA. Does cigarette smoking affect seminal fluid parameters? A comparative study. Oman Med J. 2013;28:12-5.
7. Walker WH, Cheng J. FSH and testosterone signaling in Sertoli cells. Reproduction. 2005;130:15-28.
8. Walker WH. Molecular mechanisms of testosterone action in spermatogenesis. Steroids. 2009;74:602-7.
9. McLachlan RI, O'Donnell L, Meachem SJ, Stanton PG, de K, Pratis K, et al. Hormonal regulation of spermatogenesis in primates and man: insights for development of the male hormonal contraceptive. J Androl. 2002;23:149-62.
10. Scobey M, Bertera S, Somers J, Watkins S, Zeleznik A, Walker W. Delivery of a cyclic adenosine 3',5'-monophosphate response element-binding protein (creb) mutant to seminiferous tubules results in impaired spermatogenesis. Endocrinology. 2001;142:948-54.
11. Low WY, Tan HM. Asian traditional medicine for erectile dysfunction. The Journal of Men's Health & Gender. 2007;4(3):245-50.
12. Madani T, Jahangiri N, Eftekhari-Yazdi P, Ashrafi M, Akhoond M. Is Coasting Valuable in All Patients with Any Cause of Infertility? Oman Med J. 2016;31:404-8.
13. Bashir A, Tahir M, Samee W, Munir B. Effects of Tribulus Terrestris on testicular development of immature albino rats. Biomedica. 2009;25:63-8.
14. Khaleghi Ghadiri M, Gorji A. Natural remedies for impotence in medieval Persia. Int J Impot Res. 2004;16:80-3.
15. Ahmed KA, Venkataraman BV. Assessment of a Polyherbal Ayurvedic Medicine for Sexual Activity in Rats. Indian Drugs. 1999;36:576-82.
16. Sharma V, Boonen J, Spiegeleer BD, Dixit VK. Androgenic and spermatogenic activity of alkylamide-rich ethanol solution extract of Anacyclus pyrethrum DC. Phytother Res. 2013;27:99-106.
17. Sharma V, Thakur M, Chauhan NS, Dixit VK. Immunomodulatory activity of petroleum ether extract of Anacyclus pyrethrum. Pharmaceutical biology. 2010;48:1247-54.
18. Dinchev D, Janda B, Evstatieva L, Oleszek W, Aslani MR, Kostova I. Distribution of steroidal saponins in Tribulus terrestris from different geographical regions. Phytochemistry. 2008;69:176-86.
19. Santos CA Jr, Reis LO, Destro-Saade R, Luiza-Reis A, Fregonesi A. Tribulus terrestris versus placebo in the treatment of erectile dysfunction: A prospective, randomized, double blind study. Actas Urol Esp. 2014;38:244-8.

20. Roaiah MF, Elkhayat YI, Abd El Salam MA, Din SFG. Prospective Analysis on the Effect of Botanical Medicine (Tribulus terrestris) on Serum Testosterone Level and Semen Parameters in Males with Unexplained Infertility. J Diet Suppl. 2017;14:25-31. Erratum in: J Diet Suppl. 2018;15:1014.
21. Martino-Andrade AJ, Morais RN, Spercoski KM, Rossi SC, Vechi MF, Golin M, et al. Effects of Tribulus terrestris on endocrine sensitive organs in male and female Wistar rats. J Ethnopharmacol. 2010;127:165-70.
22. Kamenov Z, Fileva S, Kalinov K, Jannini EA. Evaluation of the efficacy and safety of Tribulus terrestris in male sexual dysfunction-A prospective, randomized, double-blind, placebo-controlled clinical trial. Maturitas. 2017;99:20-6.
23. Pavin NF, Izaguirry AP, Soares MB, Spiazzi CC, Mendez ASL, Leivas FG, et al. Tribulus terrestris Protects against Male Reproductive Damage Induced by Cyclophosphamide in Mice. Oxid Med Cell Longev. 2018;2018:5758191.
24. Salgado R, MarquesiSilva M, Gonçalves E, Mathias A, Aguiar J, Wolff P. Effect of oral administration of Tribulus terrestris extract on semen quality and body fat index of infertile men. Andrologia. 2017;49:e12655.
25. Gauthaman K, Adaikan PG, Prasad RN. Aphrodisiac properties of Tribulus Terrestris extract (Protodioscin) in normal and castrated rats. Life Sci. 2002;71:1385-96.
26. Gauthaman K, Ganesan AP. The hormonal effects of Tribulus terrestris and its role in the management of male erectile dysfunction--an evaluation using primates, rabbit and rat. Phytomedicine. 2008;15:44-54.
27. Patel D, Kumar R, Prasad S, Hemalatha S. Pharmacologically screened aphrodisiac plant-A review of current scientific literature. Asian Pacific Journal of Tropical Biomedicine. 2011;1:131-8.
28. Sujith K, Darwin R, Suba V. Toxicological evaluation of ethanolic extract of Anacyclus pyrethrum in albino wistar rats Asian Pacific Journal of Tropical Disease. 2012;2:437-41.
29. Kostova, D. Dinchev. Saponins in Tribulus terrestris – Chemistry and Bioactivity. Phytochemistry 2005, 4, 111–137.
30. Elahi RK, Asl S, Shahian F. Study on the Effects of Various Doses of Tribulus Terrestris Extract on Epididymal Sperm Morphology and Count in Rat. Global Veterinaria. 2013;10(1):13-7.
31. Singh S, Nair V, Gupta YK. Evaluation of the aphrodisiac activity of Tribulus terrestris Linn. in sexually sluggish male albino rats. Journal of pharmacology & pharmacotherapeutics. 2012;3:43-7.
32. Amin A, Lotfy M, Shafiullah M, Adeghate E. The protective effect of Tribulus terrestris in diabetes. Ann N Y Acad Sci. 2006;1084:391-401.
33. Smith KD, Rodriguez-Rigau LJ, Steinberger E. Relation between indices of semen analysis and pregnancy rate in infertile couples. Fertil Steril. 1977;28:1314-9.
34. Almabhouh FA, Osman K, Siti Fatimah I, Sergey G, Gnanou J, Singh HJ. Effects of leptin on sperm count and morphology in Sprague-Dawley rats and their reversibility following a 6-week recovery period. Andrologia. 2015;47:751-8.
35. Fouad AA, Jresat I. Thymoquinone therapy abrogates toxic effect of cadmium on rat testes. Andrologia. 2015;47:417-26.
36. Alves MG, Rato L, Carvalho RA, Moreira PI, Socorro S, Oliveira PF. Hormonal control of Sertoli cell metabolism regulates spermatogenesis. Cell Mol Life Sci. 2013;70:777-93.
37. Vijaya Bharathi B, Jaya Prakash G, Krishna KM, Ravi Krishna CH, Sivanarayana T, Madan K, Rama Raju GA, et al. Protective effect of alpha glucosyl hesperidin (G-hesperidin) on chronic vanadium induced testicular toxicity and sperm nuclear DNA damage in male Sprague Dawley rats. Andrologia. 2015;47:568-78.
38. Shalet SM. Normal testicular function and spermatogenesis. Pediatr Blood Cancer. 2009;53:285-8.
39. Solomon MC, Erasmus N, Henkel RR. In vivo effects of Eurycoma longifolia Jack (Tongkat Ali) extract on reproductive functions in the rat. Andrologia. 2014;46:339-48.
40. Spruill WA, Zysk JR, Tres LL, Kierszenbaum AL. Calcium/calmodulin-dependent hosphorylation of vimentin in rat sertoli cells. Proc Natl Acad Sci U S A. 1983;80:760-4.
41. Wu GY, Deisseroth K, Tsien RW. Activity-dependent CREB phosphorylation: convergence of a fast, sensitive calmodulin kinase pathway and a slow, less sensitive mitogen-activated protein kinase pathway. Proc Natl Acad Sci U S A. 2001;98:2808-13.
42. Haywood M, Spaliviero J, Jimemez M, King NJ, Handelsman DJ, Allan CM. Sertoli and germ cell development in hypogonadal (hpg) mice expressing transgenic follicle-stimulating hormone alone or in combination with testosterone. Endocrinology. 2003;144:509-17.
43. Simoni M, Weinbauer GF, Gromoll J, Nieschlag E. Role of FSH in male gonadal function. Ann Endocrinol (Paris). 1999;60:102-6.

44. Meroni SB, Riera MF, Pellizzari EH, Cigorraga SB. Regulation of rat Sertoli cell function by FSH: possible role of phosphatidylinositol 3-kinase/protein kinase B pathway. J Endocrinol. 2002;174:195-204.
45. Nechamen CA, Thomas RM, Cohen BD, Acevedo G, Poulikakos PI, Testa JR, et al. Human follicle-stimulating hormone (FSH) receptor interacts with the adaptor protein APPL1 in HEK 293 cells: potential involvement of the PI3K pathway in FSH signaling. Biol Reprod. 2004;71:629-36.
46. Dohle GR, Smit M, Weber RF. Androgens and male fertility. World J Urol. 2003;21:341-5.
47. Devaangam SS; Satyanarayana S; Eswar Kumar K; Vivek B; Velmurugan C, Kumar A. The effect of amantadine on clomipramine induced sexual dysfunction in male rats. Oman Med J. 2011;26:404-9.

Correspondence address:
Mahmoud Mahmoudi, MD
Immunology Research Center,
BuAli Research Institute,
Department of Immunology and Allergy,
School of Medicine,
Mashhad University of Medical Sciences, Mashhad, Iran
Fax: +98 511 711-2596
E-Mail: mahmoudim@mums.ac.ir

EDITORIAL COMMENT

Vol. 45 (5): 1055-1056, September - October, 2019
doi: 10.1590/S1677-5538.IBJU.2018.0843.1

Editorial Comment: Improvement of fertility parameters with Tribulus Terrestris and Anacyclus Pyrethrum treatment in male rats

Diogo Benchimol de Souza [1], Gabriela Faria Buys-Gonçalves [1]

[1] *Unidade de Pesquisa Urogenital Universidade Estadual do Rio de Janeiro - Uerj, Rio de Janeiro, RJ, Brasil*

Herbal medicine is as old as the history of mankind, and is still a topic of interest in current days. The article of Haghmorad et al. (1) reports promising results with two herbal extracts for improving fertility parameters. Both herbs showed positive results when used individually, but (what was more interesting) a synergic effect seems to occur when used together. The extract of *Tribulus terrestris* were more prominent in raising LH and Testosterone levels (which was already reported (2,3)) while *Anacyclus pyrethrum* showed more impressive results in raising FSH and improving sperm parameters. Thus, the combined use may improve fertility parameters by two different endocrine ways. One limitation not raised by the authors is that the extracts improved fertility parameters in control animals, in which a normal testicle, hypothalamus-pituitary-gonadal axis and fertility parameters are assumed. Future studies investigating if these herbal extracts can also improve fertility parameters in infertile/subfertile models are warranted.

The mechanisms of action of these phytotherapics are poorly understood, especially for the less-studied Anacyclus pyrethrum. This herb has been proposed for different conditions (from local anesthetic to anticancer (4,5)), although no clinical study was conducted focusing on male reproductive or endocrine systems. It seems that most phytotherapeutic study focuses only on the final specific effects, putting aside the search for knowledge on the basic mechanisms of the extracts.

Since the ancient Greece Hippocrates advocated the principle of *primum non nocere* which should be always applied when proposing any therapy, including herbal therapies. When studying any treatment for a specific condition, is important to have a more global perspective, evaluating potential side-effects of the proposed medication. Specifically, for *Tribulus terrestris*, our group recently showed this herb leads to arterial blood pressure increase and renal morphology alteration with glomerular loss (6). This kind of study may add information for the physician, helping evaluating the pros and cons of each prescription for each patient.

REFERENCES

1. Haghmorad D, Mahmoudi MB, Haghighi P, Alidadiani P, Shahvazian E, Tavasolian P, et al. Improvement of fertility parameters with Tribulus terrestris and Anacyclus Pyrethrum treatment inmale rats. Int Braz J Urol. 2019;45:1043-54.
2. Salgado RM, Marques-Silva MH, Gonçalves E, Mathias AC, Aguiar JG, Wolff P. Effect of oral administration of Tribulus terrestris extract on semen quality and body fat index of infertile men. Andrologia. 2017;49. [Ahead of Print]

3. Ghosian Moghaddam MH, Khalili M, Maleki M, Ahmad Abadi ME. The Effect of Oral Feeding of Tribulus terrestris L. on Sex Hormone and Gonadotropin Levels in Addicted Male Rats. Int J Fertil Steril. 2013;7:57-62.
4. Jalayer-Naderi N, Niakan M, Khodadadi E, Mohamadi-Motlagh M. The antibacterial activity of methanolic Anacyclus pyrethrum and Pistacia lentiscus L. extract on Escherichia coli. Iran J Microbiol. 2016;8:372-6.
5. Mohammadi A, Mansoori B, Baradaran PC, Baradaran SC, Baradaran B. Anacyclus Pyrethrum Extract Exerts Anticancer Activities on the Human Colorectal Cancer Cell Line (HCT) by Targeting Apoptosis, Metastasis and Cell Cycle Arrest. J Gastrointest Cancer. 2017;48:333-40.
6. Gonçalves G, Da Silva M, Ferraz V, Sampaio F De Souza, D. Histomorphometric evaluation of normotensive and hypertensive rats' kidney treated with Tribulus terrestris. European Urology Supplements. 2018; 17: e1256

Correspondence address:
Diogo Benchimol De Souza, MD
Unidade de Pesquisa Urogenital
Univ. Estadual do Rio de Janeiro - Uerj,
Rio de Janeiro, RJ, Brasil
Fax: +55 21 3872-8802
E-mail: diogobenchimol@gmail.com

ARTICLE INFO

Diogo de Souza
http://orcid.org/0000-0003-3456-5029
Int Braz J Urol. 2019; 45: 1055-6

SURGICAL TECHNIQUE

Vol. 45 (5): 1057-1063, September - October, 2019
doi: 10.1590/S1677-5538.IBJU.2018.0652

Penile skin flap: a versatile substitute for anterior urethral stricture

Wissem Hmida [1], Mouna Ben Othmen [1], Amidou Bako [1], Mehdi Jaidane [1], Faouzi Mosbah [1]

[1] *Department of Urology, Sahloul Hospital Sousse, Sousse, Tunísia*

ABSTRACT

Purpose: Penile skin flap uretroplasty is a useful technique for a long urethral stricture due to the ample length and surgical handling characteristics. We investigated the surgical technique and initial results of uretroplasty for anterior urethral strictures using a dorsal penile skin flap.

Patients and methods: From January 2003 to January 2018, a total of 77 patients underwent substitution urethroplasty using dorsal penile skin flap for bulbar urethral strictures in our institution. All patients were assessed preoperatively, and followed postoperatively by physical examination, urinalysis, retrograde and voiding urethrography, uroflowmetry and post-void residual urine measurement. Success was defined as no requirement of additional urethral instrumentation.

Results: The mean age was 45 years (10-87). The mean stricture length was 5cm (3-10cm). The mean flap length was 6cm. Urinary fistula was the most common postoperative complication. The mean follow-up was 60 months (6-120). The overall success rate was 88%. Recurrent strictures were found in 4 patients (5%) at 1 year. At 3 year follow-up, 5 (7%) more patients had recurrences. All recurrences were managed by internal urethrotomy.

Conclusions: Substitution urethroplasty using penile skin flap appear to be a safe and efficient technique for the treatment of a long and complex anterior urethral stricture. It provides encouraging cosmetic and functional results.

ARTICLE INFO

Mouna Ben Othmen
https://orcid.org/0000-0002-2789-4206

Keywords:
Penis; Urethral Stricture; Bulbourethral Glands

Int Braz J Urol. 2019; 45: 1057-63

Submitted for publication:
September 16, 2018

Accepted after revision:
January 26, 2019

Published as Ahead of Print:
March 30, 2019

INTRODUCTION

Urethral stricture disease is a heterogeneous condition that often requires a wide array of surgical techniques for a successful repair. Treatment options include dilation, urethrotomy and reconstructive surgical techniques (1).

As clearly evident in the literature, isolated and short bulbourethral strictures inferior to 1.5-2cm are treated by excision and anastomotic repair. On the other hand, complex anterior urethral reconstruction relies on a tissue transfer technique in the form of either a free graft and/or pedicled flap (2). The challenge lies in choosing the appropriate technique for a particular stricture (3, 4). Both are successful individually (5, 6). Penile skin flap can be used in long anterior urethral strictures. Its adaptability comes from its mobile, well-vascularized pedicle and elastic skin island that can be used from the membranous urethra to the fossa navicularis.

In the current era of buccal mucosa, now considered the donor substitute of choice for aug-

mentation, the success of the penile skin flaps in the management of anterior urethral strictures should be reassessed.

The objective of this retrospective study is to describe surgical technique, indications and initial results with dorsal penile skin flap for anterior urethral strictures.

PATIENTS AND METHODS

Patients

After obtaining approval from our ethical committee (n° 212/18), we retrospectively identified a total of 77 patients (from January 2003 to January 2018) who underwent urethroplasty using dorsal penile skin flap for bulbar urethral strictures in our institution. Patients with lichen sclerosus and failed hypospadias repair were excluded.

All patients were evaluated preoperatively by physical examination, urinalysis, uroflowmetry and retrograde and voiding urethrography. In all patients, a dorsal onlay flap urethroplasty was performed using penile skin flap. Success and failure was defined respectively by the absence of or the need for any subsequent urethral procedure (dilation, internal optical urethrotomy or repeat open urethroplasty). Postoperative complications were recorded and classified as early (onset: 30d) or late (onset: > 90d) complications, depending on the date of onset. They were also graded according to the modified Clavien system (7).

All patients were followed at 1 month postoperatively, followed by 3-month intervals for the first year and annually thereafter. Follow-up consisted of physical examination, urinalysis and uroflowmetry. In case of suspicion of recurrence (clinically or on uroflowmetry: Qmax < 15mL/s), a retrograde urethrography or urethroscopy was done. Statistical analysis was conducted using chi-square and Student t tests.

Surgical technique

The operation was made under spinal anaesthesia. The patient is placed in the lithotomy position. A foley catheter is inserted through the urethra until the stricture. A midline perineal incision is made. The urethra is completely mobilized from the corpora cavernosa. Next, it is rotated 180 degrees, and is incised along its dorsal surface. The stricture is opened along its whole length.

For all patients we use dorsal penile skin for urethral reconstruction. The flap width is carefully measured using a surgical skin marker (Figure-1A). In order to avoid redundancy and succulations, the flap width should not exceed 20mm. The flap length is measured using the current formula: L = US (urethral stricture) + (US*0.2) (8). Then, the flap is raised (Figure-1B). The pedicle is mobilized proximally to an extent that allows ventral transposition of the flap without tension (Figure-1C). The dorsal penile neurovascular complex and tunica albuginea are exposed and preserved immediately beneath the plane of dissection. The superficial lamina of Buck's fascia is elevated with the pedicle flap, thereby supplying its foundation (Figure-1D).

The flap is passed through a scrotal tunnel to the bulb without torsion and without placing excessive tension on the pedicle (Figure-2A). It is then brought on to the exposed dorsally opened portion of the urethra and sutured to adjoining edges of the urethra using continuous fine sutures over a 16-F Foley catheter (Figures 2B-D). Foley catheter and suprapubic catheter, if placed preoperatively, are left indwelling for 14 weeks. A suction drain is placed on the contra lateral side of the pedicle and removed on postoperative day 2. A retrograde and voiding urethrography is performed at the time of catheter removal in all patients. Any extravasation is managed by extending the period of catheterization.

RESULTS

The mean age was 45 years (10-87). The etiology of stricture was infectious in 40 (52%), traumatic in 28 (36%) and iatrogenic in 9 (12%). Only 8 (11%) are primary, 69 patients (89%) had a history of urethral intervention in the form of dilatations, internal urethrotomy (IU) or urethroplasty (Table-1). The stricture was located at the bulbar urethra in 70 cases (90%), perineal urethra in 5 cases (7%) and at the penile urethra in 2 cases (3%). The mean stricture length was 5cm (3-10cm). The mean flap length was 6cm (4-10cm). Urinary fistula was the most

Figure 1A - The flap width is carefully measured using a surgical skin marker; Figure 1B - The flap is raised; Figure 1C - The flap is dissected to an extent; Figure 1D - Final aspect of the flap.

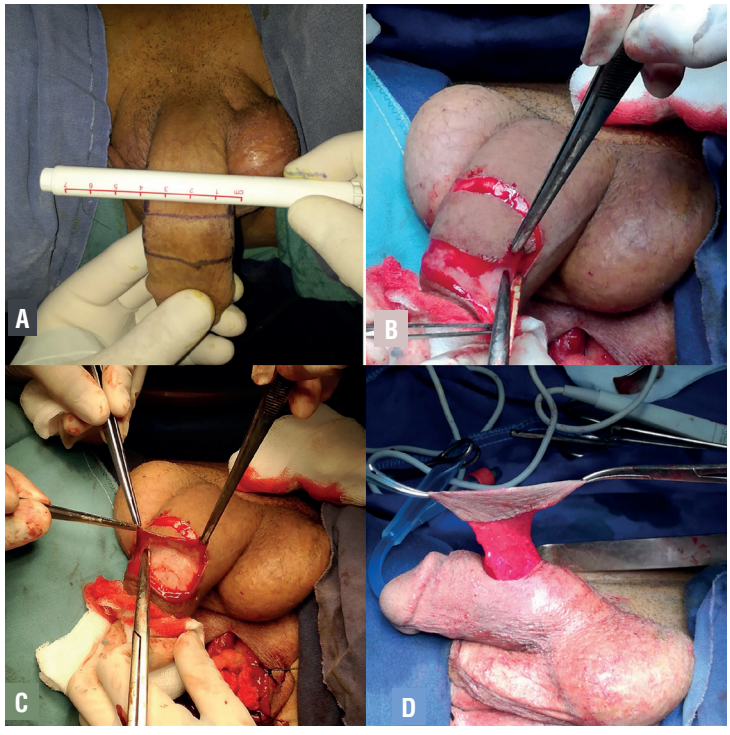
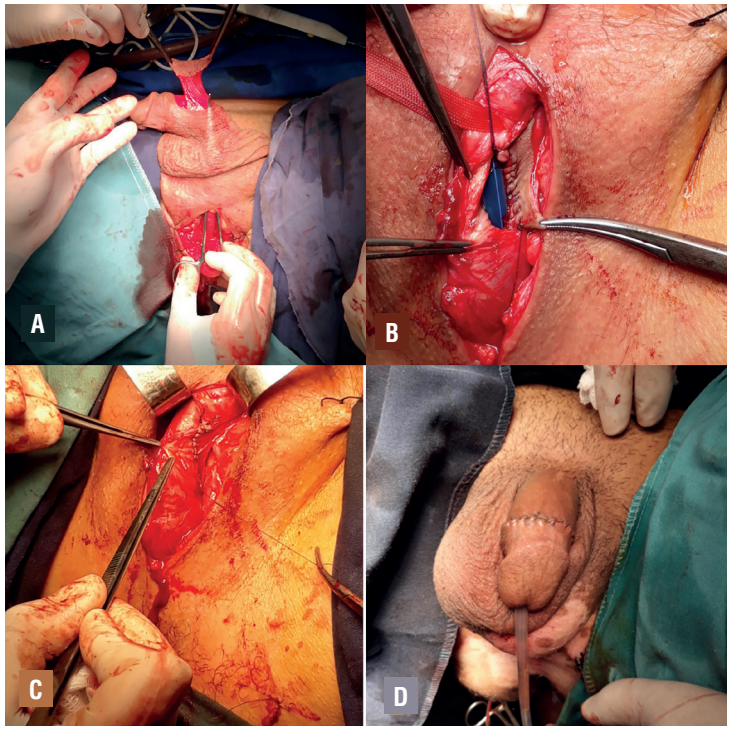


Figure 2A - The flap is passed through a scrotal tunnel to the bulb without torsion and without placing excessive tension on the pedicle; Figure 2B - The flap is sutured to the adjoining edges of urethra; Figure 2C - Final aspect of urethra, Figure 2D - Final aspect of the penis.



common postoperative complication. Postoperative complications are listed in Table-2.

The mean follow-up was 60 months (6-120). Recurrent strictures were found in 4 patients (5%) at 1 year. At 3 year follow-up, 5 (7%) more patients had recurrences. All recurrences were managed by internal urethrotomy. The overall success rate was 88%.

Table 1 - Preoperative parameters.

Parameters	Value
Age (y), mean	45 (10- 87)
Procedures performed previously, n (%)	69 (89%)
Internal urethrotomy	50 (65%)
Urethroplasty	15 (19%)
Urethral calibration	4 (5 %)
Etiology, n (%)	
Infectious	40 (52%)
Traumatic	28 (36%)
Iatrogenic	9 (12%)
Stricture location, n (%)	
Bulbar	70 (90%)
Perineal	5 (7%)
Penile	2 (3%)
Stricture (cm), mean	5 (3-10)

DISCUSSION

No single approach is appropriate for all urethral strictures. Many different reconstructive techniques have been described and are chosen based on the length, location and extent of spongiofibrous tissue contributing to the stricture. While, end to end uretroplasty is appropriate for short stricture with a high success rate, the use of flaps or grafts is mandatory in patients with longer and complex strictures (9, 10).

The controversy over the best means of reconstructing the urethra, using flap or graft, is still under debate. The current literature, however, does not clearly support the use of one technique over the other (11). In the late of 1990s, buccal mucosa became a standard for reconstructing urethral strictures due to its advantageous histological properties and high success rate (12). However, several disadvantages should be considered. It requires the need of an additional operation field and additional specialised nursing and/or surgical personnel for oral graft harvest (13). Moreover, various donor sites related complications are

Table 2 - Operative and follow-up data.

Parameters		Value
Flap length (cm), mean		6 (4-10)
Early Complications, n (%)	Clavien Grade	9 (12%)
Urethrocutaneous fistula	I	4 (5%)
Orchiepididymitis	IIS	2 (3%)
UTI	II	2 (3%)
Hematoma	I	1 (1%)
Recurrence at 1 year, n (%)		4 (5%)
Recurrence at 3 year, n (%)		5 (7%)
The overall success rate, n (%)		68 (88%)

UTI = Urinary tract infection

reported including, oral pain, oral tightness and alterations in saliva production (14, 15).

We observed that many experienced urologists are unfamiliar with the use of penile skin of urethral reconstruction. Although, penile skin flap is our preferred reconstructive technique, because of its excellent cosmetic and functional outcomes.

Penile skin has become a good urethral substitute because of ease of harvest, surgical handling characteristics, hairlessness, and compatibility in a wet environment. On the other hand, it has a flexible tissue with a rich vascular supply allowing to reconstruct long and complex urethral strictures as in our patients.

In our institution, we have adopted this technique for 14 years and we consider that harvesting of penile skin is safe and technically simple for all urologists, as it requires no special experience with oral surgery or knowledge of oral anatomy (13).

Over viewing the published reports, there is strong evidence that substitution urethroplasty using penile skin flaps has acceptable results.

Most authors have reported identical success rates for both buccal mucosa graft and penile skin flaps (16, 17). In a retrospective analysis of 299 patients, Fu Q et al. (18) reported a similar success rate with buccal mucosa (85%) as compared with penile skin flap (83%). Similarly, in a comparative study including 69 patients, the success rate was equal for both buccal mucosa graft (90%) and penile skin flap (84%) (19).

Alsikafı et al. compared the outcome of 95 buccal mucosa urethroplasty and 24 penile skin flap urethroplasty. The overall success rate of penile skin urethroplasty was 84% in a mean follow-up of 201 months (20). Similarly, Dubey et al., reported on 28 patients who underwent longitudinal penile skin flap a success rate of 85%. In this study, the stricture recurrence was described in 4 patients (17). Quartey et al., reported with transverse preputial or penile flap a success rate of 99% (21).

In our study, the overall success rate was 88%. Surgical failure was reported in 12% of our patients, wherein focal recurrence occurred mainly at the anastomotic margin showing results similar to those of previous studies (22, 23).

The best location for placing grafts (ventral or dorsal) remains controversial. Some authors, have suggested that ventral placement of the flap/graft can lead to complications like urethral diverticulum formation and succulations with postvoid dribbling and ejaculatory failure (24, 25). However, some others have reported good long-term stricture-free outcomes equal to dorsal onlay using this technique (6, 26). In our series, the transverse penile skin flaps were fashioned and rotated to be dorsally quilted into the dorsally opened strictured part of the urethra without vascular compromise. Based in our experience, it seems that the outcomes of this technique are encouraging.

A review of literature showed a variability of rate and type of complications reported. The rate of occurrence of urethrocutaneous fistula ranges from 0 to 13% (3, 17, 23). In the present study, only 4 patients (5%) developed urethrocutaneous fistula.

The incidence of pseudodiverticulum formation in flap repair ranges from 0 to 5% in various series (17, 27, 28). In our series, the incidence of pseudodiverticulum formation was 0%. In this respect, dorsal onlay flaps are more advantageous than ventral placement. Necrosis of the penile skin was less reported. It results when the vascular supply of the sub-dermal plexus was compromised. This is an inherent disadvantage of any pedicle penile skin flap, although in experienced hands its incidence is lower (24). In the present study, no case of penile skin necrosis has been reported.

Any kind of substitution urethroplasty deteriorates over time. Long-term results with skin flap urethroplasty show a decreasing success rate with time. Peterson et al. demonstrated in a multicenter study a higher failure rate when longer follow-up was considered (18.4% at 58.8 months). The high rate of recurrence can be attributed to a poor urethral quality. In our series, urethral stricture was reported in 9 patients (12%).

CONCLUSIONS

Substitution urethroplasty using penile skin flap appears to be a safe and efficient technique for the treatment of a long and complex

anterior urethral stricture. It provides encouraging cosmetic and functional results.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Barbagli G, Sansalone S, Djinovic R, Romano G, Lazzeri M. Current controversies in reconstructive surgery of the anterior urethra: a clinical overview. Int Braz J Urol. 2012;38:307-16.
2. Abdel Moneim M. Abuzeid, M. S. Abdel Kader. Reconstruction of Long Anterior Urethral Strictures by Dorsally Quilted Penile Skin Flap.ISRN Urol. 2012; 2012.
3. Bhandari M, Dubey D, Verma BS. Dorsal or ventral placement of the preputial/penile skin onlay flap for anterior urethral strictures:does it make a difference? BJU Int. 2001;88:39-43.
4. Buckley J, McAninch J. Distal penile circular fasciocutaneous flap for complex anterior urethral strictures. BJU Int. 2007;100:221-31.
5. Zinman L. Optimal management of the 3- to 6-centimeter anterior urethral stricture. Curr Urol Rep. 2000;1:180-9.
6. Kane CJ, Tarman GJ, Summerton DJ, Buchmann CE, Ward JF, O'Reilly KJ, et al. Multi-institutional experience with buccal mucosa onlay urethroplasty for bulbar urethral reconstruction. J Urol. 2002;167:1314-7.
7. Clavien PA, Barkun J, de Oliveira ML, Vauthey JN, Dindo D, Schulick RD, et al. The Clavien-Dindo classification of surgical complications: five-year experience. Ann Surg. 2009;250:187-96.
8. Da Silva EA, Sampaio FJ. Urethral extensibility applied to reconstructive surgery. J Urol. 2002;167:2042-5.
9. Kim KR, Suh JG, Paick JS, Kim SW. Surgical outcome of urethroplasty using penile circular fasciocutaneous flap for anterior urethral stricture. World J Mens Health. 2014;32:87-92.
10. Peterson AC, Webster GD. Management of urethral stricture disease: developing options for surgical intervention. BJU Int. 2004;94:971-6.
11. Andrich DE, Mundy AR. What is the best technique for urethroplasty? Eur Urol. 2008;54:1031-41.
12. Lumen N, Oosterlinck W, Hoebeke P. Urethral reconstruction using buccal mucosa or penile skin grafts: systematic review and meta-analysis. Urol Int. 2012;89:387-94.
13. Hudak SJ, Hudson TC, Morey AF. 'Minipatch' penile skin graft urethroplasty in the era of buccal mucosal grafting. Arab J Urol. 2012;10:378-81.
14. Dublin N, Stewart LH. Oral complications after buccal mucosal graft harvest for urethroplasty. BJU Int. 2004;94:867-9.
15. Jang TL, Erickson B, Medendorp A, Gonzalez CM. Comparison of donor site intraoral morbidity after mucosal graft harvesting for urethral reconstruction. Urology. 2005;66:716-20.
16. Soliman MG, Abo Farha M, El Abd AS, Abdel Hameed H, El Gamal S. Dorsal onlay urethroplasty using buccal mucosa graft versus penile skin flap for management of long anterior urethral strictures: a prospective randomized study. Scand J Urol. 2014;48:466-73.
17. Dubey D, Vijjan V, Kapoor R, Srivastava A, Mandhani A, Kumar A, et al. Dorsal onlay buccal mucosa versus penile skin flap urethroplasty for anterior urethral strictures: results from a randomized prospective trial. J Urol. 2007;178:2466-9.
18. Fu Q, Zhang Y, Zhang J, Xie H, Sa YL, Jin S. Substitution urethroplasty for anterior urethral stricture repair: comparison between lingual mucosa graft and pedicled skin flap. Scand J Urol. 2017;51:479-83.
19. Hussein MM, Almogazy H, Mamdouh A, Farag F, Rashed E, Gamal W, et al. Urethroplasty for treatment of long anterior urethral stricture: buccal mucosa graft versus penile skin graft-does the stricture length matter? Int Urol Nephrol. 2016;48:1831-5.
20. Alsikafi NF, Eisenberg M, McAninch JW. Long-Term outcomes of Penile Skin Graft Versus Buccal Mucosal Graft for Substitution Urethroplasty of the Anterior Urethra. J Urol. 2005;174:173-87.
21. Quartey JK. One-stage penile/preputial cutaneous island flap urethroplasty for urethral stricture: a preliminary report. J Urol. 1983;129:284-7.
22. McAninch JW, Morey AF. Penile circular fasciocutaneous skin flap in 1-stage reconstruction of complex anterior urethral strictures. J Urol. 1998;159:1209-13.
23. Moradi M, Moradi A. Urethroplasty for long anterior urethral strictures: report of long-term results. Urol J. 2006;3:160-4.

24. Srivastava A, Vashishtha S, Singh UP, Srivastava A, Ansari MS, Kapoor R, et al. Preputial/penile skin flap, as a dorsal onlay or tubularized flap: a versatile substitute for complex anterior urethral stricture. BJU Int. 2012;110(11 Pt C):E1101-8.
25. Rogers HS, McNicholas TA, Blandy JP. Long-term results of one-stage scrotal patch urethroplasty. Br J Urol. 1992;69:621-8.
26. Lumen N, Hoebeke P, Oosterlinck W. Urethroplasty for urethral strictures: quality assessment of an in-home algorithm. Int J Urol. 2010;17:167-74.
27. Kessler TM, Schreiter F, Kralidis G, Heitz M, Olianas R, Fisch M. Long-term results of surgery for urethral stricture: a statistical analysis. J Urol. 2003;170:840-4.
28. Xu YM, Qiao Y, Sa YL, Wu DL, Zhang XR, Zhang J, et al. Substitution urethroplasty of complex and long-segment urethral strictures: a rationale for procedure selection. Eur Urol. 2007;51:1093-8.

Correspondence address:
Mouna Ben Othmen, MD
Department of Urology, Sahloul Hospital Sousse
Route de la ceinture, Hammam sousse,
Sousse, Tunisia 4011, Tunisia
Telephone.: +216 9 881-6590
E-mail: benothmouna@gmail.com

CHALLENGING CLINICAL CASES

Vol. 45 (5): 1064-1070, September - October, 2019
doi: 10.1590/S1677-5538.IBJU.2018.0808

Novel homozygous mutation in a colombian patient with persistent müllerian duct syndrome: expanded phenotype

Mary García Acero [1], Olga Moreno [1], Andrés Gutiérrez [2], Catalina Sánchez [2], Juan Guillermo Cataño [2], Fernando Suárez-Obando [1, 3], Adriana Rojas [1]

[1] Human Genetic Institute, Pontificia Universidad Javeriana, Bogotá, Colombia; [2] Department of Urology, Hospital Universitario San Ignacio, Bogotá, Colombia; [3] Genetic Service, Hospital Universitario San Ignacio, Bogotá, Colombia

ABSTRACT

The anti-Müllerian hormone triggers the regression of uterus and fallopian tubes in male embryos; if there are problems in the synthesis or action of this protein, Müllerian structures persist in an otherwise phenotypic male. The most frequent clinical presentation of Persistent Mullerian Duct syndrome is cryptorchidism and inguinal hernia. The few cases reported in adults are incidental findings or inguinal hernias. However, we present an adult male with history of bilateral cryptorchidism with unsuccessful orchidopexy, who presents with a large abdominal mass with the finding of a seminomatous tumor and persistence of Müllerian structures, in whom the variant c.916delC (p.Leu306Cysfs*29) in the AMHR2 gene not previously reported was documented.

ARTICLE INFO

Mary García Acero
https://orcid.org/0000-0002-7109-3342

Keywords:
Mullerian Ducts; Anti-Mullerian Hormone; Persistent Mullerian duct syndrome [Supplementary Concept]; Disorders of Sex Development

Int Braz J Urol. 2019; 45: 1064-70

Submitted for publication:
December 01, 2018

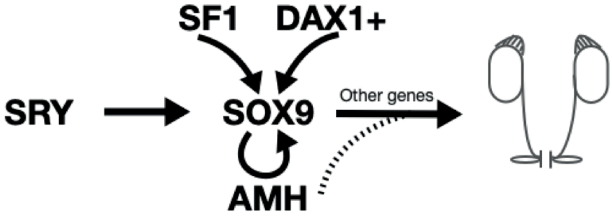
Accepted after revision:
March 17, 2019

Published as Ahead of Print:
May 30, 2019

INTRODUCTION

Male sex determination is a process determined by: 1) testis formation of the primitive gonad (sex determination) through various transcription factors, and 2) differentiation of internal and external genitalia by action of hormones secreted by the fetal testicle (sex differentiation). It starts by activation of SRY gene in the precursor cells and translocate to the nucleus and binds to the enhancer region of SOX9, to intervene in the differentiation and proliferation of Sertoli cells and the tubular organization of the testis that secret anti-Müllerian hormone (AMH) to promote the regression of Müllerian ducts (fallopian tubes, uterus and upper vagina) (Figure-1) (1). Testicular descent

Figure 1 - In normal XY males, the SRY gene begins testicular differentiation by interacting with other genes that determine sex, after which the differentiation and proliferation of Sertoli cells that secrete anti-Müllerian hormone (AMH) promote the regression of the Müllerian ducts and allow the differentiation of the Wolff ducts that will originate the vas deferens, the epididymis, seminal vesicles and ejaculatory ducts. SRY (Sex Determining Region Y), SOX9 (SRY-Box 9), DAX-1 (also know as NR0B1-Nuclear Receptor Subfamily 0 Group B Member 1), AMH (Anti-Mullerian Hormone).



eventually occurs until the end of pregnancy and is both dependent and independent of hormones.

AMH is a glycoprotein secreted by the Sertoli cells of the testes from the moment of sexual differentiation until puberty. The AMH gene, a member of the transforming growth factor-β (TGF-β) is activated by SRY but also by genes upstream and downstream as SOX9, SF-1, WT-1 and GATA415 (2-5). The defects of synthesis and action of AMH also known as Persistent Müllerian Duct Syndrome (PMDS), is an unusual disorder of sex development with autosomal recessive inheritance. In 52% of cases it happens because of mutations in the gene that encodes for anti-Müllerian hormone (AMH) and less frequently for mutations in the AMH receptor, type II (AMHR2) causing hormonal resistance (6). Mutations within these two genes have been detected in 85% of patients with PMDS, with unknown genes in the remaining 15% of cases.

AMHR2 is a membrane protein, which contains a N-terminal extracellular domain that binds AMH, a transmembrane domain, and an intracellular domain with serine/threonine kinase activity. The AMHR2 gene is located at 12q13 and contains 11 exons. The extra cellular domain of AMH binding is encoded by the first 3 exons (5); the exon 4 encodes the transmembrane domain, and the catalytic intracellular serine/threonine domain is encoded by the last 7 exons (7).

The incidence of PMDS is low, with near 200 cases documented in the world. Individuals with PMDS are phenotypical male with karyotype 46, XY with presence of Wolffian and Müllerian structures and normal virilization, consequently with normal external male genitalia. However, some individuals have infertility secondary to prolonged cryptorchidism or anomalies in the communication between the testis and the male ducts (epididymis and upper deferent duct) secondary to presence of Müllerian ducts attachments to testes preventing the normal descent. According to previous case reports, delayed diagnosis at adulthood may cause infertility (5).

The determination of serologic levels of AMH is useful when it is suspected in childhood (undetectable with mutations in AMH or normal--elevated with mutations in AMHR2) (8, 9), with certain limitations in adults because AMH levels are low and cannot discriminate between AMH and AMHR2 mutations, neither in cases of PMDS not associated with mutations in these genes. The most common symptoms of PMDS are inguinal hernia, undescended testis, abdominal mass and less frequently testes tumor (10). Also, Müllerian structures could be discovered as an incidental finding during an abdominal surgery or imaging. The treatment in men with PMDS is still under debate. Previous papers have suggested that surgery should be conducted in separate procedures: first, testis reposition into the scrotum with hernia repair and testis biopsy and second, orchidectomy upon indication for atrophic testis or when orchidopexy cannot be performed (10, 11). As in non-descended gonads, the testes of patients with this syndrome have a high risk of developing malignant tumors, calculated between 15 and 18% (12). Tumors of all types have been reported: embryonal carcinoma, teratomas, embryonal sac tumors (Yolk Sac), choriocarcinomas and seminomas. A total of 30 cases of malignant transformation have been identified in patients with PMDS (13).

Recently, we detected a mutation not previously reported in the AMHR2 gene in a male with congenital cryptorchidism with failed orchidopexy and unsuccessful surveillance who

arrived with an abdominal mass corresponding to a seminomatous tumor.

MATERIALS AND METHODS

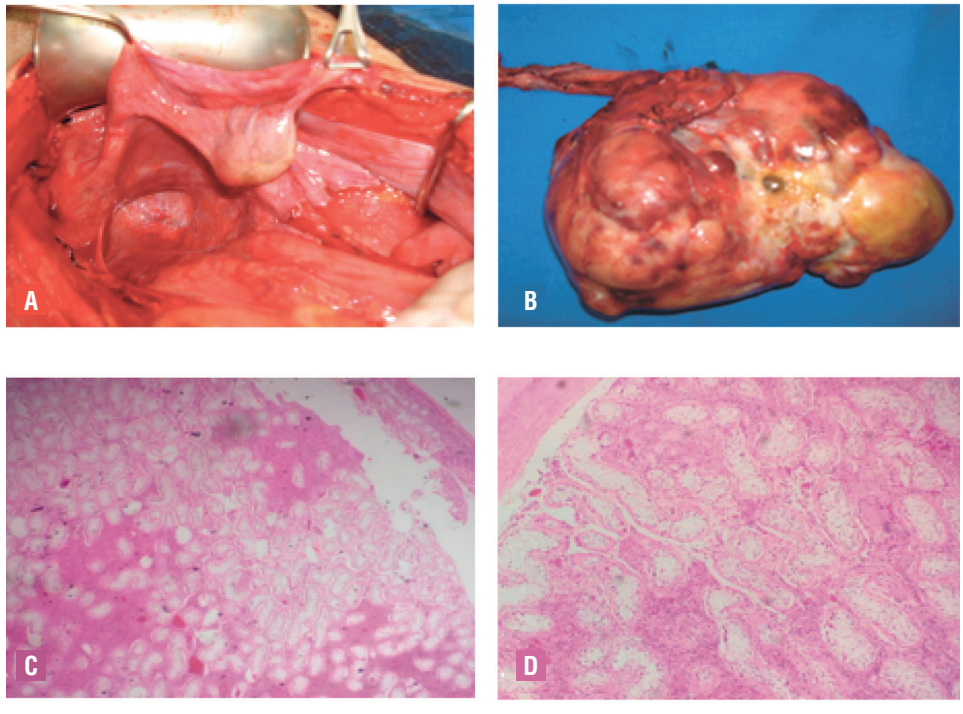
Patient

A Colombian 33-year-old married man, son of consanguineous parents, with history of orchidopexy at 4 years, without follow-up after surgery to evaluate the testicular position, was recently admitted to our clinic with complaints of urinary symptoms. Physical examination showed that the patient was phenotypically male with bilateral cryptorchidism and normal penile length. His secondary sex characteristics were normal but an abdominal mass was palpated in the hypogastrium; therefore, additional tests were done. The patient had a personal history of infertility not studied.

The hormonal evaluation of the patient at the time of consultation revealed hypergonadotropic hypogonadism FSH (2.2mUI/mL-reference: 0.2-1.4), LH (0.08ng/mL-reference: 0.01-0.5), testosterone (0.04ng/mL-reference: 0.01-0.04-: basal without alterations) and AMH: 816pg/L (reference: 360-668pg/L). Other laboratory results showed elevated tumor markers alpha-fetoprotein and human chorionic gonadotropin. The pelvic MRI showed a heterogeneous mass of tumoral aspect in the pelvis, extension to the hypogastrium and retrovesical space, with a volume of 640cc. Features of the mass suggested a mixed lesion with solid and multilocular cystic component, with some hemorrhagic foci, which contacts the dome of the bladder and base of the prostate, displacing the seminal vesicles to the left, with suspicious image of Ewing's sarcoma.

Diagnostic laparotomy and resection of the mass revealed a 20cm multi-loculated mass arising from the left undescended gonad. Right hypotrophic undescended gonad was adhered to Müllerian ducts (Figure-2). Total excision of the mass, uterus and bilateral fallopian tubes gonads was perfor-

Figure 2 - Intraoperative appearance and histopathologic findings.



A) Left intra-abdominal gonad associated with tubular structure reminiscent of uterine horn and broad ligament, **B)** multiloculated mass dependent of left gonadal tissue, **C)** pure seminomatous tumor, and **D)** atrophic tissue of right teste.

med. Pathologic examination revealed pure seminomatous tumor and right atrophic testis (Figure-2).

Sections from both fallopian tubes showed congestion and fibrosis. No ovarian tissue was seen. The patient received multidisciplinary management by endocrinology, urology, genetics, oncology and psychology. His family received genetic counseling for the risk of recurrence and the carrier status.

METHODS

Cytogenetic

Cytogenetic analyses of the patient were carried out based on phytohemaglutinin-stimulated peripheral blood lymphocyte cultures, according to standard laboratory protocols. Chromosome preparations were treated with HCl and stained with Wright for banding G. A total of 50 metaphase cells were analyzed at the 550-band resolution level.

Molecular cytogenetics using fluorescence in situ hybridization (FISH) with the following probes and probe sets was subsequently performed following the manufacturer's instructions: SRY (in Yp11.31; Cytocell-Aquarius, Cambridge, UK), DYZ1 (in Yq12; Cytocell-Aquarius, Cambridge, UK). Also, a control probe for Xp11.1-q11.1 was hybridized. As counterstain 4',6-diamidino-2-phenylindole DAPI was used. 200 metaphases and 50 nuclei were analyzed with the ZEISS ZEN© microscope software.

Molecular analysis of the AMH and AMHR2 gene

Genomic DNA was extracted from the peripheral blood of the proband, following standard salting-out protocol. DNA was amplified by PCR using specific primers for the AMH and AMHR2 genes, reported by Yumie et al. (14) (Table-1). PCR conditions were: one cycle of 95°C for 5 minutes, 35 cycles of 97°C for 45 seconds; annealing temperatures for 45 seconds; 72°C for 2 minutes; and a final cycle of 72°C for 10 minutes. The PCR products were analyzed by direct DNA sequencing on an ABI 310 sequencer (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA). The results were analyzed by comparing the sequences with those reported in Gene Bank NM_000479 and NM_020547, respectively.

RESULTS

Patient's karyotype showed 46.XY.ish Yp11.31 (SRY+). Sanger sequencing revealed the existence of a homozygous variant NM_020547 (AMHR2) c.916delC (p.Leu306Cysfs*29) in the 7

Table 1 - Primer sequences and annealing temperatures of fragments of AMH and AMHR2.

Gene exon	Forward primer (5' – 3')	Reverse primer (5' – 3')	Annealing Temp (C°)
AMH 1	F – AAACACCCCACCTTCCACTC	R –CCGGCCCACCTGAAGGAA	60
AMH 2	F – CAGGGACAGATCCCAAAGAT	R – TACTGCAGACCCTGCAACAA	60
AMH 3 – 4	F – GTAGAGCGGGGCTGGGTA	R – CGCAATTGGAGGAGTTGAGA	57
AMH 5	F – CTGGACACCGTGCCCTTC	R – TGGGGTCCGAATAAATATGG	57
AMHR2 1 – 2	F – CAGGATGCCCTGTATCTGAAG	R – acaccccaggatgtgtctgt	58
AMHR2 3 – 4	F – CTCTGTTTCCACACCCCATT	R – GGAGAGGGGTCAGAGCTTTT	58
AMHR2 5 – 6	F – GACTCCCATGACCTCTCACAA	R – CATGTAGCCCCCACCTCTAT	58
AMHR2 7	F – GGATGGATCAGCCGTCTC	R - AGGCAGAATCACAAACATAGCA	61
AMHR2 8 – 9	F - AAAAAGAGGGAGGAAGAAAATC	R – ttggggtgaacctagaatgg	54
AMHR2 10	F – CCCTTTCTACATGGTAGGCA	R – ACGTCCTTGAAGCCCATGCCCA	49
AMHR2 11	F – TTTTAACCCTGGGGCCCACT	R - GCACACCTACCCCAAGTCAC	58

exon of the AMHR2 gene, that produces a change in the amino acid that results in a pathogenic frameshift with a shorter protein of 334 amino acids, respect to the normal protein of 574 amino acids, removing a significant part of the intracellular serine/threonine kinase domain responsible of biological activity. This variant has not been previously reported. The amino acid Leucine is conserved in some mammal species (Figure-3), indicating that changes in this position can have a significant effect on the protein.

Although this variant has not been reported previously in the dbSNP, 1000G, and ExAC databases, it has been identified as pathogenic by bioinformatics analysis tools Protein Variation Effect Analyzer (provean.jcvi.org/index.php) and Polyphen (http://genetics.bwh.harvard.edu/pph2/).

DISCUSSION

To date, 4 patients with PMDS have been reported in Colombia: two children with diagnosis during laparoscopic treatment of cryptorchidism without molecular study, (15) and two adult patients with cryptorchidism and inguinal hernia were identified in pelvis MRI with uterus and fallopian tubes (16).

This case report highlights the importance of a continuous and multidisciplinary evaluation of patients with cryptorchidism, a clinical spectrum of disorder of sexual differentiation, whereas the post-operative follow-up of the patient would have contributed to the timely management of the failed orchidopexy and to avoid secondary consequences as tumor and infertility.

The initial laboratory evaluation in a phenotypically male with bilateral nonpalpable testes must include karyotype, ultrasonography of the pelvic structures, serum testosterone, gonadotropins (LH, FSH), anti-Müllerian hormone level, and exploratory surgery is usually necessary for definitive diagnosis. In males, serum levels of AMH remain high until 2 years of age and persist in measurable levels until puberty, before decreasing to undetectable levels at puberty (17).

AMH is a possible screening assay in young patients with bilateral cryptorchidism, due to the possibility of differential diagnosis of PMDS, even more in the presence of a positive history of consanguinity, bilateral cryptorchidism and male infertility, as in this case, which was susceptible to be diagnosed in childhood. An opportune diagnosis allows an adequate surgical management, and giving advice in the surveillance of the tumoral risk and in the possible compromise of the fertility, but even the molecular diagnosis allows confirming the diagnosis and providing genetic family counseling.

Mutations in the intracellular domain, like this, have been reported previously, all with lack of the kinase activity (7, 18) and alteration of the secondary catalytic activity, therefore, without functional AMH that leads to regression of the Mullerian ducts.

Likewise, the importance of long-term postoperative follow-up of orchidopexy is highlighted, due to possible complications associated with non-descendant testicular tissue (eg, testicular cancer, occult testicular torsion, infertility) as occurred in this patient, who, due to lack of knowledge and non-adherence to periodic follow-ups, had a late diagnosis of PMDS and testicular tumor.

Figure 3 - Conservation of AMHR2 protein between different species. The red box indicates the altered amino acid in the individual reported conserved in all species in which this protein is present. Bold letters indicate amino acid changes with respect to the human Wild type sequence.

Species	Match Gene	Alignment
Human		TSDWGSSL RMA L SLAQGLAFLHEERWQNGQYPGIAHRDLSSQNVLIREDGSCAIGDLGLALVLPGLTQPP
P. troglodytes	All identical	SL RMA L SLAQGLAFLHEERWQNGQYPGIAHRDLSSQNVLIREDGSCAIGDLGLALVLPGLTQPP
M. mulata	All identical	SL RMA L SLAQGLAFLHEERWQNGQYPGIAHRDLSSQNVLIREDGSCAIGDLGLALVLPGLTQPP
F. catus	Partly conserved	SDWGGSS**M** RMA L SLA**R**GLA FLHEERWQ**D**GQYPGIAHRDLSSQNVLIREDGSCAIGDLGLALVLPGLTQPP
M. musculus	Partly conserved	SL RMA L SLAEGLAFLHEERWQ**D**GQYPGIAHRDLSSQNVLIRED**R**SCAIGDLGLALVLPGL**A**QPP
G. gallus	No homologue	
T. rubripes	No homologue	
D. rerio	No homologue	
D. melanogaster	No homologue	

CONCLUSIONS

In conclusion, PMDS is a condition that is observed in men with cryptorchidism and/or inguinal hernia. It must be diagnosed and treated promptly to protect the fertility and prevent the potential risk of tumors. Our results expand the mutational spectrum of this infrequent condition and emphasize that PMDS should be included in the differential diagnosis of cryptorchidism. Genetic counselling should be considered in cases of parental consanguinity.

ACKNOWLEDGEMENT

The authors thank to the patient for his participation and cooperation in the study.

COMPLIANCE WITH ETHICAL STANDARDS

The authors have no ethical conflicts to disclose. The patient gave a signed informed consent according to the guidelines of the Research Ethical Committee of the National Research Center.

FUNDING SOURCES

This work was funded by COLCIENCIAS (grant 711/2015 - project number 120371150037); HUSI 1702 and MG was beneficiary of the grant "young researcher COLCIENCIAS" 761/2016.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Rey R, Josso N. Regulation of testicular anti-M ullerian hormone secretion. Eur J Endocrinol. 1996;135:144-52.
2. Haqq CM, King CY, Ukiyama E, Falsafi S, Haqq TN, Donahoe PK, et al. Molecular basis of mammalian sexual determination: activation of Müllerian inhibiting substance gene expression by SRY. Science. 1994;266:1494-500. Erratum in: Science 1995;267:317.
3. Shen WH, Moore CC, Ikeda Y, Parker KL, Ingraham HA. Nuclear receptor steroidogenic factor 1 regulates the müllerian inhibiting substance gene: a link to the sex determination cascade. Cell. 1994;77:651-61.
4. De Santa Barbara P, Bonneaud N, Boizet B, Desclozeaux M, Moniot B, Sudbeck P, et al. Direct interaction of SRY-related protein SOX9 and steroidogenic factor 1 regulates transcription of the human anti-Müllerian hormone gene. Mol Cell Biol. 1998;18:6653-65.
5. di Clemente N, Jamin SP, Lugovskoy A, Carmillo P, Ehrenfels C, Picard JY, et al. Processing of anti-mullerian hormone regulates receptor activation by a mechanism distinct from TGF-beta. Mol Endocrinol. 2010;24:2193-206.
6. Josso N, Belville C, di Clemente N, Picard JY. AMH and AMH receptor defects in persistent Müllerian duct syndrome. Hum Reprod Update. 2005;11:351-6.
7. Picard JY, Cate RL, Racine C, Josso N. The Persistent Müllerian Duct Syndrome: An Update Based Upon a Personal Experience of 157 Cases. Sex Dev. 2017;11:109-25.
8. Josso N, Rey RA, Picard JY. Anti-müllerian hormone: a valuable addition to the toolbox of the pediatric endocrinologist. Int J Endocrinol. 2013;2013:674105.
9. Lindhardt Johansen M, Hagen CP, Johannsen TH, Main KM, Picard JY, Jørgensen A, et al. Anti-müllerian hormone and its clinical use in pediatrics with special emphasis on disorders of sex development. Int J Endocrinol. 2013;2013:198698.
10. Farikullah J, Ehtisham S, Nappo S, Patel L, Hennayake S. Persistent Müllerian duct syndrome: lessons learned from managing a series of eight patients over a10-year period and review of literature regarding malignant risk from the Müllerian remnants. BJU Int. 2012;110(11 Pt C):E1084-9.
11. Odi TO, Abdur-Rahman LO, Nasir AA. Persistent Mullerian duct syndrome: a case report and review of the literature. Afr J Paediatr Surg. 2010;7:191-3.
12. Renu D, Rao BG, Ranganath K, Namitha. Persistent mullerian duct syndrome. Indian J Radiol Imaging. 2010;20:72-4.
13. Barad AK, Bharath NM, Narayana V, Raja VO, Jambula PR. Persistent Mullerian Duct Syndrome with Embryonal Cell Carcinoma along with Ectopic Cross Fused Kidney. J Clin Diagn Res. 2016;10:PD07-8.
14. Nishi MY, Domenice S, Maciel-Guerra AT, Zaba Neto A, Silva MA, Costa EM, et al. Analysis of anti-Müllerian hormone (AMH) and its receptor (AMHR2) genes in patients with persistent Müllerian duct syndrome. Arq Bras Endocrinol Metabol. 2012;56:473-8.
15. Perez J. Persistent Müllerian Duct Syndrome. Present and Future of Antimullerian Hormone. Revista Urologia Colombiana. 2003;12:73-83.

16. Massaro M, Montoya M, Alzate CLA. Persistent Mullerian Duct Syndrome Case Report. Revista colombiana de Radiologia. 2010;21:3053-8.
17. Bergadá I, Milani C, Bedecarrás P, Andreone L, Ropelato MG, Gottlieb S, et al. Time course of the serum gonadotropin surge, inhibins, and anti-Müllerian hormone in normal newborn males during the first month of life. J Clin Endocrinol Metab. 2006;91:4092-8.
18. Ju XB, Zhang W, Wu HF, Qian LX, Shen BX, Xu ZQ, et al. Persistent Müllerian duct syndrome: a report of 2 cases and review of the literature. Zhonghua Nan Ke Xue. 2008;14:51-4.

Correspondence address:
Adriana Rojas, MD
Human Genetic Institute,
Pontificia Universidad Javeriana, Bogotá, Colombia
Cra 7 No. 40-62 Building 32
Bogotá, 110231, Colombia
Telephone: +32 0 8320-2787
E-mail: rojas-adriana@javeriana.edu.co

VIDEO SECTION

Vol. 45 (5): 1071-1072, September - October, 2019
doi: 10.1590/S1677-5538.IBJU.2018.0421

Open anterograde anatomic radical retropubic prostatectomy technique: description of the first fifty-five procedures

Fabrício Borges Carrerette [1, 2], Emanuel Carvalho [3], Henrique Machado [3], Felipe Cassau de Sá Freire [3], Ronaldo Damião [4]

[1] Faculdade de Ciencias Médicas, Universidade do Estado do Rio de Janeiro - UERJ, Rio de Janeiro, RJ, Brasil; [2] Uromedic - Urologia, Petropolis, RJ, Brasil; [3] Departamento de Cirurgia, Universidade do Estado do Rio de Janeiro - UERJ, Rio de Janeiro, RJ, Brasil; [4] Departamento de Urologia, Universidade do Estado do Rio de Janeiro - UERJ, Rio de Janeiro, RJ, Brasil

ABSTRACT

Introduction: Robotic-assisted radical prostatectomy is the leading surgical technique and was discussed in Pasadena Consensus Panel (1). The goal of this study is to present the results of the first fifty-five patients submitted to Anterograde Anatomic Radical Retropubic Prostatectomy technique (R2PA2), without adding complexity or cost.
Materials and Methods: Fifty-five eligible men with localized prostate cancer underwent R2PA2 from January, 2016 to December, 2017. The technique was previously described (2): the main surgical steps were anterograde dissection, ligation of the dorsal vascular complex without dividing, preservation of the bladder neck, nerve sparing, preservation of Denonvilliers' fascia and confection of the running suture anastomosis. All patients were operated on by second-year residents.
Results: All procedures were completed as planned, but one converted to retrograde prostatectomy (mean duration, 163.40 minutes; hospital stay, 4 days with 4.20 days of drainage; indwelling vesical catheterization of 9.80 days). Positive surgical margin was found in six T2 staging patient (10.90%) and five T3 (9.10%). Biochemical PSA recurrence occurred in three patients (5.50%).
Twenty-four (43.60%) were continent immediately after indwelling catheter removal, seventeen (30.90%) did not wear a pad at one postoperative month while eighteen (30%) used only one safety pad. Five minor complications occurred.
Conclusion: We were able to perform R2PA2 allowing men who do not have access to this new technology to be operated on with the same technique used in robotic surgery. This method was reproducible by low-volume prostate cancer surgeons; help inexperienced surgeons to develop skills valuable to future training with robotic techniques.

ACKNOWLEDGEMENTS

This work was supported by the FAPERJ - Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro. Secretaria de Estado de Ciência, Tecnologia e Inovação do Governo do Estado do Rio de Janeiro, Brazil, and Pedro Ernesto University Hospital of the State University of Rio de Janeiro, Brazil.

CONFLICT OF INTEREST

None declared.

ARTICLE INFO

Fabricio Carrerette
https://orcid.org/0000-0002-7678-7589

Available at: http://www.intbrazjurol.com.br/video-section/20180421_Carrerette_et_al
Int Braz J Urol. 2019; 45 (Video #19): 1071-2

Submitted for publication:
July 28, 2018

Accepted after revision:
January 08, 2019

Published as Ahead of Print:
March 20, 2019

Correspondence address:
Fabrício Borges Carrerette, MD, PhD
Faculdade de Ciencias Médicas, Universidade do Estado
do Rio de Janeiro - UERJ
Av 28 de Setembro 77
Rio de Janeiro, RJ, 20551-030, Brasil
E-mail: carrerette2@gmail.com

VIDEO SECTION

Vol. 45 (5): 1073-1074, September - October, 2019
doi: 10.1590/S1677-5538.IBJU.2018.0705

Intracorporeal renal hypothermia with ice slush for robot-assisted partial nephrectomy in a highly complex renal mass

Jose Luis Bauza [1], Prithvi Murthy [2], Daniel Sagalovich [2], Riccardo Bertolo [2], Enrique Pieras [1], Pedro Piza [1], Jihad Kaouk [2]

[1] Department of Urology, Hospital Universitario Son Espases, Palma de Mallorca, Illes Balears, Spain; [2] Center for Laparoscopic and Robotic Surgery, Glickman Urological & Kidney Institute, Cleveland Clinic, Cleveland, Ohio - United States

ABSTRACT

CASE DESCRIPTION

Objective

To report our step-by-step technique for robotic partial nephrectomy using intracorporeal renal hypothermia (RPNIRH) in a highly complex renal mass. The robotic technology has allowed surgeons to recreate the principles of open surgery in a minimally invasive approach (1). With increasing experience, larger deeply infiltrative tumors can be treated with this technique (2). In complex cases, when a long warm ischemia time is expected, intracorporeal renal hypothermia can be useful to prevent permanent renal function loss (3).

MATERIALS AND METHODS

A 69 years old male with chronic kidney disease with an atrophic left kidney, appendectomy and a right ureteral reimplant due to an ureteral stenosis was incidentally found a right renal mass, 8.5 cm in diameter, cT2a, RENAL score 11p and several retroperitoneal lymph nodes <1cm. Neoadjuvant therapy with tyrosine kinase inhibitors and subsequent partial nephrectomy was recommended by the Urologic--Oncology tumor board. No tumor shrinkage was evident in the control imaging. Thus the patient underwent a RPNIRH.

RESULTS

Operative time was 185 min. Cold ischemia time was 49:50 min. Average kidney temperature was 24.3ºC. Blood losses were negligible and no postoperative complications appeared, eGFR at discharge was 14ml/min/1.73m^2. Final pathology revealed a clear cell renal cell carcinoma, pT3aN0, ISUP grade 3, involving the sinus fat. Surgical margins were negative.

CONCLUSIONS

The RPNIRH using ice slush is simple, highly reproducible and may improve postoperative renal function in the short term. Consistent experience is needed before embarking on this surgery. Reports on long-term oncological outcomes for such lesions are awaited.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Garisto J, Bertolo R, Dagenais J, Sagalovich D, Fareed K, Fergany A, et al. Robotic versus open partial nephrectomy for highly complex renal masses: Comparison of perioperative, functional, and oncological outcomes. Urol Oncol. 2018;36:471.e1-471.e9.
2. Eyraud R, Long JA, Snow-Lisy D, Autorino R, Hillyer S, Klink J, et al. Robot-assisted partial nephrectomy for hilar tumors: perioperative outcomes. Urology. 2013;81:1246-51.
3. Ramirez D, Caputo PA, Krishnan J, Zargar H, Kaouk JH. Robot-assisted partial nephrectomy with intracorporeal renal hypothermia using ice slush: step-by-step technique and matched comparison with warm ischaemia. BJU Int. 2016;117:531-6.

Correspondence address:
Jose Luis Bauza Quetglas, MD
Department of Urology, Hospital Universitario Son Espases
79 Valldemossa Rd
Palma de Mallorca, 70120, Spain
Telephone: +34 608 688-560
E-mail: peplluis15@hotmail.com

ARTICLE INFO

Jose Luis Bauza
https://orcid.org/0000-0002-8955-483X

Available at: http://www.intbrazjurol.com.br/video-section/20180705_Bauza_et_al
Int Braz J Urol. 2019; 45 (Video #20): 1073-4

Submitted for publication:
October 10, 2018

Accepted after revision:
January 08, 2019

Published as Ahead of Print:
March 22, 2019

VIDEO SECTION

Vol. 45 (5): 1075-1075, September - October, 2019
doi: 10.1590/S1677-5538.IBJU.2018.0415

Indocyanine green – guided laparoscopic renal pedicle lymphatic disconnection: A novel, targeted treatment for chyluria

Joshua Yi Min Tung [1], Kenneth Chen [1], Allen Soon Phang Sim [1]

[1] *Singapore General Hospital, Singapore*

ABSTRACT

Introduction and Objectives: Chyluria, or the passage of chyle into the urine from anomalous lymphatic connections, results in a characteristic milky urine. In severe cases, it can cause significant morbidity from nutritional losses and immune suppression. Although predominantly associated with *Wuchereria bancrofti* infections, non-parasitic cases have also been described.

Traditionally, surgical treatment has involved renal lymphatic disconnection using open or minimally invasive methods, occasionally aided by pre-operative imaging techniques like lymphangiography, or by identification of structures with laparoscopic magnification.

Materials and Methods: Here we describe a novel technique of targeted renal pelvis lympholysis using retrograde intra-ureteric Indocyanine Green (ICG) administration to accurately identify and ligate the anomalous lymphatics.

Results: ICG-guided renal pelvis lympholysis was successfully performed in a single patient, who was discharged well on post-operative day 4 and remained symptom-free at 6-month follow-up.

Conclusion: The use of intra-ureteric ICG is a new technique which allows for precise, intra-operative identification and ligation of anomalous lymphatics in chyluria, with sustainable results. Complete skeletisation of the renal hilum could be avoided, reducing procedure time and decreasing the risk of injuring hilar structures. This novel technique should be considered as a valuable addition to the urologist's arsenal.

CONFLICT OF INTEREST

None declared.

Correspondence address:
Joshua Yi Min Tung, MD
Singapore General Hospital
Outram Road Singapore 169608
Singapore
E-mail: joshua.tung@gmail.com

ARTICLE INFO

Joshua Yi Min Tung
https://orcid.org/0000-0003-4991-9790

Available at: http://www.intbrazjurol.com.br/video-section/20180415_Tung_et_al
Int Braz J Urol. 2019; 45 (Video #21): 1075

Submitted for publication:
June 19, 2018

Accepted after revision:
January 08, 2019

Published as Ahead of Print:
March 20, 2019

VIDEO SECTION

Vol. 45 (5): 1076-1077, September - October, 2019
doi: 10.1590/S1677-5538.IBJU.2018.0430

Laparoscopic nephroureterectomy as treatment in obstructed hemivagina and ipsilateral renal agenesis (OHVIRA) syndrome

María Medina-González [1], Jorge Panach-Navarrete [1], Lorena Valls-González [1], Ana Castelló-Porcar [1], Jose María Martínez-Jabaloyas [1]

[1] *Department of Urology. University Clinic Hospital of Valencia, Facultat de Medicina i Odontologia, Universitat de València, Spain*

ABSTRACT

Introduction: OHVIRA syndrome is a rare entity characterized by renal and Mullerian anomalies. The objective of the video is, through a clinical case, to discuss the importance of diagnosis, management and treatment, to avoid the complications that this syndrome entails, and to improve the long-term prognosis.
Materials and Methods: We report the case of a 10-year-old girl who consulted for abdominal pain, being diagnosed with OHVIRA syndrome. We describe the diagnosis and the surgical technique. In addition, we perform a systematic review in PubMed to report the published literature of this topic and we show the optimal management of this pathology.
Results: This syndrome is characterized by a bicornuate uterus with obstructed hemivagina, renal agenesis or dysplastic and atrophic kidney with ectopic ureter, which drains to the obstructed hemivagina. It causes clinical symptoms such as persistent vaginal drainage after resection of the vaginal septum and cyclic abdominal pain.
The diagnosis was completed with a URO-CT scan and magnetic resonance imaging, to identify the anatomical alterations and to plan a correct surgery.
In the video we show a laparoscopic left nephroureterectomy of an atrophic and ectopic kidney without incident as an effective treatment to avoid complications.
Conclusions: In OVHIRA syndrome, if an atrophic kidney exists surgical treatment is recommended, in order to avoid complications. For this, a suitable diagnosis is necessary for a good preoperative plan, as well as the knowledge of the spectrum of renal anomalies for an early diagnosis. We show the indicated procedure, by a laparoscopic approach.

CONFLICT OF INTEREST

None declared.

ARTICLE INFO

María Medina-González
https://orcid.org/0000-0002-4864-1339

Available at: http://www.intbrazjurol.com.br/video-section/20180430_ Medina-Gonzalez_et_al
Int Braz J Urol. 2019; 45 (Video #22): 1076-7

Submitted for publication:
June 25, 2018

Accepted after revision:
January 08, 2019

Published as Ahead of Print:
March 20, 2019

Correspondence address:
Jorge Panach-Navarrete, MD
Department of Urology,
University Clinic Hospital of Valencia
Facultat de Medicina i Odontologia,
Universitat de València
Av. Blasco Ibáñez, 17
Valencia, CP 46010, Spain
Telephone: +34 9619-73500
E-mail: jorge.panach@uv.es

VIDEO SECTION

Vol. 45 (5): 1078-1079, September - October, 2019
doi: 10.1590/S1677-5538.IBJU.2018.0174

One-sided anterior Urethroplasty for panurethral stricture: step-by-step

Willian Eduardo Ito [1], Marco Aurélio Rodrigues [1], Silvio Henrique Maia de Almeida [1]

[1] *Disciplina de Urologia, Universidade Estadual de Londrina Centro de Ciencias da Saude, Londrina, PR, Brasil*

CASE DESCRIPTION

INTRODUCTION AND OBJECTIVES

The management of complex urethral strictures is surgical challenging, especially for stenosis affecting the entire extension of the anterior urethra.

In this video, we present a step-by-step one-sided anterior urethroplasty for discussion about the surgical aspects of this technique.

MATERALS AND METHODS

We present a case report of a 23-year-old male patient, complaining of progressive voiding symptoms, bleeding from meatus and perineal pain, which began after a sexual intercourse four months ago. He had no previous urethral surgery, urethral instrumentation or any urethritis treatment. Retrograde urethrography showed a full length stricture of the anterior urethra. Urofluxometry showed a maximum flow of 3mL per second.

We performed the one-sided anterior urethroplasty with oral mucosal graft as described by Kulkarni (1, 2), a minimally invasive technique which preserves the neurovascular supply (3-5).

RESULTS

The patient's postoperative recovery was uneventful and the patient had no complain about his graft donor site, with minimal pain, easily managed with common analgesics. On postoperative day one, there was a penile edema, which regressed spontaneously.

After 21 days, the 16Fr Foley catheter was removed and a retrograde urethrography was performed, which has shown a successful improvement of the width of the anterior urethra and a small proximal diverticulum, but the patient referred great subjective urinary flow.

Post-operative uroflowmetry showed a maximum voiding flow of 13mL per second.

CONCLUSIONS

The Kulkarni's technique for panurethral strictures is a less invasive and smart technique which spares one side of the urethra neurovascular supply and the operation can be performed in one single stage.

CONFLICT OF INTEREST

None declared.

ARTICLE INFO

Willian Ito
http://orcid.org/0000-0002-1038-1375

Available at: http://www.intbrazjurol.com.br/video-section/20180174_Ito_et_al
Int Braz J Urol. 2019; 45 (Video #23): 1078-9

REFERENCES

1. Kulkarni S, Barbagli G, Sansalone S, Lazzeri M. One-sided anterior urethroplasty: a new dorsal onlay graft technique. BJU Int. 2009;104:1150-5.
2. Kulkarni SB, Kulkarni JS, Kirpekar DV. A new technique of urethroplasty for balanitis xerotica obliterans. J Urol. 2000; 163 (Suppl.): 352 (abstract V31).
3. Barbagli G, De Stefani S, Annino F, De Carne C, Bianchi G. Muscle- and nerve-sparing bulbar urethroplasty: a new technique. Eur Urol. 2008;54:335-43.
4. Lumen N, Hoebeke P, Willemsen P, De Troyer B, Pieters R, Oosterlinck W. Etiology of urethral stricture disease in the 21st century. J Urol. 2009;182:983-7.
5. Tavakkoli Tabassi K, Ghoreifi A. Dorsally Placed Buccal Mucosal Graft Urethroplasty in Treatment of Long Urethral Strictures Using One-Stage Transperineal Approach. Int Sch Res Notices. 2014;2014:792982.

Correspondence address:
Willian Eduardo Ito, MD
Disciplina de Urologia, Universidade Estadual de Londrina Centro de Ciencias da Saude
Av Robert Koch, 60
Londrina, PR, 86038-440, Brasil
E-mail: willianito@hotmail.com

Submitted for publication:
March 22, 2018

Accepted after revision:
January 30, 2019

Published as Ahead of Print:
March 22, 2019

VIDEO SECTION

Vol. 45 (5): 1080-1080, September - October, 2019
doi: 10.1590/S1677-5538.IBJU.2018.0495

New technologies for old procedures: when Firefly improves robotic bladder diverticulectomy

Francesca Vedovo [1], Bernardino de Concilio [2], Guglielmo Zeccolini [2], Tommaso Silvestri [1], Antonio Celia [2]

[1] Department of Urology, Azienda Sanitaria Universitaria Integrata di Trieste, Trieste, Italy; [2] Department of Urology, San Bassiano Hospital, Bassano del Grappa, Italy

ABSTRACT

Introduction: Several techniques have been described to aid in the intra-operative identification of the bladder diverticula. The video shows the peculiar advantage of using Firefly Fluorescence Imaging da Vinci System® (FFIS) during bladder diverticula detection and dissection (BD).
Material and Methods: Patient is placed in the lithotomic position. A transperitoneal access to the bladder is preferred. A flexible cystoscopy with the FFIS is performed. This procedure facilitates the diverticulum detection. This near-infrared technology can be usefully utilized to facilitate the diverticulum dissection. Using sharp and blunt dissection, the diverticulum is totally resected. Bladder is sutured in two absorbable layers. Drainage is placed in the Retzius space and a peritoneum reconstruction is performed.
Results: Between 2016 and 2017, 4 BDs with intraoperative FFIS were performed in our Center. Median operative time was 110 minutes. Mean time of postoperative catheterization was 11 days and mean length of stay was 4 days. No significant post void residual neither urine extravasation after catheter removal occurred. No Clavien-Dindo post-operative complications ≥2 have been reported. Several approaches have been described for intra-operative diverticulum identification and its dissection: Parra used a cystoscopic transillumination of diverticulum; Das proposed the use of a Foley 50 mL balloon inserted in the diverticulum, while Nadler used a balloon catheter, placed in the diverticulum and bloated with 180 cc saline solution.
Conclusions: In our experience, intra-operative use of FFIS enhances the transillumination effect. The identification and dissection of the diverticulum is more rapid, safe and effective with no additional cost.

ARTICLE INFO

Francesca Vedovo
https://orcid.org/0000-0002-9791-6888

Available at: http://www.intbrazjurol.com.br/video-section/20180495_Vedovo_et_al
Int Braz J Urol. 2019; 45 (Video #24): 1080

Submitted for publication:
July 18, 2018

Accepted after revision:
February 27, 2019

Published as Ahead of Print:
May 10, 2019

Correspondence address:
Francesca Vedovo, MD
Department of Urology,
Azienda Sanitaria Universitaria Integrata di Trieste,
Trieste, Italy
Strada di Fiume , Trieste 34128, Italy
E-mail: francesca.vedovo@gmail.com

INTERNATIONAL
BRAZ J UROL

INFORMATION FOR AUTHORS

Manuscripts submitted for publication should be sent to:

Sidney Glina, M.D, PhD
Editor, International Braz J Urol

by e-mail with attached text files and figures to:
submission@brazjurol.com.br

Manuscripts must be written in current English or Portuguese. Non-native English speakers should ask a native specialist in medical English for checking the grammar and style. Either American or British English may be used but should be consistent throughout the manuscript.

A submission letter signed by all authors must accompany each manuscript. This letter must state that: a)- the paper or portion thereof have not been previously published and are not under consideration by another Journal, b)- that all authors have contributed to the information or material submitted for publication, and that all authors have read and approved the manuscript, c)- that the authors have no direct or indirect commercial financial incentive associated with publishing the manuscript, d)- that the source of extra-institutional funding, specially that provided by commercial companies, is indicated, e)- that the study had been reviewed and approved by a certified Ethical Board or Committee, including the nmeber of the approval dociment and the date of the approval, f)- a non-plagiarism statement (I (We) declare that all material in this assignment is my (our) own work and does not involve plagiarism). g)- Clinical trials must be registered on any Clinical Trials Registry and the letter must bring the number of registration and the name of the registry. After accepted for publication, the manuscript will become property of the **International Braz J Urol.**

Conflict of Interest – Any conflict of interest, mainly financial agreement with companies whose products are alluded to in the paper, must be clearly disclosed when submitting a manuscript for review. If accepted, a disclosure will be published in the final manuscript.

The requirements for authorship and the general rules for preparation of manuscripts submitted to the **International Braz J Urol** are in accordance with the Uniform Requirements for Manuscripts Submitted to Biomedical Journals (International Committee of Medical Journal Editors. Uniform Requirements for Manuscripts Submitted to Biomedical Journals. Ann Intern Med, 126: 36-47, 1997). An electronic version of the Uniform Requirements is available on various websites, including the International Committee of Medical Journal Editors web site: www.icmje.org.

In response to the concerns of the editors of scientific medical journals with ethics, quality and seriousness of published articles, a Committee on Publication Ethics (COPE) was established in 1997 and a guideline document was published. The International Braz J Urol signed, approved, and follows the COPE guidelines. The Editor strongly encourages the authors to carefully read these guidelines before submitting a manuscript **(www.publicationethics.org.uk/guidelines or www.brazjurol.com.br**, vol. 26 (1): 4-10, 2000).

Peer Review – All submissions are subject to editorial review. Typically, each manuscript is anonymously forwarded by the Editor to 4 Reviewers (at least 2). If the Editor receives conflicting or inconclusive revisions, the manuscript is always sent to 1 or 2 additional Reviewers before the Editor's decision. If considered necessary by the Editor or by the Reviewers, statistical procedures included in the manuscript will be analyzed by a statistician.

The **International Braz J Urol** contains six sections: **Original Article, Review Article, Surgical Technique, Challenging Clinical Case, Radiology Page**

and **Video Section**. The articles should be written in Portuguese or English official orthography.

Abbreviations should be avoided, and when necessary must be specified when first time mentioned. Unusual expressions may not be used. A list of abbreviations must be provided at the end of the manuscript.

Every manuscript submitted to publication should have a cover page containing the title, short title (up to 50 characters), authors and institution. Up to six key words should be provided. These words should be identical to the medical subject headings (MeSH) that appear in the Index Medicus of the National Library of Medicine **(http://www.nlm.nih.gov/mesh/meshhome.html)**. One of the authors should be designated as correspondent and the complete correspondence address, telephone and fax numbers and E-mail should be provided.

If any financial support has been provided, the name of the institution should be mentioned.

Original Article: Original articles should contain a Cover Page, Abstract, Introduction, Materials and Methods, Results, Discussion, Conclusions, References, Tables and Legends, each section beginning in a separate page and numbered consecutively. Original articles should cover contemporary aspects of Urology or experimental studies on Basic Sciences applied to urology. The manuscript text should contain no more than 2500 words, excluding the Abstract. The number of authors is limited to five. References should contain no more than 30 citations, including the most important articles on the subject. Articles not related to the subject must be excluded.

Review Article: Review articles are accepted for publication upon Editorial Board's request in most of the cases. A Review Article is a critical and systematic analysis of the most recent published manuscripts dealing with a urological topic. A State of the Art article is the view and experience of a recognized expert in the topic. An abstract must be provided.

Surgical Technique: These manuscripts should present new surgical techniques or instruments and should contain Introduction, Surgical Technique, Comments and up to five References. An abstract must be provided. At least five cases performed with the technique must be included.

Challenging Clinical Case: These manuscripts should present relevant clinical or surgical situations which can bring or consolidate our understanding of genesis, natural history, pathophysiology and treatment of diseases. *Structure of the articles*

Abstract (maximum 200 words) and should contain

- **Main findings:** Report case(s) relevant aspects
- **Case(s) hypothesis:** Proposed premise substantiating case(s) description
- **Promising future implications:** Briefly delineates what might it add? Lines of research that could be addressed

Full text (maximum 2000 words):

- **Scenario:** Description of case(s) relevant preceding and existing aspects;
- **Case(s) hypothesis and rational:** precepts, clinical and basic reasoning supporting the case(s) hypothesis and the raised scenario. Why is it important and is being reported?
- **Discussion and future perspectives:** what might it add and how does it relate to the current literature. 'Take-home message' - lessons learnt;
- **Table and/or Figure limits:** 2 (plates aggregating multiple images are encouraged) each exceeding table or figure will decrease 250 words of the full text;
- **Number of references:** 10-15.

Radiology Page: Will be published upon the Section Editor decision.

Video Section: The material must be submitted in the appropriate local, in the Journal's site, whe-

re all instructions may be found (Video Section link) Letters to the Editor: The letter should be related to articles previously published in the Journal, should be useful for urological practice and must not exceed 500 words. They will be published according to the Editorial Board evaluation.

ILLUSTRATIONS:

The illustrations should not be sent merged in the text. They should be sent separately, in the final of the manuscript.

1) The number of illustrations should not exceed 10 per manuscript.
2) Check that each figure is cited in the text.
3) The legends must be sent in a separate page.
4) The legends of histological illustrations should contain the histological technique and the final magnification.
5) The International Braz J Urol encourages color reproduction of illustrations wherever appropriate.
6) All histological illustrations should be supplied in color.

ELECTRONIC SUBMISSION:

1) Do not embed the figures in the text, but supply them as separate files.
2) For Submitting Photographs Electronically, please:
Supply photographs as TIFF (preferable) or JPG files. The TIFF of JPG should be saved at a resolution of 300 dpi (dots per inch) at final size. If scanned, the photographs should be scanned at 300 dpi, with 125mm width, saved as TIFF file and in grayscale, **not embed in Word or PowerPoint.**
3) For Submitting Line Artwork Electronically please note that:
Line drawings must be supplied as EPS files (give an EPS extension, e.g. Fig01.eps). Use black text over light to mid grey and white text over dark grey or black shades. Use lower case for all labeling, except for initial capitals for proper nouns and necessary mathematical notation. Centre each file on the page and save it at final size with the correct orientation. We recommend a minimum final width of 65 mm, but note that artwork may need to be resized and relabeled to fit the format of the Journal.
4) IMPORTANT - Avoid - Do Not

a) **DO NOT** embed the images in the text; save them as a separate file
b) **DO NOT** supply artwork as a native file. Most illustration packages now give the option to "save as" or export as EPS, TIFF or JPG.
c) **DO NOT** supply photographs in PowerPoint or Word. In general, the files supplied in these formats are at low resolution (less than 300 dpi) and unsuitable for publication.
d) **DO NOT** use line weights of less than 0.25 point to create line drawings, because they will nor appear when printed.

TABLES: The tables should be numbered with Arabic numerals. Each table should be typed on a single page, and a legend should be provided for each table. Number tables consecutively and cites each table in text in consecutive order.
REFERENCES: The References should be numbered following the sequence that they are mentioned in the text. The references should not be alphabetized. They must be identified in the text with Arabic numerals in parenthesis. Do not include unpublished material and personal communications in the reference list. If necessary, mention these in the body of the text. For abbreviations of journal names refer to the "List of Journals Indexed in Index Medicus" (http://www.nlm.nih.gov). The authors must present the references according to the following examples; the names of **all authors** must be included; when exist more than six authors, list the first six authors followed **by et al.** The **initial** and the **final** pages of the reference should be provided:

Papers published in periodicals:

▪ Paterson RF, Lifshitz DA, Kuo RL, Siqueira Jr TM, Lingeman JE: Shock wave lithotripsy monotherapy for renal calculi. Int Braz J Urol. 2002; 28:291-301.

▪ Holm NR, Horn T, Smedts F, Nordling J, de la Rossete J: Does ultrastructural morphology of human detrusor smooth muscle cell characterize acute urinary retention? J Urol. 2002; 167:1705-9.

Books:

▪ Sabiston DC: Textbook of Surgery. Philadelphia, WB Saunders. 1986; vol. 1, p. 25.

Chapters in Books:

▪ Penn I: Neoplasias in the Allograft Recipient. In: Milford EL (ed.), Renal Transplantation. New York, Churchill Livingstone. 1989; pp. 181-95.

The Int Braz J Urol has the right of reject inappropriate manuscripts (presentation, number of copies, subjects, etc.) as well as proposes modifications in the original text, according to the Referees' and Editorial Board opinion.

THE EDITORS SUGGEST THE AUTHORS TO OBSERVE THE FOLLOWING GUIDELINES WHEN SUBMITTING A MANUSCRIPT:

The **Ideal Manuscript** may not exceed 2500 words.

The **Title** must be motivating, trying to focus on the objectives and content of the manuscript.

Introduction must exclude unnecessary information. It should briefly describe the reasons and objective of the paper.

Materials and Methods should describe how the work has been done. It must contain sufficient information to make the study reproducible. The statistical methods have to be specified.

The **Results** should be presented using Tables and Figures whenever possible. Excessive Tables and Figures must be avoided. The tables should not be repeated on the text.

The **Discussion** must comment only the results of the study, considering the recent literature.

Conclusions must be strictly based on the study findings.

References should contain no more than 30 citations, including the most important articles on the subject. Articles not related to the subject must be excluded.

The Abstract must contain up to 250 words and must conform to the following style: Purpose, Materials and Methods, Results and Conclusions. Each section of the manuscript must be synthesized in short sentences, focusing on the most important aspects of the manuscript. **The authors must remember that the public firstly read only the Abstract, reading the article only when they find it interesting.**

NOTE:

Recent issues of the International Braz J Urol must be observed concerning the presentation form of the manuscript.

INTERNATIONAL
BRAZ J UROL

MANUSCRIPT CHECKLIST

The authors should observe the following checklist before submitting a manuscript to the **International Braz J Urol**

- The sequence of manuscript arrangement is according to the Information for Authors.
- The Article is restricted to about 2,500 words and 6 authors.
- Abbreviations were avoided and are defined when first used and are consistent throughout the text.
- Generic names are used for all drugs. Trade names are avoided.
- Normal laboratory values are provided in parenthesis when first used.
- The references were presented according to the examples provided in the Information for Authors. The references were numbered consecutively, following the sequence that they are mentioned in the text. They were identified in the text using Arabic numeral in parenthesis. The names of all authors were provided. When exist more than six authors, list the first sixauthors followed by et al. The initial and the final pages of the reference should be provided. The number of references must be accordingly to the informed in the Instructions for Authors, depending on the type of manuscript.
- The staining technique and the final magnification were provided for all histological illustrations. The histological illustrations are supplied in color.
- Legends were provided for all illustrations, tables, and charts. All tables and charts were in separate pages and referred to in the text. All illustrations and tables are cited in the text.
- An Abstract was provided for all type of articles. The length of the Abstract is about 250 words.
- A corresponding author with complete address, telephone, Fax, and E-mail are provided.
- A submission letter and a disclosure form, signed by all authors, are included.
- The authors should included written permission from publishers to reproduce or adapt a previously published illustrations or tables.
- **Conflict of Interest** – Any conflict of interest, mainly financial agreement with companies whose products are alluded to in the paper, is clearly disclosed in the manuscript.
- **Check that each figure is cited in the text. The illustrations are not merged in the text.**
- The photographs are supplied as TIFF or JPG files and saved at a resolution of 300 dpi (dots per inch) at final size.
- The photographs should be scanned at 300 dpi, with 125mm width, saved as TIFF file and in grayscale, not **embed in Word or PowerPoint.**
- A list of abbreviations is provided.