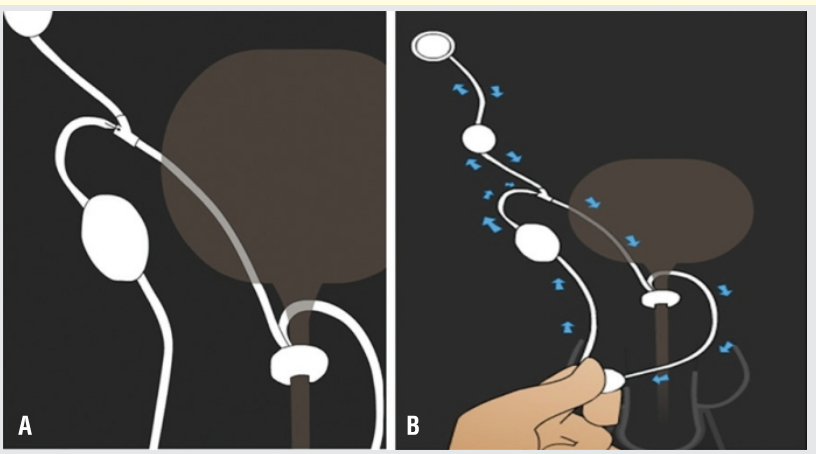
ISSN 1677-5538

INTERNATIONAL BRAZ J UROL

OFFICIAL JOURNAL OF THE BRAZILIAN SOCIETY OF UROLOGY
VOLUME 44, NUMBER 6, NOVEMBER – DECEMBER, 2018



(A). During pumping, the fluid present in the apparatus is displaced from the urethral cuff to the reservoir located in the peritoneal cavity and through a flow reducing system it slowly returns to the cuff, causing it to remain deflated for about three minutes allowing urination (B).

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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.

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EDITORIAL
IN THIS ISSUE

Vol. 44 (6): 1057-1062, November - December, 2018
doi: 10.1590/S1677-5538.IBJU.2018.06.01

The current status of renal cell carcinoma and prostate carcinoma grading

Brett Delahunt [1], Lars Egevad [2, 3], John Yaxley [4, 5], Hemamali Samaratunga [5, 6]

[1] Department of Pathology and Molecular Medicine, Wellington School of Medicine and Health Sciences, Wellington, New Zealand; [2] Department of Pathology, Karolinska Institute, Stockholm, Sweden; [3]Department of Oncology-Pathology, Karolinska Institute, Stockholm, Sweden; [4] Wesley Hospital, Brisbane, Queensland, Australia; [5] University of Queensland School of Medicine, Brisbane, Queensland, Australia, 6 Aquesta Uropathology , Brisbane, Queensland, Australia

INTRODUCTION

Grading is an important prognostic indicator for tumors and for most malignancies provides information additional to staging. As with staging, grading criteria for individual tumors are subject to change, with developments reflecting contemporary advances in our understanding of the behavior of tumors. In the field of urological pathology, the grading classifications most commonly utilized for both renal cell carcinoma (RCC) and prostate adenocarcinoma (PCa) have undergone radical change. This evolution has, most recently, led to the establishment of novel grading systems for both of these tumors, under the auspices of the International Society of Urological Pathology (ISUP) (1, 2). The release of the Fourth Edition of the World Health Organization (WHO) Bluebook on the Classification of Tumours of the Urinary Tract and Male Genital Organs in 2016 (3), followed on from the development of these contemporary grading classifications. In this publication these novel classifications, relating to the two most common morphotypes of RCC and for PCa, were endorsed for international implementation. Subsequently both grading classifications have been incorporated into the reporting datasets issued by the International Collaboration on Cancer Reporting (4, 5).

Renal cell carcinoma grading

Numerous grading systems for renal malignancies have been proposed with validation studies often providing conflicting results (6). While a variety of grading parameters for RCC have been proposed the concept of nuclear grading for these tumors was established 50 years ago by Myers et al. This was followed three years later by the publication of a comprehensive nuclear grading system by Skinner; however, in 1972 this was modified into the four tier grading system of Fuhrman, Lasky and Limas (7-9). Although Fuhrman grading has been utilized internationally for almost 50 years, it is now widely recognized that the system is hampered by uncertainties relating to reproducibility and its validity as a prognostic marker (10). Among the criticisms applied to Fuhrman grading is the fact that it relies on the simultaneous assessment of nuclear shape and size, as well as nucleolar prominence. This implies that these parameters increase in parallel with increasing grade. Unfortunately, in reality, these parameters

are discordant in over 20% of clear cell RCC and as a consequence Fuhrman grading cannot be applied to these cases (10, 11).

These issues were addressed at the Consensus Conference of ISUP convened in Vancouver in 2012 (1). The meeting adopted a grading system derived from assessment of series of clear cell, papillary and chromophobe RCCs (12-14). These studies demonstrated that for clear cell RCC nuclear size and nucleolar prominence, based on the high power field showing the highest grade features, was significantly associated with outcome. For papillary RCC only nucleolar prominence correlated with outcome, while for chromophobe RCC not one of the three parameters of the Fuhrman grading system was found to have prognostic significance. Informed by these findings the novel grading system adopted by the ISUP was based upon nucleolar prominence (1). In this grading classification grade 1 tumors show inconspicuous to small basophilic nucleoli visible at 400x magnification; In grade 2 tumours nucleoli are eosinophilic and prominent at 400x magnification, but inconspicuous at 100x magnification. Grade 3 tumors have nucleoli clearly seen as prominent at 100x magnification. Features required for tumours to be classified as grade 4 are any of the following: 1. sarcomatoid morphology (sarcoma-like mesenchymal to epithelial translocation), 2. rhabdoid morphology, 3. extreme nuclear pleomorphism and 4. anaplastic tumour giant cells. The grading system was formally recommended by the ISUP for both clear cell and papillary RCC. In the absence of evidence that grading was of prognostic significance for chromophobe RCC, it was agreed that this tumor type should not be graded (1). The literature relating to the ISUP grading system for RCC was considered at the 2014 meeting of the WHO Renal Tumour Classification Panel. At this meeting the grading system was endorsed by the WHO and was incorporated into the fourth edition WHO renal tumour classification being designated the WHO/ISUP Grading System (3).

While the WHO/ISUP grading system is applicable only to clear cell and papillary RCC it was agreed that it may also be utilized for descriptive purposes for other morphotypes of RCC (4). If grading is applied to morphotypes other than clear cell and papillary RRC, it is recommended that it be clearly stated in the pathology report that grading is provided for descriptive purposes only and that grading has not been validated for any specific type of renal cell neoplasia, other than clear cell and papillary RCC (15).

WHO/ISUP grading has been validated in a number of studies for both clear cell and papillary RCC (11, 16-18). An interesting, but perhaps not surprising feature of the new grading system, is that when WHO/ISUP graded cases of both clear cell and papillary RCC were compared to those cases in the same series for which Fuhrman grading could be applied, there was a down-grading, with an increase in cases assigned into WHO/ISUP grades 1 and 2 (11, 18). This is a reflection of the fact that for WHO/ISUP grades 2 and 3 tumors, the degree of nucleolar prominence required is greater than that needed to assign tumors into grades 2 and 3 of the Fuhrman system.

While the WHO/ISUP grading classification provides prognostic information for clear cell RCC, studies have suggested that this may be improved if the presence/absence of tumor-related necrosis is incorporated into a revised grading system (16, 19, 20). This specific form of necrosis must be differentiated from thrombo-embolic coagulative necrosis, being characterized by loss of architecture and has been shown to have prognostic significance independent of both tumor grade and TNM staging category (10). In a modified grading system, in which WHO/ISUP grade was sub-stratified on the basis of absence/presence of tumor-related necrosis it was shown that WHO/ISUP grade 2 tumors with necrosis had an outcome similar to WHO/ISUP grade 3 tumors without necrosis and similarly that WHO/ISUP grade 3 tumors with necrosis had an outcome similar to grade 4 tumours without necrosis (16, 19, 20). These results suggest that this modified grading system has an enhanced positive predictive value when compared to WHO/ISUP grading alone.

Prostate adenocarcinoma grading

Numerous grading systems have been proposed for PCa, of which the system that achieved worldwide acceptance for decades has been that of Donald Gleason (20, 21). Since the introduction of

the Gleason system in 1966, a number of modifications have been proposed, with the latest being in 2016 (22). Gleason based his grading purely on architecture without taking cytological atypia into consideration. Five grades were created from the lowest grade of 1 to the highest grade of 5. The dominant and subdominant grades were added to create the Gleason score of 2 to 10. In Gleason's series, which pre-dated the introduction of thin core needle biopsy of the prostate, 88.5% of patients presented with extra-prostatic disease, with 36% having metastases (23). This situation changed dramatically with the introduction of prostate specific antigen (PSA) testing in 1994 (24). Since then, the number of patients presenting with metastatic PCa has decreased markedly (25). During this time, many other aspects of PCa diagnosis and treatment have also evolved. It also became apparent that not all tumor patterns in the Gleason system were classified correctly and in particular the designation of cribriform glands as grades 2 and 3, as well as single cells and solid cords and masses as grade 3, was inappropriate as these patterns are now recognized as features of high-grade disease.

While initial modifications to the Gleason system were made by Gleason himself in 1974 and 1977 (26, 27), other major changes were introduced in 2005 at a Consensus Conference convened by the International Society of Urological Pathology (28). At this meeting, it was decided that Gleason grade 1 cancer represented adenosis, and therefore should not be diagnosed irrespective of the type of prostate specimen. It was also decided that grade 2 should be diagnosed rarely, if ever, in needle biopsies and that cribriform glands were indicative of, at least, grade 3 tumor. Cribriform cancer, which consisted of small round uniform glands with regular round lumina, were considered grade 3, while all other patterns of cribriform glands were considered grade 4. As recommended by Gleason, it was agreed that the presence of comedonecrosis was a feature of grade 5, while Grade 4 was expanded to include poorly formed acini. There was agreement that the grading of all variants of PCa, other than mucinous tumors, should be based upon architecture, ignoring cellular changes. At this meeting, there was no consensus as to how mucinous PCa should be graded (29). A further major change related to scoring of needle biopsies containing a higher grade tertiary pattern. Here it was decided that the Gleason score should be the sum of most common grade (primary grade) and the highest grade present. This change was recommended to avoid sample bias inherent in needle biopsies leading to apparent downgrading in contrast to grading of radical prostatectomy specimens, where the entire tumor is available for assessment. Subsequent studies have confirmed the validity of these modifications in improving the predictive value of needle biopsy grading in relation to grading of radical prostatectomy specimens, as well as biochemical recurrence free survival and overall survival rates (30-32).

More recent studies have highlighted difficulties in differentiating grade 3 cribriform PCa from cribriform cancers with a grade 4 morphology and it is now recognized that all cribriform PCa has a uniformally unfavourable prognosis (33, 34). A further issue related to the assignment of an overall grade to a cancer as in the 2005 modified system Gleason scores ranged from 6 to 10. From a management viewpoint it was apparent that there was value in differentiating cases into prognostic categories such as low, intermediate and high-grade. In 1977, Gleason suggested grouping scores of 2-3, 4-5, 6, 7 and 8-10 would be clinically valid (27, 35). While more recently others have proposed a variety of different combinations of Gleason scores to produce valid prognostic groups (22, 36-39).

To formulate changes to PCa grading, the ISUP convened a further Consensus Conference in 2014 attended by 82 experts from 19 countries (2). At this conference additional amendments to the 2005 Gleason grading criteria were recommended. In particular all cribriform and glomeruloid patterns of tumour were classified as Gleason grade 4 and it was agreed that mucinous adenocarcinoma grading should be based upon the underlying architecture. It was also decided that intraductal carcinoma should not be assigned a grade. Five prognostic categories labelled ISUP grades were created. Gleason score 3+3 were reclassified as Grade 1, Gleason score 3+4 as Grade 2, Gleason score 4+3 as Grade 3, Gleason score 8 (4+4, 3+5,

5+3) as Grade 4, and Gleason scores 9 and 10 as Grade 5. It was decided that, if present, a higher tertiary pattern would continue to be applied to grading as the secondary pattern in needle biopsies. In contrast, there was no agreement as to how tertiary patterns should be dealt with in radical prostatectomy specimens, which means that ISUP grading cannot be strictly applied to these specimens. Several studies have subsequently validated this new ISUP grading system with respect to patient outcome (40-42).

It has subsequently been suggested that tertiary patterns in radical prostatectomy specimens should be treated as a high-grade component in the Gleason score if > 5% of tumor volume. This recommendation has not been validated and was not a consensus decision of the 2014 ISUP meeting. Other recent recommendations are that the percentage of pattern 4 and 5 in both needle biopsies and radical prostatectomies should be recorded as they appear to provide additional prognostic information (43, 44). Currently, the optimum method for evaluating the volume of a higher-grade PCa, is uncertain at it could be based on measurement of surface area or length of the biopsy core. There is also debate as to whether percent pattern 4/5 should be reported for individual cores or for the entire case. Since the introduction of ISUP grading another issue that has been highlighted is the validity of grouping of Gleason score 4+4, 3+5 and 5+3 tumors into ISUP grade 4 category, as subsequent studies have suggested that each of these differ in outcome (45). While the introduction of the ISUP grading system for prostate core biopsies has resulted in significant improvements in outcome prediction for PCa, it remains a system in evolution.

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Brett Delahunt, PhD

Department of Pathology and Molecular Medicine
Wellington School of Medicine and Health Sciences,
University of Otago
PO Box 7343, Wellington, New Zealand
Fax: (64) 4 385 5930
E-mail: brett.delahunt@otago.ac.nz

DIFFERENCE OF OPINION

Vol. 44 (6): 1063-1066, November - December, 2018
doi: 10.1590/S1677-5538.IBJU.2018.06.02

Microdissection is the best way to perform sperm retrieval in men with non-obstructive azoospermy? | *Opinion: Yes*

Renato Fraietta [1]

[1] *Setor Integrado de Reprodução Humana, Universidade Federal de São Paulo, São Paulo, SP, Brasil*

Keywords: Azoospermia; Microdissection; Sperm Retrieval; Fertility

Non-obstructive azoospermia (NOA) is the diagnosis of one percent of all men and 10% of men complaining about infertility (1, 2). All NOA patients should be evaluated with complete history and physical examination, with genetic testing (karyotype analysis and Y chromosome microdeletion testing) being offered and performed, which will identify the causes of NOA in up to 17% of men (3, 4). Hormonal profile is also important as up to 47% of men that have impaired spermatogenesis with NOA were found to have hypogonadism (4,5).

For this situation and after the breakthrough of intracytoplasmic sperm injection (ICSI) in 1992, a man with NOA can be a genetic father if it is possible to obtain viable sperm directly from his testis. Classically, there are two ways to perform it: percutaneously (TESA – TEsticular Sperm Aspiration) through fine needle aspiration (FNA) - which is dependent on a small amount of sampling - or surgically. Nowadays, there are two basic surgical techniques to retrieve sperm: conventional TEsticular Sperm Extraction (cTESE) and testicular microdissection (or also micro--TESE), which is the topic of this session Difference of Opinion.

Until recently, cTESE was considered gold standard for retrieving sperm in men with NOA (6). During a cTESE procedure, the testis is exposed through a small incision and one or multiple biopsies - more commonly - are taken randomly under direct sight (6). According to Donoso et al., cTESE has an average retrieval rate around 50% in NOA men (6, 7). This procedure is performed under general or regional anesthesia in a daily-basis clinical center.

Testicular microdissection was first introduced in 1999 (6, 8). Micro-TESE consists of an equatorial testicular opening in order to retrieve engorged seminiferous tubules that are more likely to contain active spermatogenesis, with the use of a surgical microscope (6). This procedure requires admittance to a hospital and general or regional anesthesia.

Microdissection-TESE (micro-TESE) versus conventional TEsticular Sperm Extraction (cTESE)

Some authors have shown that microdissection is the best way to perform sperm retrieval in men with NOA, not only in terms of sperm retrieval (SR) but also when considering complications to the technique itself. Three recent studies are more representative of this subject, a systematic review and meta-analysis (2), a systematic review (6) and a review (4), which deserve the following considerations:

Sperm retrieval

Bernie et al. performed a systematic review and meta-analysis that identified fifteen studies of 1,890 total patients, published between 1997 and 2012 (2). In a direct comparison of cTESE to micro-TESE, the unadjusted SR was 35% for cTESE (95% CI 30%-40%) and 52% for micro-TESE (95% CI 47%-58%) (2). Performance of micro-TESE was 1.5 times more likely (95% CI 1.4-1.6) to result in successful SR as compared with cTESE (2).

The aim of the study by Deruyver et al. was to compare the outcome of cTESE with micro-TESE through a systematic review of the literature comparing these two methods (6). Primary outcome was sperm retrieval rate in the micro-TESE group and in the cTESE group. Secondary outcome was other clinical predictors of positive sperm retrieval. Seven studies were included: two were prospective, non-randomized studies (Schlegel, 1999; Amer et al., 2000) (8, 9). Three studies were retrospective (Okada et al., 2002; Tsujimura et al., 2002; Ramasamy et al., 2005) (10-12) and the two remaining studies were pseudo-randomized controlled studies (Colpi et al., 2009; Ghalayini et al., 2011) (13, 14). The SR in the cTESE group ranged from 16.7 to 45% and in the micro-TESE group from 42.9 to 63%. Five of the seven studies showed a significant difference ($p < 0.05$) in favor of micro-TESE.

Sertoli cell only syndrome, a histological condition characterized by absence of germ cells with only normal Sertoli cells lining the seminiferous tubules predicted a significant better result in the micro-TESE group according to two studies (Okada et al., 2002; Ghalayini et al., 2011) (10, 14). Results ranged from 22.5 to 41% in the micro-TESE and from 6.3 to 29% in the cTESE group. No safe clinical predictors of sperm retrieval were demonstrated for both procedures (6).

In their review, Schlegel et al. reported an overall experience result with micro--TESE of 52% (607/1176) sperm retrieval rate including post-chemotherapy, Klinefelter's syndrome, cryptorchidism and AZFc deletion patients (4). According to the authors, for men who undergo cTESE and fail to have sperm retrieval, a repeat cTESE causes further testicular damage with limited success (4). In case of a failed cTESE, a salvage micro-TESE can be offered and sperm retrieval is possible in 45% of times (4, 15).

Complications

Aside from better sperm retrieval results, micro-TESE represents the technique with lower chances of complication. Comparing with micro-TESE, possible complications after cTESE are low but include loss of significant amount of testicular tissue, hematoma, inflammatory changes and permanent devascularization (6, 16). With this in mind, a possible advantage of the micro-TESE technique is a better identification of sub-tunical vessels and, as a consequence, reducing the risk of devascularization (6).

Although fewer sonographic complications may occur after micro-TESE, clinical complication rate between both procedures seems not to differ (6). Three of the included studies systematically compared the sonographic changes at different months of follow-up (Amer et al., 2000; Okada et al., 2002; Ramasamy et al., 2005) (9, 10, 12). Hematoma was less frequent in the microTESE group after 1 and 3 months. Fibrosis and decreased testicular volume (> 2 mL) were also less frequent in the micro-TESE group at 6 months. In the study of Okada et al., a significant decrease in serum testosterone after 6 months was observed in two patients in the cTESE group, whereas none occurred in the micro-TESE, although this was not statistically significant (9). Ramasamy et al. reported no significant difference in return to baseline testosterone levels between the two procedures (12).

Limitations

Histological findings from the testis of men with NOA vary and may include Sertoli cell only syndrome, maturation arrest (precocious or late) or hypospermatogenesis.

According to Bernie et al., the difference in sperm processing, the patient heterogeneity that exists in the population of men diagnosed with NOA, and the practice patterns and differing surgeon skill levels often

make it difficult to know the true differences between the extremely varied SRs for these procedures (2).

The choice of SR technique to perform in a man with NOA is not only dependent on the predicted SR, but also should be oriented by previous procedure history, knowledge of testicular pathology, potential for postoperative complications, cost of the procedure, and knowledge and skill of the surgeon (2).

A considerable number of cases of surgeon experience are necessary to reach a relative plateau level of SR, and at least 50 cases are needed to pass the steepest portion of the learning curve. Subtle continued increases in SRs appear to occur as a surgeon exceeds experience with more than 500 micro-TESE procedures (4).

When it comes to duration of the procedure and cost, in comparison with cTESE, micro-TESE procedures are much more time-consuming and require the use of an operating microscope, which increases the cost of the technique (6).

In the review published by Schlegel et al., the mean operative time was 1.8 h (range 0.5-6.6 h) for successful micro-TESE and 2.7 h (range 0.8-7.5 h) for attempts in which sperm were not found (4). Besides, a higher number of embryologists is necessary to look for sperm during the whole attempt.

Final considerations

As already known, it is very difficult to consider pregnancy rates after intervening in the male factor because they involve female potential impact which is not always evaluated in the studies. According to Bernie et al., because of incomplete reporting, analysis of other patient characteristics and outcomes (e.g., pregnancy) was not possible in their work (2). On the other hand, Schlegel et al. reported a pregnancy rate of 48% out of 1,414 overall experience NOA men cycles (4).

Furthermore, so far no clinical studies have compared birth rate between cycles using spermatozoa retrieved through cTESE and micro-TESE procedures (6).

A reason for bias in the studies that compare cTESE and micro-TESE is the fact that the latter is usually indicated in more severe situations.

Therefore, SR through micro-TESE may actually be artificially lowered by the fact that many men undergoing micro-TESE have failed a previous TESA or cTESE, suggesting that if all men treated with NOA were randomized from the very beginning, the difference between micro-TESE and cTESE might be even more pronounced (2).

CONCLUSIONS

Recent studies have shown that testicular microdissection is the best way to retrieve sperm from men with non-obstructive azoospermia.

Although micro-TESE provided the highest SR in these analyzes, the authors do not necessarily recommend that this be the only method of SR performed in men with NOA. Studies with standardized reporting are necessary that may allow for a better understanding of the true differences in SRs for each technique in men with NOA, as well as help to guide when it may be reasonable to perform a particular procedure (2).

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Renato Fraietta, M.D, PhD

Setor Integrado de Reprodução Humana,
Universidade Federal de São Paulo
Rua Sena Madureira, n.º 1.500
Vila Clementino, 04021-001, São Paulo, SP, Brasil
E-mail: fraietta@uol.com.br

DIFFERENCE OF OPINION

Vol. 44 (6): 1067-1070, November - December, 2018
doi: 10.1590/S1677-5538.IBJU.2018.06.03

Microdissection is the best way to perform sperm retrieval in men with non-obstructive azoospermy? | *Opinion: No, there are other options*

Marcelo Vieira [1, 2]

[1] Membro Titular da Sociedade Brasileira de Urologia, Rio de Janeiro, RJ, Brasil; [2] Urologista do Projeto ALFA, São Paulo, SP, Brasil

Keywords: Azoospermia; Microdissection; Sperm Retrieval; Fertility

In the last 23 years, Intracitoplasmic Sperm Injection (ICSI) has given non-obstructive azoospermic man the opportunity to become biological fathers, if sperm could be found in their testicles. These men present the biggest challenge in the routine of infertility clinics around the World, since there are no positive, clinical or laboratory, prognostic factors for sperm recovery. Once testicular sperm has been regularly used for ICSI, discussion about which technique for testicular sperm retrieval has been done. Sperm can be harvest from testicular parenchyma by: open biopsy (Testicular Sperm Extraction-TESE), percutaneous aspiration (Testicular Sperm Aspiration), open guided biopsy by previous cytology (Testicular fine-needle Aspiration) and open biopsy using microsurgery technique (Testicular Microdissection). The proposed techniques have the same objective, to find sperm with minimal testicular damage and in a reproducible way (1).

TESE can be done by a large longitudinal incision on the testicular albuginea and excision of a representative large testicular fragment where multiple tubule samples can be examined for sperm presence. A variation is a multiple biopsy approach by incision of multiple sites and searching for sperm in each fragment. Both techniques have similar results once spermatogenesis is diffusely distributed, but sometimes very sparse, making it difficult to find sperm by random biopsies (2).

Testicular fine needle aspiration (TFNA) had been defined for histologic testis examination, and brought to infertility use as a tool to identify spermatogenesis foci and guide sperm retrieval for ICSI. A simple procedure done under local anesthesia that correlates almost 90% with histology, although it is necessary a second intervention for sperm retrieval (3).

Testicular Microdissection described by Schlegel brought a new concept for sperm retrieval by using optical magnification to identify spermatogenesis foci based on the morphological testicular tubules characteristics and initial better results with minimum parenchyma amount excised (4).

The key questions are: what sperm recovery rate (SRR) is considered good and how to find spermatogenesis foci with minimal testicular damage? Multiple sampling, fine needle citology or magnification? The present techniques have positive and negatives aspects, but the debate about those aspects was interrupted after testicular microdissection, despite different experiences.

Sperm recovery rate for TESE varies among published data:Silber et al. 1997, 51%; Ostad et al. 1998, 58%; Tournaye 1999, 48%; Amer et al. 1999, 49%; Silber 2000, 55%; Bettella et al. 2005, 59%. All papers report SRR around 50%, and varieing according histologic findings (2, 5-9).

Testicular microdissection SRR was reported in a review from 42 to 63% and Schlegel's group showed 52% after 1,414 cases (10, 11).

Comparison between the different techniques is quite difficult because of the differences in histologic paterns, but published data shows an advantage for Microdisscetion TESE, although SRR for TESE in analized papers was bellow 50% (16.5-45%) (12, 13). A prospective study done by Ghalayini et al. showed significant difference in SRR for testicular microdissection compared with conventional TESE, but in other studies the median SRR for TESE was under 40%; this could be explained by taken only 3 samples from upper, median and lower testicular portion. FSH and testicular volume were prognostic factors for sperm retrieval adding some more discussion on the theme; papers differ on that opinion, and the classification chosen by the authors for FSH levels and testicular volume may have justified the results (14). One fundamental aspect discussed by authors was the fact that histological evaluation showed 61% of sperm on tissue retrieved for anatomical exam, enlightening the importance of micromanipulation laboratory for better SRR.

Microdissection TESE also have showed published irregular results as reported in one study conducted in Japan between 2014-2015 with 83% response rate from 47 infertility centers in the country analyzing the treatment results of 7,268 patients. Azoospermia was present in 1185 patients. Conventional TESE was performed in 231 patients with 98.3% sperm retrieval rate (SRR) and 56.2% pregnancy rate. Testicular microdissection was performed in 695 patients with 34% SRR and 11.8% pregnancy rate. The question with these data is the absence of clear azoospermia classification, once they showed good results for conventional TESE, probably because they were treating obstructive azoospermia. The most important conclusion about these data was the low SRR for testicular microdissection among Japanese certified specialists revealing some difficulty in finding classic dilated seminal tubules which sustains spermatogenesis (15).

Testicular damage is caused by injury of sub albugineal vessels and may be verified by symptoms, ultrassonographic changes and hormonal levels. Ultrasonographic evaluation after TESE showed parenchyma hematomas and acute inflammatory alterations (82% and 64% after 3 and 6 months respectively) and 2 patients complained about unilateral testicular atrophy (3%); unfortunately about 50% of the initial patients had not the ultrasonography done (16). Post TESE testosterone levels data were unconclusive in two studies showing divergents results (17, 18). The excision of a large sample or multiple biopsies are hypothetic more harmful to sub albugineal arteries and the use of magnification may avoid the damage and subsequently scars and testicular atrophy, but requires surgical microscope (2). TFNA also can diagnose sperm in testicular parenchyma, with minimal damage showed by immediate post-operative ultrasound and with a good cytology/histology correlation but demands a second intervention for sperm recovery (3).

We propose a different technique that was inspired on testicular fine needle aspiration (TFNA) together with TESE. The idea is to associate mapping from TFNA and the better amount of tissue for analysis provided by TESE, with no need for a second **procedure on ICSI day, that we called Open Testicular Mapping (OTEM).** Under sedation and cord block the testicle is delivered through a median scrotal incision and multiple testicular punctures are made in the tunica albuginea using a 19-gauge needle. The needle is used to open a tiny hole in the tunica. With compression of the testicle a portion of testicular tissue protrudes and is pulled out with the help of two microsurgical tweezers. The testicular samples are placed on a sterile Petri dish containing 0.6 mL of culture medium, minced with micro scissor and finally passed through a 24-gauge angiocatheter. The analysis is done by FIV la-

boratory personal under inverted microscope using 400 X magnification for sperm presence after each collection; if enough sperm to inject oocytes is found, the procedure is concluded. If no sperm is found, a new hole is performed with a new testicular tissue sampling. Six holes are made on upper, middle and lower portion of the testis, when necessary. One sample from the middle portion is sent for histological examination. OTEM iss usually performed the afternoon before ovary aspiration, first on the right testis, upper portion, from medial to lateral, except when left testis is larger or the right absent. If no sperm are found on the first testis, the contra lateral is approached in the same way.

We presented at European Society of Human Reproduction and Embryology 2018 meeting a retrospective study of patients presenting with non-obstructive azoospermia from 2008 to 2016 evaluating 92 patients submitted to OTEM and ICSI. SRR was 54% (50/92). The most frequently found histologic pattern was maturation arrest (43), followed by Sertoli cell only (23), hypospermatogenesis (15) and testicular atrophy (11). SRR for each pattern was 48% (21/43) for maturation arrest, 43% (10/23) for Sertoli cell only, 86% (13/15) for hypospermatogenesis and 54% (6/11) for testicular atrophy.

We are comfortable in offer to our patients a SRR above 50%, theoretically with minimal vascular damage once the albuginea isn´t open and without extended parenchyma dissection. The tactic of use of a needle for perforation in a non- vascularized area and not a tunica incision may prevents hematomas and parenchyma's devascularization. We are confident that for better sperm retrieval the extrusion of a group of tubules and pulling them out entirely is preferred rather than a multiple cross tubule section. We do hope to enhance our experience for large sample results for publishing.

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Marcelo Vieira, MD

Rua Cincinato Braga 37
São Paulo, SP, 01333011, Brasil
E-mail: marcelovieira.uro@uol.com.br

REVIEW ARTICLE

Vol. 44 (6): 1071-1080, November - December, 2018
doi: 10.1590/S1677-5538.IBJU.2017.0693

A review of the possibility of adopting financially driven live donor kidney transplantation

Aline Adour Yacoubian [1, 2], Rana Abu Dargham [1, 2], Raja B. Khauli [1, 2]

[1] Department of Surgery and [2] Division of Urology and Renal Transplantation, American University of Beirut Medical Center, Beirut, Lebanon

ABSTRACT

Kidney transplantation for end-stage renal disease remains the preferred solution due to its survival advantage, enhanced quality of life and cost-effectiveness. The main obstacle worldwide with this modality of treatment is the scarcity of organs. The demand has always exceeded the supply resulting in different types of donations. Kidney donation includes pure living related donors, deceased donors, living unrelated donors (altruistic), paired kidney donation and more recently compensated kidney donation. Ethical considerations in live donor kidney transplantation have always created a debate especially when rewarding unrelated donors. In this paper, we examine the problems of financially driven kidney transplantation, the ethical legitimacy of this practice, and propose some innovative methods and policies that could be adopted to ensure a better practice with accepted ethical guidelines.

ARTICLE INFO

Keywords:
Kidney Transplantation; Kidney Diseases; Review [Publication Type]

Int Braz J Urol. 2018; 44: 1071-80

Submitted for publication:
December 21, 2017

Accepted after revision:
May 23, 2018

Published as Ahead of Print:
June 20, 2018

INTRODUCTION

Living related kidney donation evolved significantly between 1960s and 1970s and became a routinely acceptable practice (1). With the improvements and availability of maintenance dialysis in the 1980s and 1990s, deceased donor kidney transplantation led to enhanced numbers but with limited success (1). Traditional cultural beliefs continue to persist in some countries like in China wherein dead bodies should be kept intact and no organ should be used for donation (2). The gap between supply and demand of kidneys continues to rise and is expected to rise more with a clear inconsistency between the number of transplants and the number of patients on the waiting list (3). For instance, in China, around 1.5 million Chinese patients are placed on the organ waiting list every year, while less than 1% receive an organ, because only relatives are allowed to donate (2). Absence of donors in Qatar has obliged most patients with end-stage renal disease to seek commercial donors abroad and return with high postoperative complications (4). Anecdotal evidence also shows that commercial kidney transplants take place in third world countries such as in India, Pakistan, Cambodia, Sri Lanka wherein potential recipients or patients may seek poor donors (5).

The most commonly accepted method of live kidney donation, altruism, remains insufficient since it does not help halt the illegal buying and selling of kidneys (1). Altruism occurs very rarely due to its challenges in trying to find such donors. Although many countries, like China, have initiated the deceased donor organ donation, the issue of shortage has not been solved (2). Additionally, the paucity of deceased donor organs has recently contributed to the surge of living unrelated transplants (1) as evidence shows that even if supposedly all kidneys were supplied from deceased donors, the supply would still not be enough to satisfy the increasing demand (6). However, the solution of living unrelated donation especially with commercialization has resulted in ethical dilemmas.

In 2008, the Transplantation Society in Turkey organized the International Summit on Transplant Tourism and Organ Trafficking. The Summit released the Istanbul Declaration which emphasizes the importance of preventing organ trafficking and transplant commercialism and encourages legitimate transplantation protocols (1). However, severe organ scarcity along with increasing suffering and death of patients on waiting lists have overpowered the rejection of commercialization and altruistic paradigm (7). On another note, living donation seems promising as it aims to add the number of donor organs and enhance the overall efficacy of transplants (6); it can also reduce trafficking, but waiting lists continue to grow (8). As a result, a great focus has been put on integrating financial rewards to increase the number of unrelated living donations rather than relying solely on altruistic donors.

The American Society of Transplantation's Live Donor Community of Practice organized a Consensus Conference on Best Practices in Live Kidney Donation in 2014 (9). The group generated the following guidelines: assign resources for standardized reimbursement of lost wages and incidental costs for live kidney donation; pass legislation to propose employment and insurability protections to live kidney donations; generate live kidney donation financial toolkit to deliver standardized and evaluated education to donors and providers about options to increase donor coverage and reduce financial effect within the current climate; and endorse additional research to recognize possible barriers to living donation and live kidney donation to ensure the creation of potential strategies (9).

In this review, we highlight the different types of kidney donation and emphasize the ethical dilemmas in financial rewards for living kidney donors, and discuss the reasons for the emerging of compensation in donation with a focus on some known models of compensation for unrelated kidney donation as practiced by some countries.

MATERIALS AND METHODS

A comprehensive search was made on Pubmed for studies, review papers and meta-analyses discussing different types of kidney donation, financially driven kidney transplantation and the ethics revolving around compensation. The inclusion criteria were based on the most relevant, most recent and most cited studies present in Pubmed. A summary table of the studies is presented in a Table-1.

DISCUSSION

The Middle East region features some insufficiencies in the transplantation mechanism. These comprise inadequate preventive medicine, uneven health infrastructure, and poor awareness in the general public and medical community about organ donation. Severe organ shortage and political instability have resulted in major unethical practices such as transplant tourism and organ trafficking (1), even though the Consensus Conference on Best Practices in Live Kidney Donation aimed to achieve financial neutrality for live kidney donations (9).

Several studies were conducted to poll the opinion of the general public, health professionals and patients in regards to this issue. Questionnaires completed by medical and nursing staff at West London Renal Transplant Center showed that the highest acceptable mode of donation reported by the participants was from blood related donors (100%) followed by non-blood relatives and friends (92.6%) and strangers (47.2%). Direct financial rewards were not considered an important motive

Table 1 - Summary of discussed studies.

Author and year of publication	Sample size (if present)	Findings
Ghods, 2009 (1)	Not applicable	Iran has a 20-year experience with a compensated and regulated living unrelated kidney donation program. This transplantation model was adopted in 1988 and was able to eliminate kidney transplant waiting list in 1999.
Wu & Fang, 2013 (2)	Not applicable	Financial compensation policy initiated in five pilot provinces and cities in China helped increase the concept of organ donation.
Ghahramani et al., 2013 (3)	Survey of 1280 nephrologists from 74 countries.	Thirty-seven percent agreed with the provision of free life-long health insurance to donors. Forty-nine percent agreed with some form of compensation, and 26% agreed with direct financial compensation for living donors. Thirty-one percent believed that living unrelated donors should receive financial rewards, while 23% favored rewards to related donors. Twenty-seven percent were in favor of financial rewards for families of deceased donors.
Alkuwari et al., 2014 (4)	Not applicable	Hamad Medical Corporation initiated the Doha Donation Accord (DDA) in 2010 to develop deceased organ donation and live related kidney transplantation prohibiting trade in human organs and financial rewards for organ donation. It covers expenses throughout the whole process.
Chapman, 2018 (5)	Not applicable	A review paper discussing organ trafficking and transplant commercialism.
Akkina et al., 2011 (6)	Not applicable	A review discussing donor exchange programs.
Schweda & Schicktanz, 2009 (7)	Focus group discussions with 66 European citizens.	The group resisted organ commercialization. Many respondents stated that the altruistic form of donation is not a one-way relationship, but is based on mutual exchange.
van Buren et al., 2010 (8)	Survey of 250 living kidney donors.	Almost half of the respondents were in favor of financial compensation for living donors by the government. The majority of the living donors would not have wanted any financial reward for themselves, because they donated a kidney out of love for the recipient or altruistic principles.

Tushla et al., 2015 (9)	Not applicable	Consensus Conference on Best Practices in Live Kidney Donation took place in 2014. The following recommendations were established: (1) allocate resources for standardized reimbursement of living kidney donors' lost wages and incidental costs; (2) pass legislation to offer employment and insurability protections to living kidney donors; (3) create an living kidney donor financial toolkit to provide standardized, vetted education to donors and providers about options to maximize donor coverage and minimize financial effect within the current climate and (4) promote further research to identify systemic barriers to living donation and living kidney donor transplantation to ensure the creation of mitigation strategies.
Mazaris et al., 2009 (10)	Survey completed by 108 medical and nursing staff in a Renal and Transplant center in London.	Live donor kidney transplant was considered ethically acceptable between blood relatives (100%), non-blood relatives and friends (92.6%) and strangers (47.2%). Around 34.3% believed there should be no financial reward, not even compensation for expenses, for donors; 8% favored direct financial rewards for donors known to recipients and 18% favored rewards for donors not known to recipients, while 57.4% of respondents supported compensation for expenses incurred for donors known to the recipient and 50.0% supported this kind of compensation when the donor was a stranger.
Mazaris et al., 2011 (11)	There were 464 participants (63.8% patients and 36.2% health-care professionals).	Around 80% were willing to donate to children, siblings, parents; around 70% to non-blood relatives or friends and around 15% to strangers. Around 50% were willing to receive a kidney from a stranger versus 80% from parents, siblings, children or relatives and friends. Around 29% did not approve financial reward for donors and 60% approved covering expenses for donors.
Peters et al., 2016 (12)	There were 1011 respondents from the US (427 males and 584 females).	Around 65% were willing to donate a kidney to anyone and around 59% were willing if a payment of $50,000 was made.
Kute et al., 2014 (13)	There were 56 patients and 140 KPDs in a single center in India between 2000 and 2013.	For the 56 KPD transplantations, graft survival was 97.5%. KPD was done to avoid blood group incompatibility (n = 52) or positive cross-match (n = 4).
Mierzejeweska et al., 2013 (14)	Not applicable	A review paper discussing improvement in transplant numbers in several countries that have adopted KPD.
Pham et al., 2017 (15)	Not applicable	A review discussing KPD and desensitization.
Kute et al., 2017 (16)	There were 3616 living donor kidney transplantations, 561 deceased donor kidney transplantations.	There were 300 transplants done by KPD in a single center in India between January 2000 and July 2016.

Catwell et al., 2015 (17)	Not applicable	A review paper discussing the four years' experience of KPD in Australia.
Kute et al., 2017 (18)	There were 380 KPD transplantations.	There were 77 transplants done by KPD in a single center in India between 1 January, 2015 and 1 January, 2016. The reasons for KPD were ABO incompatibility (n = 45), sensitization (n = 26) and better matching (n = 6).
Ghods and Savaj, 2006 (19)	Not applicable	A review paper discussing the Iranian model which was adopted in 1998 to regulate and compensate living-unrelated donor renal transplant program and has helped decrease the number of patients on the waiting list.
Bailey et al., 2016 (20)	Semi-structured interviews with UK 32 deceased-donor kidney transplant recipients.	The following themes were identified for those who were against altruistic donation: Prioritizing other recipients above self; fear of acquiring an unknown donor's characteristics and concern for the donor for unnecessary risk. For those willing to accept a non-directed altruistic living donor kidney transplantation the following themes were identified: Prioritizing known above unknown persons, belief that they are as deserving as other potential recipients, and advantages of a living donor kidney transplantation.
de Castro, 2003 (21)	Not applicable	A review paper discussing commodification of human organs.
Ghods et al., 2001 (22)	There were 1000 patients in Iran (500 living unrelated donors and 500 recipients).	The majority of living unrelated donors (84%) were poor and no single wealthy individual was listed in the category.
Friedman, 2006 (23)	Not applicable	A review paper discussing the need to legalize payment for living organ donation to prevent exploitation of organs.

by the majority of participants wherein a substantial minority favored direct financial returns (10). Another study exploring attitudes towards live kidney donation and commercialism was conducted among 1105 participants from health professionals and patients. Most participants accepted the idea of alternative types of donation while the minority (10%) of the participants found that commercialism is acceptable (11). An international survey investigated the attitudes and perceptions of nephrologists. The study showed that 49% of the physicians expressed agreeable attitudes towards some form of compensation with the higher number from the Middle East region, while 66% mentioned that financial rewards will contribute to an increase in living kidney donation (3). Another study in the Netherlands conducted on 250 donors showed that 20% would have wanted some forms of financial compensation for their donation and 47% wanted a decreased fee or a free health insurance premium (8). In a survey completed by 1011 participants in the United States (US), 68% were willing to donate and 59% would be more compelled to donate if a monetary sum was given (12).

Since the greatest obstacle in kidney transplantation is the limited availability of deceased / living kidney donors, all possible solutions to increase access to kidney transplant should be taken into consideration before resorting to paid dona-

tion. This has given rise to another type of donation known as kidney paired donation (KPD) that has been established to prevent commercial transplantation (13). It takes place when a potential kidney recipient who has a willing but incompatible live donor receives a kidney from the donor of another incompatible pair and vice versa (13). KPD programs have been established to increase availability of organs and to overcome several obstacles such as blood group incompatibility, tissue compatibility, highly sensitized recipients to donors, and improvement in transplant quality such as graft size and age difference (14). The first KPD took place in South Korea in 1991 (15). This model has been proven to be practical, legal, cost-saving and time-saving for facilitating living donor related transplant for patients who are incompatible with their healthy and willing living donor (13).

Numerous transplant centers have adopted this program with amounted increase in transplant numbers. For instance, in the US the number of paired kidney exchanges has increased from 2 in 2000 to 400 transplants annually (14). In India, there were 3616 living donor kidney donations and 561 deceased donor kidney donations in one center between January 2000 and July 2016, while 300 of these were through KPD. The success of the program was due to maintaining registry for incompatible pairs, good teamwork and counselling on KPD (16). In Australia, the KPD program was established in 2010 and the four years' experience has proven to achieve a sufficiently large pool of donors and recipients to be able to find the best match. It also helped highly sensitized patients by combining KPD with antibody removing strategies (17). KPD was also proven to be beneficial to unpaired patients since by removing all patients with potential living donors from the waiting list, and it will decrease their waiting time (17).

The KPD program requires a national database that is absent in some countries like in India, in addition to lack of coordination between transplant centers (18). There are still inefficiencies in this system; high number of patients waiting on the list (more than 500 patients in one center in India). Another hurdle is that blood group O recipients had lower rate of transplantation and high mortality rate due to financial incapability to afford dialysis (13). The authors also discussed the issue of putting more pressure on women to donate since KPD eliminates the excuse of incompatibility as there is gender imbalance with women donating more than men and receiving less donations than men (13). Moreover, desensitization has arisen for highly sensitized patients such as patients in need for repeat kidney transplants (15). Although this technique involves plasmapheresis, it is costly and there is the risk of increase in antibody levels. However, there are not many studies discussing the outcome of desensitization (15).

The past decade has witnessed an upsurge in innovative mechanisms for living kidney donation such as altruistic donation, KPD, donor chains and exchange programs (3). However, these strategies, especially the last three were not capable of increasing the kidney donor pool nor capable of alleviating renal transplant waiting lists (19). As a result, altruistic donation may seem ideal for kidney transplantation, yet safety and ethical concerns remain. Living donors are at risks without any direct medical benefit to themselves (1) putting their safety and welfare at risk. Another note to take into account is whether these altruistic donors have health insurance and can maintain their health insurance following donation (3) or whether their medical expenses can be secured by another party (1). Thus, what if a healthy and altruistic individual is willing to donate but there is no available center that offers free medical tests or post-op follow-up for any complications, especially in developing countries? Altruistic donation provokes a feeling of indebtedness and guilt to the recipients (7). These recipients may be willing to discover who their donor is when sometimes the system does not allow them or they may express worries about the nature of the kidney donated or about the characteristics of the donor. A qualitative study examined the attitudes of 32 renal patients (17 females and 15 males) towards non-directed altruistic living donation (20). Concerns were raised over transmission of donor characteristics, feeling responsible for the risks donors would be exposed to and feeling guilty (20). Nevertheless, providing compensation for altruistic donors does not essentially lead to exploitation, but may help minimize the level of exploitation that already exists in current organ procurement systems (21).

If it is already taken for granted that financial rewards for organ donation are pernicious and accordingly prohibited by laws worldwide, why do these rewards still receive heightened attention? Why are they practiced clandestinely? What are the reasons behind the rise of compensation in kidney donation? Possible explanations include: surge of commercial kidney transplants even in the presence of strict rules against them, limited success of altruistic donations and incapability of governments to secure organs through organized networks. Only under exceptional conditions, does the human being exhibit willingness for uncompensated transfers and generosity to others? (19). Altruistic donation failed to eliminate severe organ shortage and lessen the number desperate recipients on the waiting list (21). These desperate patients refer to other means to secure a kidney in developing countries whether they are natives or coming from developed countries (1). Therefore, altruistic donation has not been successful in providing kidneys to those in need and in shortening the waiting period (21). In developing countries, altruistic living unrelated kidney donations are less commonly experienced and these donors are usually secured through commercial transplants (1). Kidney markets are regularly seen in developing countries, since dialysis is usually not funded by the governments and deceased donor kidney transplantation is scarce due to poor and inefficient procurement mechanisms (1). However, this does not mean that all kidneys that have been purchased go through unfair practices. Many recipients rely on more 'negotiable' and 'democratic' ways for receiving kidneys wherein they try to financially secure kidneys from their relatives, friends or colleagues without imposing serious or direct harms to these donors (19).

There is a major argument against compensation of living unrelated kidney donation. It revolves around the concept of commodification. This argument assumes that there are limits to what can be sold and since organs are valuable and priceless then this means that they cannot be sold. Once a kidney is put into marketplace, it denies the definite valuable organ of a human being and denies his / her dignity and worth (21). The argument, at first glance, may seem convincing, but many objections follow. It is true that organs in general should not be sold or purchased, but in most cases the individual who has made the choice of donation is actually doing so for a valid reason and humane excuse. No one can imagine the circumstances that force the person to make a vehement decision of giving up one of his / her kidney (21). These persons have probably no other means of securing money and the reasons would not necessarily include luxury goods. Most donors actually decide to sell their kidneys either to provide financial support to their families or to secure money for other reasons (21). Commercialization seems to be a win-win-situation wherein both the donor and recipient benefit (7) provided that the donor has been compensated fairly and has not been abused. If commercialization is prohibited, not only will the recipient be put at disadvantage, but also the donor who was willing to make some money. However, the recipient may still win by travelling to another country to look for another donor or by trying to pay the same donor furtively in the same center by pretending to be relatives or friends. This shows how difficult it is to stop commercialization even when it is prohibited by law in the host country since recipients may travel to other countries to secure kidney.

What would seem a good solution to end the malevolent practice of kidney trafficking? In an attempt to explore the reasons donors propel to sell their organs, a primary overall factor appears: poverty. To compensate for such unethical practices, adopting a regulated system incorporated with financial incentives can help eliminate commercial transplants. This can be done when KPD has failed for a specific donor. An example of such a successful program is the Iranian model, initiated in 1988 and governed by a charitable organization called the Dialysis and Transplant Patients Association (DATPA). The DATPA provides compensation for those who provide kidneys without the need for a broker or mediator since the government covers for the hospital expenses, immunosuppressive drugs and provides award and health insurance to the unrelated donor (19). As a result, the number of renal transplants performed increased noticeably in Iran and the renal transplant waiting list was completely eliminated in 1999 (1). Prior to this model, most patients in Iran used to travel to India to undergo paid transplantation and many of these

transplants were associated with transmission of hepatitis and surgical complications (19). A controlled living unrelated donor renal transplant program was introduced in Iran in 1998 wherein volunteered living unrelated donors introduce themselves to the Dialysis and Transplant Patients Association without any need for middle men or agencies (22).

The concept of financial incentives was grouped in three categories. The first class focuses on the subject of compensation, wherein the donor is covered for the incurred detriments such as medical tests and examinations, health insurance, etc. This is important because many of the donors may not have insurance to cover for their tests and out-of-pocket expenses are linked to more extensive medical care in the long term (9). The second category is known as rewarded gifting that involves monetary or non-monetary form of appreciation without direct intention to encourage donation. Lastly, the market model is based on procurement and allocation of organs. Such a system will help control the existence of black markets, organ trafficking and coercive methods targeting underprivileged and economically disadvantaged persons (7). Donors should be competent enough to understand the risks and benefits of kidney donation and undergo psychological evaluation and sign an informed consent (1). In China, the Red Cross is the third party that is responsible for implementing the donation policy wherein they raise and manage funds for the financial compensation in a fair manner (2). This includes covering funeral expenses and financial assistance for the family of the donor in case the family was classified as a low-income family by the local Bureau of Civil Affairs (2).

What would be the benefits of a system that adopts a model similar to the Iranian model? Ghods elaborates this topic by addressing several concerns that could be raised by opponents of this type of donation. Patient and graft survival rates, as well as donor morbidity and mortality in Iran have comparable results when compared to conventional transplant centers in the country, since all donors are positively selected and screened for diseases (19). The Iranian model has not forced poor citizens to donate their kidneys due to its rewarding nature, as one study has indicated that among 500 participants, 50.4% of kidneys from paid donors were given to poor individuals (22). The model has not even eliminated the number of kidneys from deceased persons, as deceased-donor organ transplantation has increased steadily since its legislation from 1.8% in 2000 to 12% in 2004 and 2005 (19). As mentioned previously, this model also helped reduce the number of commercial or illegal transplants that used to take place prior to its introduction and the model also prohibits foreigners to benefit from this system (19). It is also important that donors display utmost understanding of the possible risks and complications of the surgery.

On the other hand, the Hamad Medical Corporation (HMC) has initiated the Doha Donation Accord (DDA) in 2010 which aims at meeting the needs for transplantation as to discourage patients from undergoing unsafe practices abroad (4). The DDA provides a broad health insurance for life for the donor, covers the whole incurred expenses and prioritizes the donor in case of end-stage organ failure. While this seems to be encouraging and has actually resulted in the reduction of transplant travel, the rise of recipients on the waiting list has increased remarkably from 21% in 2010 to 73% in 2014 (4). The National Living Donor Assistance Center (NLDAC) was initiated in the US in 2007 wherein donors were provided financial support to travel to the transplant center for eligible living donation (9). The program received 3918 applications until August 2013 and approved 89% of them and 74% of donors mentioned that they would have not donated without the support of NLDAC (9). The question remains whether the country is able to fulfill this high demand when solely relying on living related donors. Although the DDA seems to be more ethical than the Iranian model, it is inevitable to state that the DDA model is not applicable to most countries since they cannot afford to have such a system especially noting that Qatar is a wealthy country with a high Human Development Index.

If countries decide to adopt a model that provides financial reward to donors, payment should be governed by a balanced, objective and multidisciplinary body which determines standardized protocols for donors and recipients as well as a uniform fee (23). If countries are not ready to accept such a model due to cultural reasons or financial difficulties, it these rely heavily on raising awareness about organ donation. When asked about ways to encourage living donation, 50% of the 250 donors mentioned that the media

plays a major role especially when living donors discuss their experiences to the public (8). Media can a play a robust role in encouraging deceased organ donation or altruistic organ donation.

CONCLUSIONS

Kidney transplantation is currently the accepted mode of renal replacement therapy, which provides long-term and robust survival advantage (10). A drawback represents the shortage of organ availability whether cadaver or living. This has given rise to the implementation of other strategies, each with its own challenges. Unethical practices still take place in countries with refugees or poor and displaced people (23). Many centers are not even able to detect the secret planned financial reward wherein they pretend to be relatives in order to get accepted for transplantation. It is important to start considering financial compensation in order to protect the indigent donors and avoid organ trafficking. Thus, the idea of initiating governmental supervision with regulated compensation to living donors should be revisited, especially that no alternative solution is available until today (23).

ACKNOWLEDGEMENTS

Aline Yacoubian and Rana Abu Dargham contributed similarly as first author.

CONFLICT OF INTEREST

None declared.

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Correspondence address:
Raja B. Khauli, MD
American University of Beirut Medical Center
P.O. Box 11-0236; Riad El-Solh / Beirut 1107 2020
Lebanon, Beirut, 1107 2020, Lebanon
E-mail: rkhauli@aub.edu.lb

ORIGINAL ARTICLE

Vol. 44 (6): 1081-1088, November - December, 2018
doi: 10.1590/S1677-5538.IBJU.2017.0328

Lowering positive margin rates at radical prostatectomy by color coding of biopsy specimens to permit individualized preservation of the neurovascular bundles: is it feasible? a pilot investigation

Leslie A. Deane [1], Wei Phin Tan [1], Andrea Strong [1], Megan Lowe [1], Nency Antoine [1], Ritu Ghai [1], Shahid Ekbal [1]

[1] *Department of Urology, Rush University Medical Center, Chicago, IL, USA*

ABSTRACT

Objective: To evaluate whether color-coding of prostate core biopsy specimens aids in preservation of the neurovascular bundles from an oncological perspective.
Materials and Methods: MRI guided transrectal ultrasound and biopsy of the prostate were performed in 51 consecutive patients suspected of being at high risk for harboring prostate cancer. Core specimens were labeled with blue dye at the deep aspect and red dye at the superficial peripheral aspect of the core. The distance from the tumor to the end of the dyed specimen was measured to determine if there was an area of normal tissue between the prostate capsule and tumor.
Results: Of the 51 patients undergoing prostate biopsy, 30 (58.8%) were found to have cancer of the prostate: grade group 1 in 13.7%, 2 in 25.5%, 3 in 7.8%, 4 in 7.8% and 5 in 3.9% of the cohort. A total of 461 cores were analyzed in the cohort, of which 122 showed cancer. Five patients opted to undergo robotic assisted laparoscopic radical prostatectomy. No patients had a positive surgical margin (PSM) or extra prostatic extension (EPE) on radical prostatectomy if there was a margin of normal prostatic tissue seen between the dye and the tumor on prostate biopsy.
Conclusion: Color-coding of prostate biopsy core specimens may assist in tailoring the approach for preservation of the neurovascular bundles without compromising early oncological efficacy. Further study is required to determine whether this simple modification of the prostate biopsy protocol is valuable in larger groups of patients.

ARTICLE INFO

Keywords:
Robotic Surgical Procedures; Prostatectomy; Laparoscopy

Int Braz J Urol. 2018; 44: 1081-88

Submitted for publication:
January 08, 2018

Accepted after revision:
April 25, 2018

Published as Ahead of Print:
June 20, 2018

INTRODUCTION

Prostate cancer remains the most common solid organ malignancy in men globally with almost 181.000 new cases in the United States in 2016 (1). Albeit a topic of tremendous controversy, there have been significant advances in screening methods utilizing novel imaging, serum and urinary biomarkers. However, the diagnostic hallmark remains a transrectal ultrasound guided biopsy of the prostate for microscopic tissue and histopathological diagnosis (2, 3). A major advancement in this field was the introduction of multiparametric magnetic resonance imaging (mpMRI) of the prostate gland, conferring the ability to detect lesions with low, intermediate or high suspicion of being malignant (4). This development permitted targeted biopsies to be taken, focusing on these

suspicious areas in addition to standard sextant template biopsies.

However, the ability of mpMRI to distinguish extracapsular extension (ECE) from organ-confined disease when a lesion appears to be in contact with the capsule remains poor and has very limited sensitivity (5). There are several surrogates indicative of ECE including tumor contact length and PIRADS score of the lesion in question, perineural invasion and Gleason score, but determination of extension at a microscopic level is not possible at this time (6, 7). A multitude of nomograms have been validated to estimate the risk of ECE, seminal vesicle invasion, lymph nodal involvement, organ confined disease and the clinical decision whether to spare or resect the neurovascular bundles (NVB) is largely based on these combinations and the judgment and experience of the surgeon.

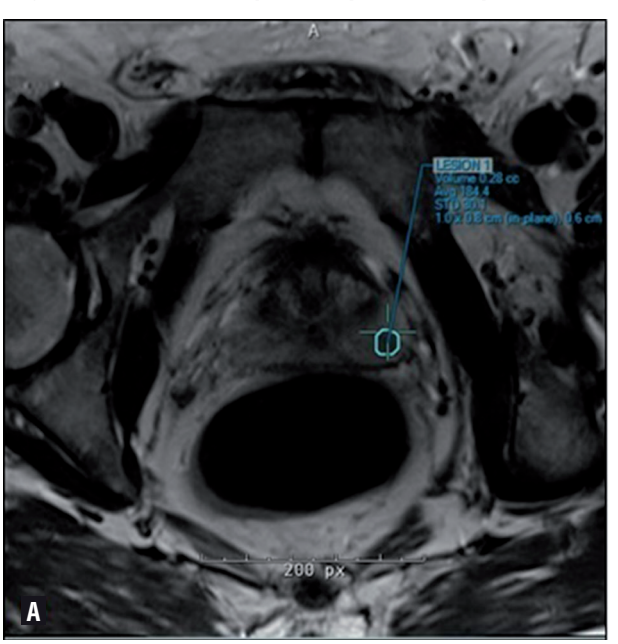
Unless peri-prostatic fat, extra-prostatic neural tissue or rectal mucosa remain adherent to the biopsy core, it is difficult to orient the specimen into superficial versus deep regions. Currently, to our knowledge, no attempts have been made to localize the site of the cancer within individual biopsy cores nor to quantify its distance from the capsule. This could have significant implications for altering the surgical plan especially now that mpMRI is gaining wide acceptance. We sought to evaluate whether a system for color-coding transrectal mpMRI guided fusion prostate biopsy cores could reliably show where cancer was located in relation to periphery (i.e capsular aspect of the sample) in comparison to the deep aspect of the sample (i.e distant from the capsule).

MATERIALS AND METHODS

We performed an Institution Review Board (IRB) approved retrospective analysis of a prospectively collected and maintained database consisting of all patients undergoing mpMRI transrectal ultrasound guided fusion prostate biopsy performed by a single urologist (LAD) for clinical features concerning the presence of prostate cancer. mpMRI of the prostate was performed using a Siemens MAGNETOM 3T machine (Munich, Germany) as previously described (8). Each MRI scan was reviewed and reported by an attending radiologist.

Each patient received prophylactic oral antibiotics (fluoroquinolone) prior to the procedure and an augmented regimen with an intramuscularly administered antibiotic if deemed high risk. Standard fleet enema was also administered the night before and at the morning of the procedure. All biopsies were performed transrectally using 3-dimensional modeling software (Invivo Corporation, Gainesville, FL, USA) and mpMRI / US fusion biopsy of the prostate was performed with an end-fire Philips iU22 transrectal ultrasound probe and sonographic system (Amsterdam, The Netherlands). A total of 5 cc of 0.5% bupivacaine was injected into the tissue in the angle of the seminal vesicle and prostate, and the periprostatic nerve plexus under transrectal ultrasonic guidance for intra-procedural patient comfort. Each biopsy sample was taken using an 18-gauge Bard MaxCore (Bard Medical Division, Covington, GA) and immediately handed to the circulating nurse or medical assistant. A minimum of 3 cores was taken from each suspicious lesion, largely dependent on the size of the volume hotspot on mpMRI (Figure-1). When obtaining the sample, a concerted effort was made to ensure that the needle insertion

Figure 1 - Volume hotspot on mpMRI of the prostate.



site was as close to a perpendicular entry across the capsule for peripheral zone lesions. The sample was placed onto a small square (3 x 4 cm) of Telfa (Kendall Telfa, Tyco Healthcare, Mansfield, MA) and verbally confirmed to be an intact core of adequate length. Pre-drawn insulin syringes with red dye (capsular aspect) and blue dye (deep aspect) (Davidson Tissue Marking System, Bradley Products Inc, Bloomington, MN) were used to color code each sample and the correct orientation was verbally affirmed prior to placing the sample into the specimen container, which was pre-labeled. A single drop of each color dye was applied to the core in its respective orientation. Each sample was allowed 30 seconds for the dye to dry prior to placing it into formalin in the specimen container and sent for pathological analysis.

Images from the mpMRI of the sample sites of each biopsy core were archived and saved for future reference and review (Figure-2). These were both 3-dimensional renderings and gray-scale images to demonstrate the path of the biopsy needle relative to each targeted lesion.

Each prostate biopsy core was read by a team of genitourinary pathologists and reviewed with the treating urologist. Each sample was reported as either benign or malignant, the latter being assigned a Gleason Score and allocated to Grade Groups 1 through 5 (Figure-3).

Figure 2 - 3-D image of prostate core acquisition.

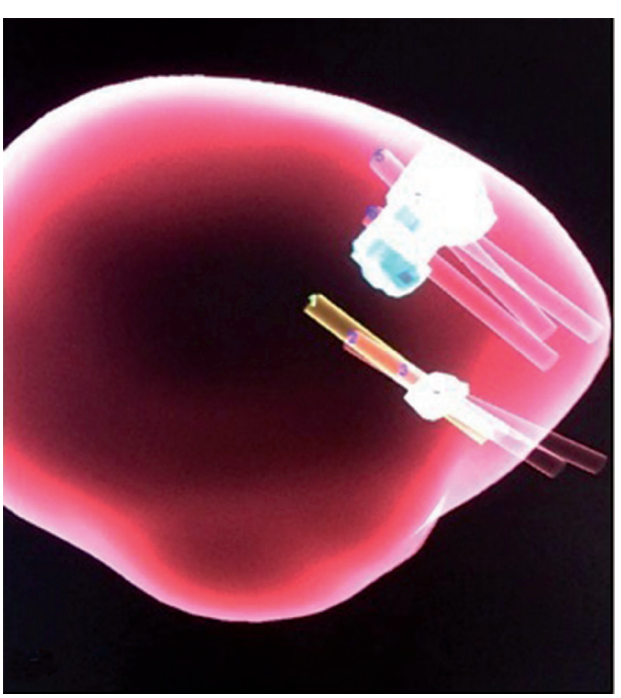
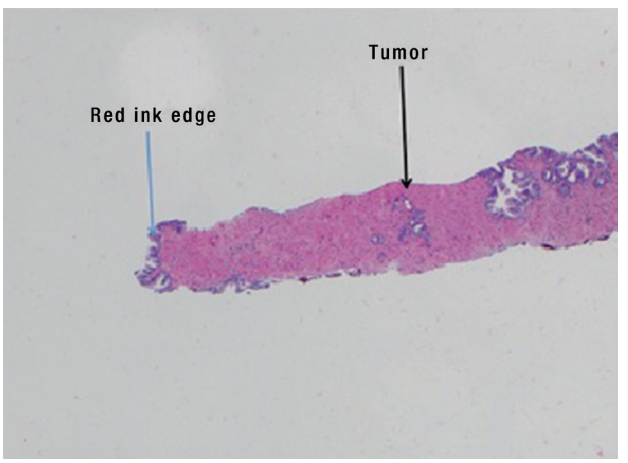
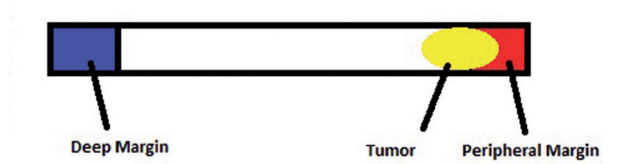


Figure 3 - Relationship of cancer to peripheral margin.



If cancer was present, the distance from the tumor to the end of the dyed specimen was measured to determine if there was an area of normal tissue between the prostate capsule and tumor (Figure-4).

Figure 4 - Example of tumor distance from end of dyed specimen.



Patients who were found to have cancer were informed of all treatment options available. For patients electing to undergo robotic radical prostatectomy, the decision for nerve sparing was

deemed based on established and accepted clinical standards and clinician oncologic judgment, irrespective of the distance of cancer from the dyed peripheral margin of the biopsy core. On all patients, on the involved side, complete neurovascular bundle excision was performed as per standard of care. On patients that did not have any cancer involving a particular side on mpMRI, only complete neurovascular bundle preservation was performed.

RESULTS

Of the 51 patients undergoing prostate biopsy, 30 (58.8%) were found to have cancer of the prostate. This was Grade Group 1 in 13.7%, 2 in 25.5%, 3 in 7.8%, 4 in 7.8% and 5 in 3.9% of the cohort. 21 patients (41.2%) did not have cancer on biopsy. A total of 461 cores were analyzed in the cohort, of which 122 showed cancer. The mean distance of tumor from the most superficial aspect of the red dye was 5.2 mm (Range 0 - 23 mm) (Table-1) (Figure-5). There were 10 cores with cancer < 1 mm from the red dye margin. 5 patients opted to undergo robotic assisted laparoscopic radical prostatectomy (RALP), 7 patients opted for radiation therapy, 1 patient switched provider and 17 patients were placed on active surveillance.

After reviewing these 5 patients in detail, all 5 patients had a lesion that appeared to be abutting or distorting the prostatic capsule (Table-2). Two patients had a lesion that was positive for cancer at the apex of the prostate and 3 patients had a lesion that was located in the lateral peripheral zone. Of the 2 patients that were positive for cancer at the apex, one patient had a tumor that distorted the prostatic capsule at the apex concerning of extracapsular extension seen on mpMRI while the other patient had a tumor abutting the prostate capsule on mpMRI. Both these patients had tumors involving the blue dye (deep) on the MRI / US guided fusion biopsy specimen and both these patients had ECE on the apical region on final pathology. This is because both these lesions were anterior and given the biopsy needle was activated from posterior to anterior, the blue dye represents the superficial aspect of the anterior prostate. Of the three patients who had tumors involving the red dye (superficial), one patient had tumor involving the edge of the

Table 1 - Details of Cohort.

PATIENT CHARACTERISTICS	
Age, median (first/third quartile)	67 (62/70) years
Ethnicity, n (%)	
Caucasian	17 (33)
African American	28 (55)
Hispanic	5 (10)
Indian	1 (2)
PSA, median (first/third quartile)	6.7 (5.0-10) ng/mL
Clinical Stage, n (%)	
No cancer	21 (41.2)
T1c	27 (52.9)
T2a	3 (5.9)
Gleason Grade Group, n (%)	
No cancer	21 (41.2)
1	7 (13.7)
2	13 (25.5)
3	4 (7.8)
4	4 (7.8)
5	2 (3.9)
Distance from red dye, median (first/third quartile)	4 (2-7) mm
PIRADS score (first/third quartile)	3 (3/4)

Figure 5 - Distance from peripheral margin to malignant cells.

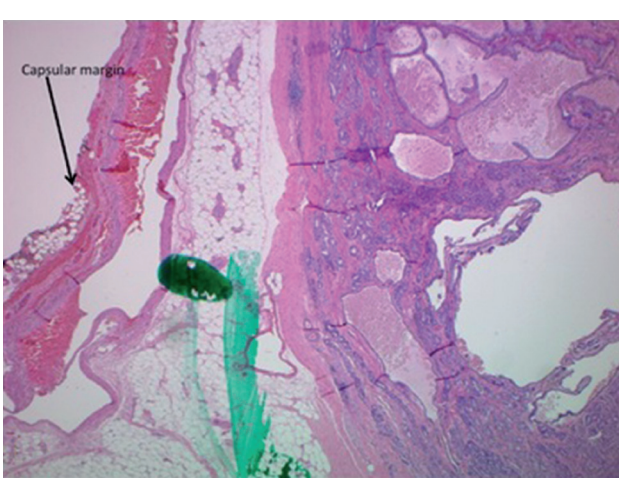
Table 2 - Pathological features of patients who underwent a radical prostatectomy.

Patient	Location of lesion on mpMRI	Distance of red dye from the edge of the biopsy specimen (mm)	Distance of blue dye from the edge of the biopsy specimen (mm)	Gleason Score on Biopsy	Gleason Score on Final Pathology	Extra Capsular Extension
1	Anterior Peripheral Zone		0; 0	4+3	3+4	Yes
2	Anterior Peripheral Zone		0; 0; 0; 0	4+5	4+5	Yes
3	Left Peripheral Zone	12; 9; 12; 1		4+4	4+3	No
4	Left Lateral Peripheral Zone	9; 8; 0		4+3	3+4	Yes
5	Left Lateral Peripheral Zone	3; 5; 5		3+4	3+4	No

red dye on the prostate biopsy specimen and he was found to have ECE on RALP. The other two patients that had a lesion in the lateral peripheral zone were found to have a margin between the red dye (superficial) and cancer. These two patients were subsequently found to be negative for ECE on radical prostatectomy, indicating that theoretically, the neurovascular bundle could have been spared.

No patients had a positive surgical margin (PSM) or extra prostatic extension (EPE) on radical prostatectomy if there was a margin of normal prostatic tissue seen between the dye and the tumor on prostate biopsy (Figure-6). The first post-operative prostate specific antigen level PSA was undetectable (< 0.01 ng / mL) in all patients undergoing surgery.

Figure 6 - Capsular margin seen at radical prostatectomy - Distance from capsule to malignant cells demonstrated.



The cost of the dye is $27.50 per 2 oz vial and this lasted throughout this entire initial pilot cohort. The total amount of dye used per biopsy was < 0.5 cc. The cost per box of insulin syringes is $17.38 and two syringes were used per patient at an additional cost of $0.35. As a result, the overall additional cost per biopsy was < $1.00, nominal when one considers the potential impact on surgical risk stratification and operative outcome, both oncologic and functional.

We have also confirmed that color coding the specimen does not alter the cellular architecture, nor does it affect the integrity of DNA / RNA material, thus having no potential for impacting genomic assessment of the tissue for further clinical risk analysis and stratification. There was 1 / 467 (0.002%) specimen core that was dyed incorrectly where the superficial and deep region of the core was inadvertently inverted. We realized this on pathology as there were fat cells and neural tissue seen on the specimen, thus confirming the true peripheral aspect of the core. The mpMRI images were reviewed in detail and showed the lesion in the central peripheral zone, and confirmed the trajectory of the biopsy needle.

DISCUSSION

The technique of prostate biopsy continues to evolve and the specialty has progressed from finger guided, hand activated tru-cut sampling, through ultrasound guided sextant sampling with a trigger activated biopsy gun, and now mpMRI transrectal ultrasound fusion guided biopsy (9, 10). Despite significant advances in the ability to image the prostate, definitive imaging evidence of ECE on mpMRI remains elusive, unless gross tumor extension is observed. The accurate identification and reporting of biopsy specimens and their orientation is clinically relevant when it pertains to in office breast biopsy, frozen section in the operating room, and surgical specimens in all specialties (11-13). However, this concept has not been applied for prostate cancer biopsy specimens. This pilot study sought to assess the feasibility of color-coding prostate biopsy core samples with a view to determining how close the tumor was to the capsule cancer and if indeed the capsule was involved.

We obtained early evidence to show that a "normal tissue" interface between capsule (most peripheral aspect of the core dyed red) and malignant cells may be associated with a similar "normal tissue" interface at radical prostatectomy. This may have potential implications for surgeons in the decision-making algorithm regarding NVB sacrifice versus preservation.

Employing clinical risk nomograms such as the Memorial Sloan Kettering Prostate Risk Stratification Nomogram (MSKCC Nomogram), Cancer of the Prostate Risk Assessment tool (CAPRA), Partin tables, and the Stephenson Nomogram have characterized the risks of such entities as ECE and positive margins based on large cohorts of patients submitted to radical prostatectomy with pathologic and long-term clinical data and outcomes (14). In each of these nomograms, there is a defined risk of the pathological entity being present and similarly an inverse risk of the entity not being present. However, with the burgeoning field of genomic profiling of tumors and outcomes based on this information independent of clinical nomograms, we are aware that despite lesions having the same Gleason characteristics on biopsy and volume of cancer, they have biologically different behaviors. We are also cognizant that the nomograms in current use have not taken the spatial tumor location into consideration, as many were developed in an era pre-dating advanced imaging.

It is currently unclear as to what the peritumoral region on mpMRI is home to and what zone of mpMRI invisible tissue is positive surrounding the volume hot spot (15). One may argue that this is irrelevant as it relates to Gleason hot spot, however, and that any tumor outside of the hot spot (which represents high grade disease) that is not seen is likely to be lower grade disease. But we do not know this to be always true.

With this in mind, an ancillary pathologic correlate which is easily obtainable from the biopsy tissue, namely color-coding, and increased accuracy of tumor localization may be useful to incorporate into future nomograms which would also include mpMRI findings such as PIRADS score, overall tumor volume, tumor location, tumor-capsule contact length and a combination of single or multi-genomic data. Conceptually, this approach could also serve as a forewarning for the use of technologies such as confocal microscopy, optical coherence tomography and confocal laser endo-microscopy from the perspective of pre-emptively alerting the surgeon that malignant cells are known to be at a predetermined distance from the given plane of dissection (16-18). These factors may enable surgeons to take a more individualized approach to sparing of the NVB in a patient specific and disease-centric manner.

We acknowledge that our data is limited due to its small sample size and the few patients that went on to have radical prostatectomy as definitive therapy. It may also be best applied to posterior peripheral zone lesion but can also be applied to anterior zone lesions by careful study of the mpMRI lesion and the tract of the biopsy needles relatively to the anterior capsule (in this instance the blue dye would be the most peripheral as seen in 2 of our patients). Another limitation is this approach does not account for anteriorly based lesions and the propensity for a PSM at this site (19, 20). Handling this area at the time of prostatectomy remains a clinical / surgical judg-

ment heavily reliant on imaging and intraoperative cues.

Despite the aforementioned shortcomings, we are enthused that this data represents an early proof of concept and have expanded the study to collect more patient data using this simple modification to the well-established biopsy protocol. It is our intention to incorporate that "normal tissue" interface concept into an algorithm with other nomograms, the goal being to predict the likelihood of cancer at the margin and ECE on a more individualized basis. This data may not be applicable in patients undergoing a transperineal biopsy.

CONCLUSION

Color-coding of prostate biopsy samples obtained by mpMRI transrectal ultrasound and fusion biopsy is a simple adjunct to standard biopsy techniques, which may yield useful information regarding the proximity of malignant cells to the capsule and may provide useful information to complement surgical planning. Further study, with a larger patient cohort and pathological outcomes from radical prostatectomy, is needed to validate whether this approach may be beneficial when tailoring preservation of the NVB on an individualized basis.

CONFLICT OF INTEREST

None declared.

ACKNOWLEDGEMENTS

Leslie A. Deane and Wei Phin Tan contributed similarly as first author.

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Correspondence address:
Wei Phin Tan, MD
Rush University Medical Center
1725 W Harrison St, Suite 348
Chicago, 60612 Illinois, USA
Fax: + 1 312 942-4005
E-mail: tanweiphin@yahoo.com

ORIGINAL ARTICLE

Vol. 44 (6): 1089-1105, November - December, 2018
doi: 10.1590/S1677-5538.IBJU.2017.0339

Predictive factors for prolonged hospital stay after retropubic radical prostatectomy in a high-volume teaching center

Rafael F. Coelho [1], Mauricio D. Cordeiro [1], Guilherme P. Padovani [1], Rafael Localli [1], Limirio Fonseca [1], José Pontes Junior [1], Giuliano B. Guglielmetti [1], Miguel Srougi [1], William Carlos Nahas [1]

[1] *Divisão de Urologia, Instituto do Câncer de Estado de São Paulo, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brasil*

ABSTRACT

Objective: To evaluate the length hospital stay and predictors of prolonged hospitalization after RRP performed in a high-surgical volume teaching institution, and analyze the rate of unplanned visits to the office, emergency care, hospital readmissions and perioperative complications rates.

Materials and Methods: Retrospective analysis of prospectively collected data in a standardized database for patients with localized prostate cancer undergoing RRP in our institution between January/2010 - January/2012.

A logistic regression model including preoperative variables was initially built in order to determine the factors that predict prolonged hospital stay before the surgical procedure; subsequently, a second model including both pre and intraoperative variables was analyzed.

Results: 1011 patients underwent RRP at our institution were evaluated. The median hospital stay was 2 days, and 217 (21.5%) patients had prolonged hospitalization. Predictors of prolonged hospital stay among the preoperative variables were ICC (OR. 1.40 p=0.003), age (OR 1.050 p<0.001), ASA score of 3 (OR. 3.260 p<0.001), prostate volume on USG-TR (OR, 1.005 p=0.038) and African-American race (OR 2.235 p=0.004); among intra and postoperative factors, operative time (OR 1.007 p=0.022) and the presence of any complications (OR 2.013 p=0.009) or major complications (OR 2.357 p=0.01) were also correlated independently with prolonged hospital stay. The complication rate was 14.5%.

Conclusions: The independent predictors of prolonged hospitalization among preoperative variables were CCI, age, ASA score of 3, prostate volume on USG-TR and African-American race; amongst intra and postoperative factors, operative time, presence of any complications and major complications were correlated independently with prolonged hospital stay.

ARTICLE INFO

Keywords:
Prostatectomy; Therapeutics; Retrospective Studies

Int Braz J Urol. 2018; 44: 1089-105

Submitted for publication:
June 01, 2017

Accepted after revision:
August 12, 2018

Published as Ahead of Print:
September 10, 2018

INTRODUCTION

The number of new cases of prostate cancer worldwide is higher than 1.1 million each year, which represents 15.3% of all incident cancer cases in developed countries and 4.3% of the cases in developing countries (1). For patients with a clinically localized disease, a series of alternative treatment options is currently available. However, for patients with a life expectancy exceeding 10

years, radical prostatectomy (RP) continues to be one of the standard treatments (2).

After its first description in 1905 by H.H. Young (3), RP was initially associated with significant peri and postoperative morbidity. However, the technique of retropubic radical prostatectomy (RRP) was subsequently standardized by Walsh et al. (4), with significant improvement in perioperative, oncological, and functional outcomes (5). The practices of hospitalization and convalescence time post-RRP have also clearly accompanied the evolution in the refinements of the RRP technique. With improvements in the anesthetic technique and intra-operative care, the mean hospitalization time has decreased (6-8); subsequently, this time was further reduced by the establishment of optimized strategies for perioperative care after RRP, which resulted in short hospitalization, without an associated increase in the postoperative complication rates (9).

This perioperative care pathway can be further optimized by determining potentially modifiable predictive factors for prolonged hospitalization after RP. The importance in evaluating those predictors becomes even clearer when we observe that the primary definitive treatment has the highest impact in the overall costs of prostate cancer care, both in the short and long term (10, 11). Therefore, determining the factors that predict longer hospitalization after RRP may impact significantly in hospital costs, in patient management and, finally, in the governmental spending plans in public institutions involved in prostate cancer care.

However, studies evaluating predictors for prolonged hospitalization after PRR are still rare in the literature (12) and non-existent in our setting - a public high volume teaching hospital. We cannot overemphasize that the real benefits of minimally invasive RP over RRP are still unclear in the literature (13) and that in many areas of the World open RP is still the most common surgical approach to treat clinically localized prostate cancer (14).

We sought in this study to evaluate the length of hospital stay and the impact of pre, intra, and post-operative factors on the incidence of prolonged hospitalization in patients who underwent RRP in a high-volume teaching hospital.

MATERIALS AND METHODS

Study Design

The study was a retrospective analysis of prospectively collected data in a standardized database for patients with clinically localized prostate cancer who underwent surgical treatment in our institution. The surgical procedures were performed by residents during their last year of training under the supervision of one of the staff members; the rotation of each resident in the Urology Oncology Department lasts 5 months, and over this period each resident performs on average 300 surgical procedures, being 100 PRRs.

Patient Selection

We included in this study all patients with clinically localized or locally advanced prostate cancer, who underwent RRP in the instituting between January 2010 and January 2012. All patients with PSA>10mg/mL and/or Gleason score ≥8 underwent preoperative bone scans, in order to excluded the presence of bone metastases. All patients included in the study signed an informed consent form authorizing data collection.

Pre-operative Evaluation

All patients had demographic and clinical data collected and underwent a standardized pre-operative evaluation, including Digital Rectal Exam (DRE), ultrasound-guided trans-rectal biopsy (TRUS), evaluation of the serum PSA level and bone scan when necessary. Prostate magnetic resonance imaging (MRI) was done in select cases, at the discretion of the attending physician. The preoperative clinical risk stratification was done by cardiologists or general practitioners following the American Cardiology Association guidelines (16).

Surgical Technique - RRP

RRP was done using the technique standardized by Srougi et al. (17, 18). The preferred anesthetic modality was the combination of general and epidural anesthesia. RR|P was performed through a medial infra-umbilical incision; the pre-peritoneal retropubic space was dissected to expose the anterior aspect of the prostate and

the vesico-prostatic transition. When indicated, pelvic lymph node dissection (PLND) was performed prior to RP including only the obturator fossa (level I). As a rule, PLND was performed in intermediate and high risk patients according to NCCN criteria. A standard retrograde approach to RP was performed in every case including opening of the endopelvic fascia, ligation of the dorsal venous complex, retrograde dissection of the prostatic apex and neurovascular bundles, bladder neck dissection (without sparing), seminal vesicle dissection, bladder neck closure in "tennis racket" and, finally, vesicourethral anastomosis. A Penrose drain was left in place and removed before patient discharge.

Intraoperative Data Collection

The data collected intra-operatively included: type of anesthesia, operative time, estimated blood loss (EBL) (calculated through the weighting of surgical sponges and volume of blood aspirated from the surgical field), blood transfusion, neurovascular bundle preservation (unilateral/bilateral, partial, or complete), presence or absence of a medium lobe, lymph node dissection and extension, intraoperative complications (vascular injury, rectal injury, obturator nerve injury, etc.).

Perioperative Care and Hospital Discharge Criteria

A sole dose of an intravenous first generation cephalosporin was administered during anesthesia induction. Intermittent compression stockings were routinely used in the trans-operative period. Pharmacological prophylaxis of thromboembolic events was not done routinely.

In the immediate post-operative period (iPO) the patients were offered oral liquids freely; regular diet was offered in the morning after the surgery. Walking was also started in the first postoperative day. Analgesia was done routinely with common painkillers combined with anti-inflammatory medication intravenously in the iPO and orally in the first day after surgery. The Penrose drain was removed usually due to hospital discharge.

The patients received hospital discharge within 2 days post-operatively as long as they fulfilled the following criteria: were afebrile, no signs of orthostatic hypotension, pain controlled with oral medication, were able to walk with minimal discomfort, tolerated the diet without nausea or vomiting, did not present post-operative complications or intercurrences that required daily medical evaluation (Table-1).

Anatomopathological Evaluation

The surgical specimens were processed according to the recommendations of the American Society of Clinical Pathologists (19). Clinical staging was done according to the TNM system (15). Histopathological findings were analyzed as potential predictors for prolonged hospitalization including prostate weight, Gleason score, pathological stage, tumor volume, PSMs and its location and number of lymph nodes removed.

Post-operative Evaluation

The length of the hospital stay was calculated by subtracting the date of admission from the date of discharge. Patients who were re-hospitalized within 28 days after the surgery were considered "readmissions" (20). Since there is no standardized definition, neither national nor international, for prolonged hospitalization, it was defined in this study as a hospital stay longer than the upper quartile of hospitalization time in our series (>2 days).

Complications that occurred during the surgical procedure or within 90 days after the surgery were analyzed and classified according to the modified Clavien-Dindo system (21).

Definition of Complications

Perioperative blood transfusion was generally indicated for patients with symptomatic anemia and serum hemoglobin levels < 7g/dL. For intermediary levels of hemoglobin (7-10 g/dL), blood transfusion was indicated in case of real or potential continuous hemorrhage, or in the presence of risk factors for secondary complications to insufficient oxygenation (for example, ischemic heart disease).

Paralytic ileus was defined as nausea, vomiting and/or abdominal distension post--operatively requiring hospitalization for longer than 2 days in the absence of mechanical intestinal obstruction.

Table 1 - Peri-operative Care and Hospital Discharge Criteria.

	Immediate post-operative	First/second days P.O.	Hospital Discharge Criteria
Activity	- Sit on the armchair 4 hours after surgery -Respiratory Physiotherapy in cases with higher risk of pulmonary complications, at the surgeon's discretion	- Walking in the morning on the first P.O. -Motor and respiratory Physiotherapy, at the surgeon's discretion	- Able to walk with minimal discomfort, being confident and comfortable to leave the hospital.
Diet	Liquid diet	Laxative general diet	-Tolerating general diet without nausea or vomiting
Analgesia and medication	- NSAIDs + common painkillers in routine (opioids orally if necessary) -Prophylactic antibiotic therapy until 24 hours after the procedure - Proton pump inhibitors	- NSAIDs + common painkillers in routine (opioids orally if necessary) - Mass forming laxatives orally - Proton pump inhibitor -Reintroduction of the usual medications	-Afebrile, without orthostatic hypotension, pain controlled with oral medication
Care and recommendations	- Recommendations about care with the drain, incision, collecting pouch, fall prevention No lab exams were ordered routinely	-Penrose drain removed - Recommendations about the use of the urine collector at home (leg collector offered to the patients)	Attested ability of the patient, family member, or companion to understand the guidelines about physical activity, medication, pain control, constipation prevention, care with the incision, care with the Foley, return at the clinic

Symptomatic lymphocele was defined as a pelvic fluid collection (especially along the iliac vessels) in patients who underwent lymphadenectomy associated with pelvic pain or pressure, lower limb edema, hydronephrosis, deep vein thrombosis, or infection/sepsis.

Statistical analysis

Descriptive statistics was used initially to analyzed the frequency of the variables included in the study. Univariate analysis was performed to select the potential predictors to be included in the logistic regression model. Logistic regression was then used to determine the factors that independently predict prolonged hospitalization after RRP in our institution. A model including only preoperative variables was initially built to determine the factors that predict hospitalization time before surgery; subsequently a second model, including pre, intra, and postoperative variables were analyzed. Preoperative variables included in the model were age, race, BMI, PSA, Charlson comorbidity index (CCI), ASA score, previous abdominal surgery, clinical stage, prostate volume, biopsy (Gleason score) and percentage of positive cores, NCCN risk stratification. Intra and postoperative factors included in the analysis were: type of anesthesia, operative time, EBL, transfusion, nerve-sparing approach, lymph node dissection, prostate weight, tumor volume, Gleason score specimen, positive margin rates, pathologic stage and post-

operative complications. For statistical analyses purposes, CCI was analyzed as a binary (0 vs. ≥1 comorbidities) and also as a continuous variable, in order to ensure that the categorization of CCI did not affect the results. The data were analyzed using Stata® 13.1 software. P-values lower than 0.05 were considered statistically significant.

RESULTS

Clinical and demographic data and pre-operative tumor characteristics

Between January 2010 and January 2012, 1011 patients underwent a RRP in our institution and were included in the study; no patient who underwent RRP for primary treatment of a localized prostate cancer during the period was excluded from the study. However, 110 patients undergoing laparoscopic RP and 5 patients submitted to salvage RP after radiotherapy in this time frame were excluded from the study.The pre-operative clinical, demographic, pathological characteristics of the patients are presented in Table-2.

Perioperative Outcomes

The most common type of anesthesia used was general anesthesia associated with epidural (92.2%), which is the standard at our institution for RRP. The median operative time was 130 minutes (IQR, 110-160); the median EBL was 600 mL (IQR, 300-1000) and 52 patients (5.14%) received blood transfusions peri-operatively. PLND was performed in 63% of patients; no patient underwent extended PLND, since it was not the standard at our institution during the period of this study.

The median hospitalization time was 2 days (IQR, 1-2; mean 1.86±1.27 days); 217 (21.5%) patients presented prolonged hospitalization according to the definition adopted in this study (>2 days). Figure-1 shows a Box Plot graph of the hospitalization time in this study.

Hospital readmission was necessary in 28 (2.7%) patients, while 74 (7.3%) patients had non-scheduled visits to the emergency room or clinic without need for hospitalization.

Perioperative results are shown in Table-3.

Table 2 – Pre-operative clinical, demographic, and pathologic characteristics.

Characteristics	N=1011
Age (years) - median (IQR)	65.4 (60.2-69.7)
Race (%)	
White	825 (81.6%)
Black	73 (7.2%)
Yellow	20 (2%)
Other	93 (9.2%)
BMI, kg/m²- median (IQR)	26.6 (24.2-29.4)
Charlson Comorbidity Index (CCI)	
0	738 (73%)
≥1	273 (27%)
CCI continuous variable – median (IQR)	0 (0-1)
ASA Score	
1	304 (30.1%)
2	656 (64.9%)
3	51 (5%)
Previous abdominal surgery	193 (19.1%)
PSA (ng/mL) - median (IQR)	9.1 (6-14.3)
Prostate volume TRUS (mL) - median (IQR)	42 (30-68.2)
Biopsy Gleason score	
≤6	672 (66.5%)
7	263 (26%)
≥8	76 (7.5%)
Clinical Stage	
T1c	539 (53.3%)
T2	404 (40%)
T3	68 (6.7%)
Risk stratification - NCCN	
Low or very low	296 (29.3%)
Intermediate	497 (49.1%)
High or very high	218 (21.6%)

Figure 1 - Hospitalization Time.

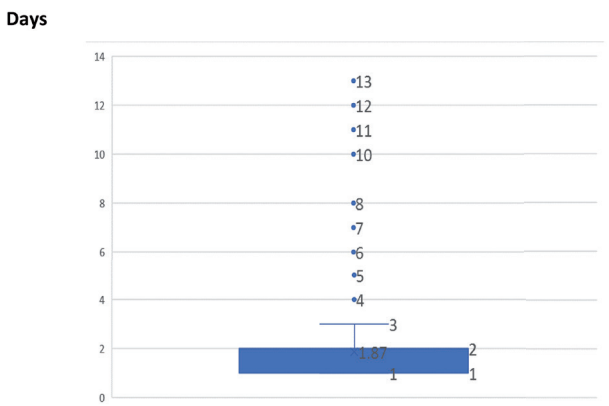

Table 3 - Peri-operative outcomes.

Perioperative variables	N=1011
Anesthesia	
General	62 (6.1%)
Peridural + general	932 (92.2%)
Rachianesthesia + general	17 (1.7%)
Obturatory fossa lymphadenectomy	
Yes	638 (63%)
No	373 (37%)
Operative time (min) - median (interquartile variation)	130 (110-160)
Estimated bleeding (mL) - median (interquartile variation)	600 (300-1000)
Rate of blood transfusion	5.14%(52/1011)
Hospital stay - median (interquartile variation)	2 (1-2)
≤2 days	794 (78.5%)
>2 days	217 (21.5%)
Non-scheduled visits to the ER or clinic	74 (7.3%)
Hospital readmissions	28 (2.7%)

Surgical Complications

We observed 156 postoperative complications in 141 patients (14.5%). Intra-operative complications included 5 rectal injuries and one obturator nerve injury. These complications were not classified according to the Clavien system, since it applies solely to post-operative complications.

The classification of complications according to the Clavien System is presented in Table-4. There were no deaths within 90 days after surgery and/or due to the surgical procedure (Grade 5).

Histopathological Findings

The histopathological findings are presented in Table-5.

The median prostate weight was 43 (34-68.2) g. The majority of patients (74.2%) presented organ-confined disease; 15.1% were pT2a, 7.2% were pT2b, and 51.8% were pT2c. Seminal vesicle invasion (pT3b) was identified in 10.2% of the patients and extra prostatic extension (pT3a) was found in 15.6%.

The most common Gleason score in the surgical specimen was 7, which corresponded to 52.8% of the patients (396 patients Gleason 3+4, 39.1%; 138 patients Gleason 4+3, 13.6%). The median tumor volume was 15 (10-30)%.

The overall PSM rate was 26.6%; in patients with pT2 disease the PSM rate was 23.3% while in patients pT3 disease the rate was 36.2%. The most common location of positive margins was in the prostate apex (74/1011, 7.3%).

Predictors of prolonged hospitalization – Univariate analysis

We initially conducted a univariate analysis of predictors of prolonged hospitalization including pre, intra, and post-operative variables. In this analysis, among the pre-operative variables, the following were correlated with prolonged hospitalization: age, race, ASA score, CCI both as a binary and continuous variable, and prostate volume in the TRUS (Table-6A).

Considering intra and post-operative variables, the following were correlated with prolonged hospitalization: EBL, blood transfusion rate, operative time, presence of post-operative compli-

Table 4 – Complications (1).

Clavien Classification	Number of Patients	Percentage of Patients, %	Incidence of Complications
I	21	14.3%	2.0%
II	66	44.9%	6.5%
IIIa	23	15.6%	2.3%
IIIb	18	12.2%	1.8%
IVa	11	7.5%	1.1%
IVb	2	1.4%	0.2%
V	0	0	0
Total	147	100	14.5% (147/1011)
Minor post-operative complications (Grades I + II) *	87	61.7%	8.5%
Major post-operative complications (Grades III + IV) *	54	38.3%	5.4%

(1) - When patients presented more than one complication, the higher Clavien Score was considered.
*- . Excluding intra-operative complications.

cations (of any grade, minor and major complications), and prostate weight in the histopathological evaluation (Table-6B).

Predictors of prolonged hospitalization including pre-operative variables

In this first logistic regression model, only preoperative variables were included (Table-7A). CCI was initially analyzed as a binary variable (0 vs. ≥1); subsequently, we also performed a sensitivity analysis including CCI as continuous variable, to ensure that our categorization of CCI did not affect the results. The independent predictors of prolonged hospitalization in this model were age (OR 1.050, CI 95% 1.023-1.078, p<0.001), CCI as a binary (ICC ≥1 vs, ICC 0, OR. 1.818, IC95% 1.272-2.6, p=0.001) and as continuous variable (OR 1.401, CI 95% 1.118-1.756, p=0.003), TRUS prostate volume (OR 1.006, CI 95% 1.001-1.011, p=0.033) and black race (OR 1.910, CI 95% 1.103-3.307, p=0.021).

Predictors of prolonged hospitalization including pre, intra, and post-operative factors combined

In the second logistic regression model we analyzed predictors of prolonged hospitalization based on pre, intra, and post-operative factors combined. Similarly to the first model, we evaluated CCI as a binary and as a continuous variable. The independent predictors of prolonged hospitalization in this model were age (OR 1.042, CI95% 1.016-1.070, p=0.002), CCI both as a binary variable (CCI ≥1 vs. CCI 0, OR 1.832, CI 95% 1.277-2.682, p=0.001) or as a continuous variable (OR 1.461, CI 95%, 1.150-1.855, p=0.002), ASA score 3 (OR 3.192, CI 95% 1.585-6.430, p=0.001), black race (OR 1.788, CI 95% 1.037-3.083, p=0.036), operative time (OR 1.006, CI 95% 1.001-1.011, p=0.019), presence of any grade of post-operative complications (OR 1.7945, CI 95% 1.072-3.008, p=0.026) or major complications (OR 2.104, CI 95% 1.100-4.025, p=0.0125), and pathology prostate weight (OR 1.009, CI 95% 1.003-1.015, p=0.006) (Table-7B).

DISCUSSION

Hospital admission and discharge practices represent an important indicator of the quality and efficiency of medical care and have a clear impact on the healthcare costs. In an effort to minimize these costs without compromising clini-

Table 5 – Histopathological findings.

Histopathological findings	N=1011
Prostate weight (g) – median (IQR)	43 (34-68.2)
Pathological staging	
pT2a	153 (15.1%)
pT2b	74 (7.3%)
pT2c	524 (51.8%)
pT3a	157 (15.6%)
pT3b	103 (10.2%)
Tumor volume (%) – median (IQR)	15 (10-30)
Positive surgical margin rates - overall	269 (26.6%)
pT2	175/751(23.3%)
pT3	94/260 (36.2%)
Positive surgical margins – location	
Apex	74 (7.3%)
Bladder neck	11 (1%)
Circumferential	121 (12%)
Multifocal	63 (6.2%)
Gleason Score – Specimen	
≤6	375 (37.1%)
7	534 (52.8%)
≥8	102 (10.1%)

cal outcomes, physicians and hospital managers have increasingly focused on reducing the hospital stay following surgical procedures (12). Thus, the identification of factors that correlate with longer hospitalization time not only helps to plan expenditures, but also can help in the modification of variables that potentially increase the admission period of the patients. Our study demonstrated several interesting points regarding perioperative care, practices to minimize hospitalization and modifiable factors correlated with prolonged hospitalization.

The feasibility of promoting early hospital discharge after RRP has been demonstrated in several previous studies and different approaches have been described in order to optimize the perioperative recovery. Abou-Haidar Het al. (22) recently described a multidisciplinary approach which involves meeting with nurses prior to surgery for perioperative instructions, provision of an appropriate booklet to reinforce perioperative care, visit by an internist before and after surgery for optimization of medications, early mobilization, respiratory physiotherapy, use of PCA pump in the iPO with transition to oral medications on the first postoperative day. By adopting this standardized strategy of care, the authors were able to reduce hospital stay from a median of 3 (IQR 3 to 4 days) to a median of 2 days (IQR, 2 to 3 days, p<0.0001). The complication rates, emergency room visits and hospital readmissions were not significantly different in the pre and post-intervention groups (17% vs. 21%, p=0.80, 12% vs. 12%, p=0.95, and 3% vs. 7%, p=0.18, respectively). The perioperative care post-RRP at our institution is very similar to the one presented by these and some authors (23-25), as shown in Table-1. While hospitalization time is considered a marker of efficiency, hospital readmission is a prominent marker related to the quality of health services (26). A recent study evaluating Medicare patients, estimated that the annual cost with hospital readmissions reaches $ 17.4 billion (26-28). The impact of early hospital discharge on readmission rates has also been accessed in recent studies. Kaboli et al. (26) evaluated 4,124,907 admissions to 29 hospitals of the American Veterans Affairs (VA) system and demonstrated that the reduction in length of hospital stay does not necessarily occur at the expense of increased readmission rates; In the last 14 years, the mean length of stay in the VA system decreased by 27% (1.46 days) without any significant increase in the incidence of hospital readmission. However, in the same study, hospitals that tended to discharge earlier than expected (considering baseline disease and overall length of hospital stay in the VA system) had significantly higher rates of readmission (growth rate of 6% for each day less hospitalization than expected). It is clear, therefore, that an ideal balance between hospitalization and readmission rates should be the ultimate goal, in order to obtain the highest degree of efficiency without impairing the quality of patients' care. To this end, the adoption of strict hospital discharge

Table 6 - A) Univariate analysis – Predictors of prolonged hospitalization amongst pre-operative variables.

Pre-operative variables	Hospital stay		P-value
	≤2 days	>2 days	
Age (years) - median (IQR)	64.86 (59.59- 69.36)	67.22 (63.51-71.13)	<0.001
BMI (kg/m²)- median (IQR)	26.7 (24.27 -29.40)	26.40 (23.80-29.30)	0.531
Race			
White	670 (84.4%)	155 (71.4%)	<0.001
Black	45 (5.6%)	28 (13%)	
Asian	16 (2%)	4 (1.8%)	
Other	63 (8%)	30 (13.8%)	
ASA score			
1	241 (30.8%)	53 (24.4%)	0.001
2	513 (65.4%)	143 (65.9%)	
3	30 (3.8%)	21 (9.7%)	
CCI			
0	602 (75.8%)	136 (62.7%)	<0.001
≥1	192 (24.2%)	81 (37.3%)	
CCI as continuous variable - median (IQR)	0 (0-1)	0 (0-1)	0.001
Previous abdominal surgery			
Yes	158 (19.9%)	35 (16.1%)	0.248
No	636 (80.1%)	182 (83.9%)	
PSA (ng/mL) - median (IQR)	9.25 (6.00-14.25)	8.55 (5.7-14.30)	0.469
Clinical stage			
T1c	429 (54%)	110 (50.7%)	0.671
T2	313 (39.4%)	91 (42%)	
T3	52 (6.6%)	16 (7.3%)	
Gleason Score – biopsy			
≤6	533 (67.1%)	139 (64%)	0.624
7	201 (25.3%)	62 (28.6%)	
≥8	60 (7.6%)	16 (7.4%)	
Risk stratification– NCCN			
Low or very low	236 (29.7%)	60 (27.6%)	0.783
Intermediate	386 (48.6%)	111 (51.2%)	
High or very high	172 (21.7%)	46 (21.2%)	
Prostate volume TRUS (mL) - median (IQR)	40 (30 - 50)	43 (30-60)	0.006

Table - 6B) Univariate analysis – Predictors of prolonged hospitalization amongst intra and post-operative variables.

Intra and post-operative variables	Hospital stay		P-value
	≤ 2 days	>2 days	
Intra-operative factors			
Anesthesia			
General	45 (5.7%)	17 (7.8%)	
Peridural + general	735 (92.6%)	197 (90.8%)	0.47
Rachianesthesia + general	14 (1.7%)	3 (1.4%)	
Estimated bleeding (mL) - median (IQR)	600 (300-900)	700 (350 -1100)	0.02
Blood transfusion rate			
Yes	30 (3.8%)	22 (10.1%)	<0.001
No	764 (96.2%)	195 (89.9%)	
Operative time (min) – median (IQR)	130 (110 -155)	140(120-180)	0.004
Type of preservation of the neurovascular bundle			
No preservation	68 (8.6%)	22 (10.2%)	
Bilateral	629 (79.2%)	157 (72.7%)	0.104
Unilateral	97 (12.2%)	37 (17.1%)	
Obturator lymphadenectomy			
Yes	500 (63%)	138 (63.6%)	0.929
No	294 (37%)	79 (36.4%)	
Post-operative factors			
Any complication (All Clavien grades) (1)			
Yes	95 (12%)	52 (24%)	<0.001
No	699 (88%)	165 (76%)	
Minor complications (Clavien I and II)			
Yes	59 (7.4%)	28 (13%)	0.016
No	735 (92.6%)	189 (87%)	
Major complications (Clavien III and IV)			
Yes	30 (3.8%)	24 (11%)	<0.001
No	764 (96.2%)	193 (89%)	
Rate of positive surgical margins			
Overall	205 (25.8%)	64 (29.5%)	0.157
pT2	134/591(22.7%)	41/160 (25.6%)	0.498
pT3	71/203 (35%)	23/57 (40%)	0.555
Pathological staging			
pT2a	121 (15.3%)	32 (14.7%)	
pT2b	64 (8%)	10 (4.7%)	
pT2c	406 (51.1%)	118 (54.4%)	0.523
pT3a	122 (15.4%)	35 (16.1%)	
pT3b	81 (10.2%)	22 (10.1%)	
Specimen Gleason score			
≤6	312 (39.3%)	71 (32.7%)	
7	405 (51%)	125 (57.6%)	0.342
≥8	77 (9.7%)	21 (9.7%)	
Tumor volume(%) median (IQR)	16 (10-30)	15 (10-30)	0.944
Prostate weight (g) median (IQR)	41 (34-54)	43.5 (34-62)	0.006

1-Included intra-operative complications, but were not classified in the Clavien system

Table - 7A) Multivariate analysis – Predictors of prolonged hospitalization including solely pre-operative factors.

Pre-operative variables		Odds ratio	CI 95%	P value
Age	Continuous variable	**1.050**	**1.023 - 1.078**	**<0.001**
CCI	Continuous variable	**1.401**	**1.118 - 1.756**	**0.003**
CCI (Binary)	0	Reference	-	-
	≥1	**1.818**	**1.272 - 2.600**	**0.001**
ASA score	1	Reference	-	-
	2	1.175	0.791 - 1.744	0.425
	3	**3.192**	**1.616 - 6.308**	**<0.001**
Prostate volume (TRUS) – (mL)	Continuous variable	**1.006**	**1.001 - 1.011**	**0.033**
Race	White	Reference	-	-
	Black	**1.910**	**1.103 - 3.307**	**0.021**
	Asian	1.546	0.789 - 3.031	0.204
	Other	1.317	0.410 - 4.228	0.644

criteria respecting not only the clinical conditions, but also the logistics and patient safety are fundamental.

Direct comparisons of complication rates among different RRP series are limited due to the variations in definitions and methods of classification of surgical complications in the studies currently available in literature; additionally, very few studies evaluated the correlation between postoperative complications and hospitalization time (29). Donat et al. (30) recently analyzed the quality of the available studies evaluating complications after different urological surgeries. A total of 109 studies were identified of these, only 36 studies reported the severity of the complications and only 7 studies used some numerical classification. Furthermore, Martin et al. (31) lately proposed some strict criteria that should be followed in high quality studies reporting surgical complications including: data collection methods, duration of follow-up, outpatient information, definitions of complications, mortality and morbidity rates, specific complications rate for each procedure, graduation system and length of hospital stay. Our study is one of the rare publications on RRP outcomes available in the literature which complies with all Martin criteria (32).

We initially built a regression model including only preoperative variables; the aim of this model was to explore factors that may predict prolonged hospitalization based only on clinical parameters and tumor characteristics, which are available through medical history and clinical staging prior to the surgical procedure. Therefore, the findings of this model could be used for accurate preoperative patients counseling and could aid hospitals and healthcare payment sources in managing cost- effectiveness, hospital bed availability and healthcare resources according to the characteristics of patients awaiting RRP in each institution. In this preoperative model, we identified as independent predictors of prolonged hospital stay age, ICC (as a binary or continuous variable), ASA 3 score, TRUS prostate volume and black race. The independent predictors for prolonged hospital stay identified in our study are similar to those observed in other recent studies.

The correlation between comorbidities and age with hospitalization time after RRP has been confirmed in two recent studies. Kelly et al.(33) evaluated 2411 RRPs of the Irish Cancer Registry between 2002 and 2009. The median length of hospital stay was 8 days and in the adjusted analysis the main predictors of prolonged hospitalization

Table - 7 B) Multivariate analysis – Predictors of prolonged hospitalization including combined pre, intra, and post-operative factors.

Combined pre, intra, and post-operative variables		Odds ratio	CI 95%	P value
Age	**Continuous variable**	1.042	1.016 - 1.070	0.002
CCI	**Continuous variable**	1.461	1.150 - 1.855	0.002
CCI (binary)	0	Reference	-	-
	≥1	1.832	1.277 - 2.628	0.001
ASA score	1	Reference	-	-
	2	1.028	0.702 1.506	0.887
	3	3.192	1.585 -6.430	0.001
Race	White	Reference	-	-
	Black	1.788	1.037 - 3.083	0.036
	Asian	1.189	0.604 - 2.338	0.616
	Other	1.639	0.565 - 4.756	0.363
Estimated bleeding (mL)	Continuous variable	1.000	1.000 - 1.001	0.552
Operative time (min)	Continuous variable	1.006	1.001 - 1.011	0.019
Blood transfusion rate	No	Reference	-	-
	Yes	1.312	0.562 - 3.063	0.530
Any complication (any Clavien Grade)	No	Reference	-	-
	Yes	1.795	1.072 - 3.008	0.026
Minor complications (Clavien I and II)	No	Reference	-	-
	Yes	1.180	0.581 - 2.398	0.647
Major complications (Clavien III and IV)	No	Reference	-	-
	Yes	2.104	1.100 - 4.025	0.025
Prostate weight (g)-specimen	Continuous variable	1.009	1.003 - 1.015	0.006

were: presence of comorbidities (OR = 1.64, 95 % 1.25-2.16), advanced stage (III-IV, OR 2.19, 95% CI 1.44-3.34), and marital status single (OR = 1.71 CI 95% 125-2.34). In addition, patients submitted to treatment in high-volume hospitals (median annual PRRs> 49) or by high-volume surgeons (annual volume> 17 PRRs) had significantly lower chances of having prolonged hospitalization (OR = 0.34, 95% CI, 26-0.45, OR = 0.55, 95% CI 0.42-0.71, respectively). Co-morbidities in the study by Kelly and colleagues were assessed using the Elixhauser index (34), which includes a broad spectrum of 31 comorbidities and have demonstrated a higher discrimination power than CCI to predict in-hospital mortality in previous studies (35). The presence of any of the comorbidities listed in the Elixhauser index in this study correlated with a 64% greater chance of prolonged hospitalization compared to patients without comorbidities. In our series, the presence of comorbidities (ICC≥1 vs. 0) correlated with an 82% greater chance of prolonged hospitalization; In turn, when assessing ICC as a continuous variable, we observed that each increase of 1 point in the index correlated with a 40% higher risk of prolonged hospitalization.

In turn, Trinh et al. (12) recently published an analysis of the Nationwide Inpatient Sample evaluating 89,883 RPs between 2001 and 2007. Prolonged hospitalization was defined as hospital admission greater than 3 days (75th percentile). In the multivariate analysis, the predictors of prolonged hospitalization were age (as a continuous variable, OR 1.01, 95% CI 1.01-1.02), year of surgery (2006-2007 vs. 2001-2003 OR 0.50 , 95% CI 0.48-0.53), surgical volume (3 tercile vs. 1 tercile, OR 0.21, 95% CI 0.20-0.23), hospital location (West vs. Northeast, OR 0 , 95% CI 0.63-0.71), presence of comorbidities (ICC≥1 vs. ICC 0, OR 1.32, IC95% <0.001), surgical approach (minimally invasive vs. RRP, OR 0, 61, 95% CI 0.54-0.69), race (black versus white, OR 1.52, 95% CI 1.42-1.62), type of health insurance (Medicare versus private, OR 1, 21 95% CI, 1.16-1.28) and presence of surgical complications (OR 6.86, 95% CI 6.54-7.19). Confirming our findings, Trihn et al. (12) also observed that both CCI and age were predictors of prolonged hospitalization; each additional year of age correlated with a 1% increase in the risk of prolonged hospitalization in the Trihn et al. (12) series and with a 5% increase in our series, while the presence of comorbidities increased by 32% the risk of prolonged hospital admission in their study and by 82% in our series.

The ASA physical status classification system has been underused in the recent literature on prostate cancer. However, the importance of this classification as a predictor of complications, length of hospital stay and mortality after RRP has been demonstrated in several prior studies (36, 37). Froehner et al. (36) evaluating 444 consecutive patients with a median follow-up of 6 years demonstrated that the ASA classification is an accurate tool to improve the prognostic classification of comorbidities in RRP patients, with a greater discriminative power than the ICC in terms of overall survival and non-cancer mortality. In turn, Dillioglugil et al. (37) evaluated 472 patients treated with RRP by a single surgeon and demonstrated that ASA score of 3 correlated with a three--fold increase in the risk of complications, prolonged hospital stay, postoperative admission to ICU and blood transfusions. Major complications were also almost 3 times more frequent in ASA score 3 (21.3%) than in score 1 or 2 (7.6%) (p<0.005). Our results confirm this strong correlation between the ASA score 3 and perioperative RRP outcomes; in our logistic regression ASA 3 patients had a 3.2 times higher risk of prolonged hospitalization compared to ASA 1 patients.

Perhaps one of the most intriguing findings in our study is the correlation between black race and prolonged hospitalization; black patients had approximately a two-fold greater odds of prolonged hospitalization than white patients in our series. Similar findings were obtained by Trinh et al. (12), in their analysis, black patients presented a 52% higher chance of prolonged hospitalization compared to white patients. In our study, this result is probably correlated with variables that are potentially linked to the black race but were not included in our regression model (confounding variables). In our country, there is still a great socioeconomic disparity between white race and African-Americans, according to a recent cen-

sus (38), a black worker earns, on average, just over half (57.4%) of the income received by white workers. Moreover, the data (38) shows that while 22% of the white population had completed high school in 2013, less 10% of African Americans had reached the same level of schooling. In this way, socioeconomic factors can justify the longer hospitalization obtained among black patients in our series. Unfortunately, data on income and/or socioeconomic status were not available in our institutional database, preventing the inclusion of these variables in our regression model

When including pre, intra and post-operative variables in the regression model, CCI (continuous or binary), age, ASA score 3, black race and prostate weight maintained their statistically significant correlation with prolonged hospitalization. All variables had only small variations in their Odds Ratios, confirming, therefore, the independent correlation of these predictors with the length of hospital stay. Additionally, in this model, operative time, the presence of complications of any grade or major complications (Clavien III and IV) were identified as independent predictors for prolonged hospitalization. The impact of surgical complications on hospital stay time has been clearly established in prior series (12, 39-41), this correlation seems to be unequivocal and obvious, since patients who suffer complications require longer hospitalization time for diagnosis, treatment, and recovery from these complications. In the already cited study of Trinh et al. (12), the presence of complications was the most important predictor of prolonged hospitalization; patients who presented perioperative complications had a 7 times greater risk of prolonged hospitalization when compared to patients without complications. In the series by Chang et al. (41), perioperative complication was also an independent predictor of longer hospital stay; the rates of complication were significantly lower (p=0.013) in the group of patients who were discharged within 2 days (2.3%) in comparison with the group which stayed hospitalized for 3 days (7.0%). In our study, the presence of complications (any complication or major complications) was associated with an approximately 2 times greater odds of prolonged hospitalization, constituting one of the most significant predictors, together with an ASA score 3. Finally, the correlation between prolonged operative time, complications and prolonged hospitalization is in line with other studies. Rabbani et al. (40) evaluated 4592 consecutive patients who underwent RRP (3458) or VLRP (1134) in a single institution. In their multivariate analysis, operative time was identified as an independent predictor of surgical complications of any grade (p=0.001), together with CCI (p<0.001), BMI (p=0.01), estimated bleeding (p=0.006), and black race (p=0.027), results which are very close to the ones obtained in the present series. In our study, each additional minute of operative time was correlated with a statistically significant increase of 0.6 to 0.7% in the odds of prolonged hospitalization.

Both the complication rates and operative time can be considered as potentially modifiable variables that could reduce the risks of prolonged hospitalization. The main factors that can determine a clear decrease of both variables, with subsequent impact on length of hospital stay, are the surgeon's experience and the hospital surgical volume. It has been demonstrated that more experienced surgeons and high volume hospitals tend to present reduced complication rates, operative time, and even costs; Judge et al. (42) recently evaluated 18,027 RRPs performed between 1997 and 2004 in hospitals of the English National Health Service. The mean length of hospital stay decreased by 2.96% (95% CI, 1.98-3.92, P<0.001) per quintile of increase in hospital surgical volume, the probability of hemorrhagic complications decreased by 6% (95% CI 1-11, P=0.02) and medical complications by 10% (CI 95% 0-19, P=0.04) per quintile increase in hospital surgical volume; also re-hospitalizations within one year decreased by 15% (95% CI 6-22, P=0.001) and genitourinary complications by 5% (95% CI 2-8, P=0.002), per quintile of increase in hospital surgical volume., Finally, Coelho et al. (29) demonstrated, in a series of 2500 RRPs performed by a single surgeon, a reduction in the complication rate from 9.3% in the first 300 cases of the series to 3.3% in the last 300 cases, highlighting the concept that more experienced surgeons have less complication rates.

Our study has a number of limitations. First of all, some postoperative complications and read-

missions may be underreported, especially minor complications and those managed in other hospitals. Second, our hospital is a high-volume cancer care center and, therefore, the outcomes presented herein may not be representative of general urologists in a community setting (limited external validity). Third, as the data on socioeconomic aspects were not available in our database we could not evaluate it as confounding variables in our prediction models; undoubtedly, residual confusion may explain at least some of the observed findings, since other complex and unknown factors involved in patient selection may have been left out of the regression model. Finally, it is important to highlight that CCI, used as one of the main instruments for the evaluation of comorbidities in this study, is an index initially designed to evaluate serious diseases in hospitalized patients; thus, this instrument does not address the full range of common comorbidities among patients with prostate cancer, such as hypertension, pulmonary disease and coronary artery disease (in the absence of myocardial infarction), hyperlipidemia and asthma.

Among the strengths of the study we highlight the comprehensiveness of the data collected; all patients were included in the regression models increasing its statistical power. Additionally, it is one of the few RRP series following not only the Clavien graduation system (28) but also fulfilling all the Martin criteria (31). As already pointed out, RRP series reporting complications and peri-operative morbidity through standardized methodology are fundamental for accurate patient counseling and to facilitate the comparison between different institutions and surgical approaches; such series are, however, scarce in the literature and our study adds important findings to the body of this literature. Undoubtedly, our data demonstrate realistic outcomes and expectations for patients undergoing RRP in our setting.

CONCLUSIONS

The independent predictors of prolonged hospitalization in our series were ICC, age, ASA score 3, prostate volume on USG-TRUS, African-American race, operative time, presence of any complications and major complications. The identification of these factors allows not only better planning of institutional costs related to RRP but also proper counseling of patients undergoing RRP. Potentially modifiable risk factors, such as OR time and complications, can be optimized to shorter length of hospital stay after RRP.

CONFLICT OF INTEREST

None declared.

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Correspondence address:
Guilherme P. Padovani, MD
Divisão de Urologia,
Instituto do Câncer de Estado de São Paulo
Faculdade de Medicina da Universidade de São Paulo
Av. Dr. Arnaldo, 251 - Cerqueira César
São Paulo, SP, 01246-000, Brasil
Telephone: +55 11 9 9683-1659
E-mail: guilhermepadovani@gmail.com

ORIGINAL ARTICLE

Vol. 44 (6): 1106-1113, November - December, 2018
doi: 10.1590/S1677-5538.IBJU.2017.0552

Comparison of Gleason upgrading rates in transrectal ultrasound systematic random biopsies versus US-MRI fusion biopsies for prostate cancer

Paulo Priante Kayano [1], Arie Carneiro [1], Tiago Mendonça Lopez Castilho [1], Arjun Sivaraman [2], Oliver Rojas Claros [1], Ronaldo Hueb Baroni [1], Rodrigo Gobbo Garcia [1], Guilherme Cayres Mariotti [1], Oren Smaletz [1], Renne Zon Filippi [1], Gustavo Caserta Lemos [1]

[1] Hospital Israelita Albert Einstein, São Paulo, SP, Brasil; [2] Memorial Sloan Kettering Cancer Center - USA, New York, NY, EUA

ABSTRACT

Purpose: Ultrasound-magnetic resonance imaging (US-MRI) fusion biopsy (FB) improves the detection of clinically significant prostate cancer (PCa).

We aimed to compare the Gleason upgrading (GU) rates and the concordance of the Gleason scores in the biopsy versus final pathology after surgery in patients who underwent transrectal ultrasound (TRUS) systematic random biopsies (SRB) versus US-MRI FB for PCa.

Materials and Methods: A retrospective analysis of data that were collected prospectively from January 2011 to June 2016 from patients who underwent prostate biopsy and subsequent radical prostatectomy. The study cohort was divided into two groups: US-MRI FB (Group A) and TRUS SRB (Group B).

US-MRI FB was performed in patients with a previous MRI with a focal lesion with a Likert score ≥3; otherwise, a TRUS SRB was performed.

Results: In total, 73 men underwent US-MRI FB, and 89 underwent TRUS SRB. The GU rate was higher in Group B (31.5% vs. 16.4%; p=0.027). According to the Gleason grade pattern, GU was higher in Group B than in Group A (40.4% vs. 23.3%; p=0.020). Analyses of the Gleason grading patterns showed that Gleason scores 3+4 presented less GU in Group A (24.1% vs. 52.6%; p=0.043).

The Bland-Altman plot analysis showed a higher bias in Group B than in Group A (-0.27 [-1.40 to 0.86] vs. -0.01 [-1.42 to 1.39]).

In the multivariable logistic regression analysis, the only independent predictor of GU was the use of TRUS SRB (2.64 [1.11 – 6.28]; p=0.024).

Conclusions: US-MRI FB appears to be related to a decrease in GU rate and an increase in concordance between biopsy and final pathology compared to TRUS SRB, suggesting that performing US-MRI FB leads to greater accuracy of diagnosis and better treatment decisions.

ARTICLE INFO

Keywords:
Prostatic Neoplasms; Magnetic Resonance Spectroscopy; Image-Guided Biopsy

Int Braz J Urol. 2018; 44: 1106-113

Submitted for publication:
October 22, 2017

Accepted after revision:
August 05, 2018

Published as Ahead of Print:
September 10, 2018

INTRODUCTION

Brazilian data shows that prostate cancer (PCa) is the most common non-cutaneous malignancy in men. The estimated incidence of PCa for 2016 is 61,200 new cases, with an estimated risk of 61.82 new cases per 100,000 men (1). PCa is commonly diagnosed by transrectal ultrasound

(TRUS)-guided random biopsies. The Gleason score of PCa has been shown to be an important criterion to predict tumour behaviour and to determine the appropriate course of treatment (2). However, the randomness and non-targeted nature of TRUS biopsy can result in inaccurate sampling of the cancer and misclassification of cancer risk. Researchers have demonstrated discrepancies in the Gleason score of TRUS biopsy compared to the final surgical specimens, with under-estimation reported in approximately 30% of cases (3).

Recently, multiparametric magnetic resonance imaging (mpMRI) of the prostate has been shown to be valuable in the detection, localization and characterization of prostatic tumour foci (4). Target biopsy of the abnormality detected by MRI was initially performed by cognitive guidance of the topographic location of the cancer, but in recent years, devices were developed to combine ultrasound and mpMRI images. The US-MRI fusion images can guide biopsy and improve the detection of clinically significant prostate cancer (5, 6). A more accurate diagnostic method is desirable to avoid misclassification, which is particularly important in appropriate decision-making for the treatment of PCa (active surveillance or focal therapy or radical treatment). It is plausible that the Gleason score misclassification and upgrading noted in radical prostatectomy specimens can be reduced by employing more accurate biopsy techniques.

The aim of this study was to compare the Gleason upgrading rates and the concordance of the biopsy versus final pathology Gleason scores in patients who underwent TRUS systematic random biopsies (SRB) versus US-MRI fusion biopsies (FB) for prostate cancer.

MATERIALS AND METHODS

Patient selection and data collection

We included all consecutive patients who underwent prostate biopsy and subsequent radical prostatectomy at our institution. The study cohort was divided into those who had US-MRI FB (from June 2013 to July 2015) (Group A) and TRUS SRB (from June 2010 to February 2015) (Group B). Pathological analyses of the prostatectomy specimens were reviewed by a single, experienced pathologist and were considered as the standard of reference. Patients who did not undergo biopsy and radical prostatectomy at our institution and those with pathologic specimens that were not reviewed by the same pathologist were excluded to avoid bias. IRB approval and a waiver for informed consent were obtained for this retrospective study using prospectively collected data from our institution database.

US-MRI FB was performed in all patients with mpMRI-detected abnormalities and Likert scores ≥3. Patients with a normal mpMRI or Likert scores <3 underwent TRUS SRB. All radical prostatectomies were performed by our institution Urology staff either by robot-assisted radical prostatectomy or by an open approach.

Data related to clinical, biopsy, histopathological and MRI characteristics were collected.

Multiparametric MRI

MRIs were performed on 3T scanners (Siemens Prisma 3T, Siemens PetRM 3T, GE 750W 3,0T, Philips 3,0T) with a phased-array coil and included high-resolution T2-weighted imaging, diffusion-weighted imaging and dynamic contrast-enhanced imaging. A Likert scale score, that is a subjective assessment on the likelihood of the presence of prostate cancer on a 5 point scale (7), was assigned by one of our uro-radiologist with years of experience in interpreting prostate MRI (median of 7 years of experience; range 5 to 15 years) and every exam were reviewed by other experienced radiologist, and if there was a discrepancy in the analyses, the score was assigned after a consensus. Only lesions classified with scores ≥3 were defined as targets for US-MRI fusion biopsy.

US-MRI Fusion Biopsy

Targeted biopsies were performed by our institution interventional radiologist team, with experience in non-elastic fusion prostate biopsy and experience in reading prostate MRI (median of 9 years of experience with TRUS SRB and 2 years with target US-MRI FB) using different US-MRI fusion systems: MyLab 60 (Esaote, Florencea, Italy), Aplio 500 Smartfusion (Toshiba, Nasu, Japan) and Logiq E9 VNav (GE Healthcare, Milwaukee).

Each biopsy was performed with the patient in a left lateral decubitus position, using endocavitary 4 to 9 MHz broadband curved array end-fire transducers and an 18-gauge side-notch cutting core biopsy needle (20-mm stroke length). Patients first underwent systematic 14-core biopsies (six from each lobe and one more from each transitional zone), followed by targeted biopsies generally consisting of 2 or 3 cores from each target.

Histopathology

Gleason scoring was performed according to the 2005 International Society of Urological Pathology consensus recommendations (8). We classified the patients according to the 2014 ISUP consensus meeting held in Chicago in 2014, which classify the Gleason scores into grade groups (Gleason score ≤6 = ISUP 1; Gleason score 3+4 = ISUP 2; Gleason score 4+3 = ISUP 3; Gleason score 4+4 = ISUP 4; Gleason score 9 or 10 = ISUP 5) (9).

Cores from each lesion were numbered and labelled according to the target, enabling radiology-pathology correlation in patients with multiple targets.

Surgical specimens were processed using a modified Stanford technique; 3- to 5-mm transverse sectioned samples were taken from the apex to the base and from the sagittal section of the distal 5 to 8 mm of the apex and base.

Statistical analysis

The primary endpoint of this study was to compare the rate of any Gleason score upgrade of RP compared to US-MRI fusion biopsy and random biopsy alone. Descriptive statistics were used for patient characteristics. An independent Student's *t*, Mann-Whitney, chi-square or Fisher's exact test was used to compare characteristics of the patients when appropriate. Gleason upgrading was compared by comparison of proportions. A multivariable logistic regression using forced entry was carried out to assess the independent predictors of Gleason upgrading. The results are presented as odds ratios (ORs) and 95% confidence intervals. Agreement between Fusion US-MRI and histopathology and Random Biopsy and histopathology was assessed using a Bland-Altman plot, and bias was calculated with their respective 95% confidence intervals.

All analyses were conducted with SPSS v.20 (IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.) or R v.2.12.0 (R Foundation for Statistical Computing, Vienna, Austria). For all analyses, two–sided $p < 0.05$ were considered significant.

RESULTS

Characteristics of the cohort

A total of 73 men who underwent US-MRI fusion biopsies and 89 who underwent TRUS systematic random biopsies were included in our analyses. In both groups, the patient demographics were similar (Table-1). There were no differences according to prostate volume (histopathology), PSA, clinical and pathologic staging and number of lymph nodes. However, there was a significant difference in biopsy Gleason score between both groups. Patients from Group A had fewer Gleason score 6 tumours (11% vs. 28%), and patients from Group B had greater total tumour volumes (15% vs. 10%) and fewer clinically significant tumours (70.8% vs. 89%) (Table-1), defined as patients with Gleason score greater or equal to 3+ 4 or greater than ISUP 1.

Primary endpoint

Gleason upgrading was higher in patients who underwent TRUS SRB compared to US-MRI FB (31.5% vs. 16.4%; p=0.027) (Table-2). When analysing Gleason upgrading according to the Gleason grade group, there was also a higher rate of Gleason upgrading in patients who underwent TRUS SRB compared to those who underwent US-MRI FB (40.4% vs. 23.3%; p=0.020) (Table-2). Analyses from separate Gleason grade groups showed that Gleason scores of 3+4 presented less Gleason upgrading in the FB group (24.1% vs. 52.6%; p=0.043) (Table-2). Finally, there was no difference in Gleason upgrading when considering only patients with Gleason scores of 6 on biopsy (Table-2).

The Bland-Altman plot analysis showed a higher bias for patients submitted to TRUS systematic random biopsy compared to those

Table 1– Patients characteristics.

	US- MRI fusion (n = 73)	Random (n = 89)	*p* value
Age (years), median (IQR)	65.0 (57.5 – 69.0)	64 (59 – 69)	0.838
PSA (ng/mL), median (IQR)	4.8 (3.7 – 6.4)	5.5 (4.2 – 7.2)	0.060
Prior biopsy status, n (%)	9 / 73 (12.3)	10 / 89 (11.2)	0.829
Biopsy Gleason grade group, n (%)			
Less or equal to 6	8 / 73 (11.0)	25 / 89 (28.1)	0.007
3+4 = 7	29 / 73 (39.7)	19 / 89 (21.3)	
4+3 = 7	21 / 73 (28.8)	23 / 89 (25.8)	
8	8 / 73 (11.0)	18 / 89 (20.2)	
9-10	7 / 73 (9.6)	4 / 89 (4.5)	
Number of total cores, median (IQR)	18.0 (12.0 - 19.5)	15.0 (14.0 - 17.5)	0.144
Number of random cores, median (IQR)	14.0 (11.0 - 18.0)	14.0 (14.0 - 17.0)	0.171
Number of targeted cores, median (IQR)	4.0 (3.0 – 5.0)	ND	
Positive cores, n (%)	61 / 71 (85.9)	13 / 20 (65)	0.007
Positive targeted cores, median (IQR)	6.0 (4.5 – 10.0)	ND	
Prostate volume at histopathology (grams), median (IQR)	42.0 (30.0 – 56.0)	40.0 (32.5 – 47.0)	0.223
Surgical specimen Gleason grade group, n (%)			
Less or equal to 6	2 / 73 (2.7)	9 / 89 (10.1)	0.205
3+4 = 7	32 / 73 (43.8)	29 / 89 (32.6)	
4+3 = 7	28 / 73 (38.4)	31 / 89 (34.8)	
8	5 / 73 (6.8)	8 / 89 (9.0)	
9-10	6 / 73 (8.2)	12 / 89 (13.5)	
Total tumor volume (%), median (IQR)	10.0 (7.0 – 20.0)	15.0 (10.0 – 20.0)	0.024
Bilateral tumor, n (%)	54 / 73 (74.0)	67 / 89 (75.3)	0.848
Multifocal tumor, n (%)	60 / 73 (82.2)	76 / 89 (85.4)	0.580
Positive lymph node, n (%)	1 / 70 (1.4)	3 / 87 (3.4)	0.424
Clinically significant tumor, n (%)	65 / 73 (89.0)	63 / 89 (70.8)	0.004
Time between biopsy and surgery (days), median (IQR)	30.0 (30.0 – 60.0)	60.0 (30.0 – 60.0)	0.224
Numbers of lesions on MRI, median (IQR)	1.0 (1.0 – 2.0)	ND	---

Table 2 – Gleason upgrading.

	Fusion (n = 73)	Random (n = 89)	*p* value
Biopsy Gleason score	7.0 (7.0 – 7.0)	7.0 (6.0 – 7.5)	0.137
Surgical specimen Gleason score	7.0 (7.0 – 7.0)	7.0 (7.0 – 7.0)	0.765
Gleason upgrading*	12 / 73 (16.4)	28 / 89 (31.5)	0.027
Gleason upgrading in patients with Gleason 6 on biopsy**	6 / 8 (75.0)	17 / 26 (65.4)	0.611
Gleason upgrading according to Gleason grade pattern	17 / 73 (23.3)	36 / 89 (40.4)	0.020
3+3	6 / 8 (75.0)	16 / 25 (64.0)	0.687
3+4	7 / 29 (24.1)	10 / 19 (52.6)	0.043
4+3	3 / 21 (14.3)	3 / 23 (13.0)	1.000
Accuracy***	51 / 73 (69.9)	56 / 89 (62.9)	0.353

* defined as the number of patients with Gleason score in surgical specimen greater then biopsy sample.

** defined as the number of patients with Gleason score 6 in biopsy sample (clinically non-significant disease) that presented a Gleason score greater than 6 in surgical specimen (clinically significant disease). Patients with Gleason score greater than 6 were excluded from this analyses.

*** defined as the number of patients who presented the same Gleason score in biopsy sample and surgical specimen.

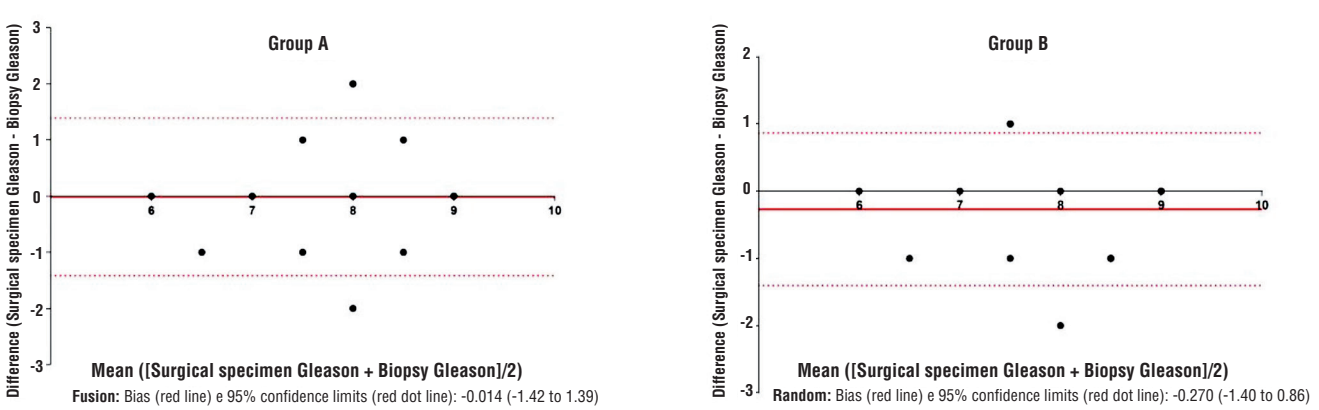
submitted to US-MRI fusion biopsy (-0.27 [-1.40 to 0.86] vs. -0.01 [-1.42 to 1.39]) (Figure-1). In the multivariate logistic regression, the use of TRUS systematic random biopsy, compared to US-MRI fusion biopsy, was the only independent predictor of Gleason upgrading (2.64 [1.11 – 6.28]; *p*=0.024) (Table-3).

The comparison analysed by the Bland--Altman plot of Group A and Group B showed that the agreement bias between Gleason score on biopsy and Gleason score on surgical specimen was lower in Group A (Figure-1).

DISCUSSION

In our study, we found a significantly lower rate of Gleason upgrading using US-MRI FB, showing that this method can improve prosta-

Figure 1 - Bland - Altman Plot Group A vs. Group B.



Fusion: Bias (red line) e 95% confidence limits (red dot line): -0.014 (-1.42 to 1.39)

Random: Bias (red line) e 95% confidence limits (red dot line): -0.270 (-1.40 to 0.86)

Table 3 – Logistic Regression (outcome: Gleason upgrading).

	Univariate		Multivariate	
	OR (95% CI)	p value	OR (95% CI)	*p* value
Age	1.01 (0.97 – 1.06)	0.596	---	---
Baseline PSA	1.05 (0.94 – 1.19)	0.371	1.03 (0.91 – 1.17)	0.632
Prostate volume	0.99 (0.97 – 1.01)	0.277	0.99 (0.96 – 1.01)	0.282
Time between biopsy and surgery	1.00 (0.99 – 1.01)	0.358	0.99 (0.97 – 1.01)	0.173
LIKERT score	1.26 (0.56 – 2.81)	0.575	---	---
Random biopsy	2.33 (1.09 – 5.01)	0.030	2.64 (1.11 – 6.28)	0.028

PSA = prostate-specific antigen; **OR =** odds ratio; **CI =** confidence interval

te cancer characterization at biopsy. All mpMRI, biopsies and surgical specimens were evaluated by the same team and methods to keep the pattern and to reduce bias in the Gleason and Likert score classifications.

The use of US-MRI FB was associated with a lower rate of Gleason upgrading compared to the use of TRUS SRB (16.4% vs. 31.5%; p=0.027). In the clinical setting, a diagnostic tool that can determine the "true" Gleason score plays a crucial role in guiding the clinician in making the best therapeutic decision, mainly for low- and intermediate-risk PCa. In the active surveillance scenario, it is imperative to decrease the risk of missing a high-grade disease and delaying a radical treatment, providing more confidence to the urologist and patient with conservative management of PCa.

For patients who will be treated by prostatectomy or radiation therapy, the biopsy Gleason score is considered a key point in most nomograms for determining the indication of extended lymphadenectomy and changes in the irradiation field or the time of hormone therapy in patients under radiation therapy, thus changing the impact on the morbidity (10-14).

Data reported in the literature show that the Gleason score is frequently lower for TRUS guided biopsies compared to that for surgical specimens, with under-estimation reported in about 30% of cases. In patients with low-grade prostate biopsies, the risk of upgrading may increase up to 50% (3).

Our study showed that patients with initial Gleason scores of 6 presented more Gleason upgrading in the US-MRI FB group (75% vs. 65.4%; p=0.611); however, this difference was not statistically significant, probably due to the relatively small number of patients. Biopsies with Gleason scores of 3+4 presented less Gleason upgrading in the US-MRI FB group (24.1% vs. 52.6%; p=0.043). The concordance was higher when the highest Gleason grading pattern was analysed between biopsy and surgical specimens, consistent with our hypothesis that US-MRI FB increases the detection of the highest-grade tumour (3, 15, 16).

Arsov et al. reported an MRI FB and TRUS random biopsy upgrading of 21.2% and 32.7%, respectively (17). In this study, Gleason upgrading was twice as frequent in patients who underwent US-MRI FB compared with TRUS SRB (31.5% versus 16.4%).

Multiparametric MRI of the prostate has shown its value in the detection, localization and characterization of prostatic tumour foci (4) and plays an important role to avoid unnecessary biopsies in patients with previously negative ones, showing accuracies of approximately 90% for the diagnosis of significant prostate cancer (18-20).

In our institution, most urologists are using mpMRI in biopsy-naïve patients, avoiding some biopsies in patients with low probability of clinically significant prostate cancer, which can explain the lower rate of Gleason 6 in patients who underwent US-MRI FB (11% vs. 28.1%)

Prostate mpMRI and target biopsy could be incorporated into active surveillance selection criteria, having a higher accuracy for risk stratification (21). Prostate mpMRI can also reduce the need for repetitive biopsies by as much as 68% through non-invasive serial monitoring for those on active surveillance (22). Disease reclassification on those in active surveillance with normal mpMRI appears to be very low, with negative predictive value ranges from 81–90% (23-25).

One limitation of this study is the non--randomized retrospective study design and the heterogeneous population studied, which might lessen the generalizability of our results because of potential selection bias. Another possible selection bias is the small number of patients with Gleason 6 biopsy scores, because we perform mpMRI in biopsy naïve patients and avoid the biopsy on those with LIKERT 1 or 2. A prospective randomized study might eliminate this bias and might confirm our hypothesis.

CONCLUSIONS

US-MRI FB appears to be associated with a lower Gleason upgrading rate and a higher concordance between biopsy and final pathology compared to TRUS SRB, leading to greater accuracy of diagnosis and therefore better treatment decisions. The routine use of MRI before biopsy is associated with a decrease in the detection of clinically insignificant tumours.

COMPLIANCE WITH ETHICAL STANDARDS

For this type of study, formal consent is not required. This study was approved by our local ethical committee (registered in number (CAAE): 61372916.4.0000.0071).

FINANCIAL SUPPORT

This study received financial support from "Sociedade Beneficente Israelita Brasileira Albert Einstein – Amigo H".

CONFLICT OF INTEREST

None declared.

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Correspondence address:
Arie Carneiro, MD
Hospital Israelita Albert Einstein
Avenida Albert Einsten, 627/701, Morumbi
São Paulo, SP, 05652-900, Brasil
Telephone: +55 11 2151-1233
E-mail: arie.carneiro@einstein.br

ORIGINAL ARTICLE

Vol. 44 (6): 1114-1121, November - December, 2018
doi: 10.1590/S1677-5538.IBJU.2017.0710

PSA kinetics before 40 years of age

Cristiano Linck Pazeto [1], Thiago Fernandes Negris Lima [1], José Carlos Truzzi [2], Nairo Sumita [2], José de Sá [2], Fernando R. Oliveira[3], Sidney Glina [1]

[1] Departamento de Urologia, Faculdade de Medicina do ABC, Santo André, SP, Brasil; [2] Fleury Medicina e Saúde São Paulo, SP, Brasil; [3] Departamento de Epidemiologia, Universidade de São Paulo, São Paulo, SP, Brasil

ABSTRACT

Purpose: The baseline PSA has been proposed as a possible marker for prostate cancer. The PSA determination before 40 years seems interesting because it not suffers yet the drawbacks related to more advanced ages. Considering the scarcity of data on this topic, an analysis of PSA kinetics in this period seems interesting.
Materials and Methods: A retrospective assay in a database of a private diagnostic center was performed from 2003 to 2016. All subjects with a PSA before 40 years were included.
Results: 92995 patients performed PSA between the ages of 21 - 39. The mean value ranged from 0.66 ng / mL (at age 22) to 0.76 ng / mL (at age 39) and the overall mean was 0.73 ng / mL. As for outliers, 3783 individuals presented a baseline PSA > 1.6 ng / mL (p95). A linear regression model showed that each year there is a PSA increase of 0.0055 ng / mL ($\beta = 0.0055$; $r^2 = 0.0020$; $p < 0.001$). A plateau in PSA between 23 and 32 years was found and there were only minimal variations among the ages regardless of the evaluated percentile.
Conclusion: It was demonstrated that PSA kinetics before 40 years is a very slow and progressive phenomenon regardless of the assessed percentile. Considering our results, it could be suggested that any PSA performed in this period could represent the baseline value without significant distortions.

ARTICLE INFO

Keywords:
Prostate-Specific Antigen; Kinetics, Prostatic Neoplasms

Int Braz J Urol. 2018; 44: 1114-21

Submitted for publication:
December 28, 2017

Accepted after revision:
July 22, 2018

Published as Ahead of Print:
September 15, 2018

INTRODUCTION

Baseline prostate-specific antigen (PSA) has been proposed as a possible marker to detect those who would be at increased risk for developing prostate cancer. The concept of baseline PSA began when Gann et al. (1995) showed the role of this test as a predictor of prostate cancer in men with PSA > 1.0 ng / mL at a median age of 62.9 (1). Similarly, in a subgroup of The European Randomized study of Screening for Prostate Cancer (ERSPC), subjects with baseline PSA > 1.0 ng / mL and > 2.0 ng / mL had an increased hazard ratio for prostate cancer specific mortality (4.0 - fold and 7.6 - fold respectively) compared with those who had < 1.0 ng / mL levels (2). Regarding PSA in young adulthood, a study enrolling 325 men have demonstrated that the fourth quartile of baseline PSA (0.56 ng / mL) was associated with an increased odds of prostate cancer before age 65 (3). Additionally, Lilja et al. reported the largest association between baseline PSA and subsequent prostate cancer in those with 40 years (4). Despite this, data on this topic is scarce which justifies

more studies. Thus, we aim to determine the PSA kinetics before 40 years.

MATERIAL AND METHODS

A retrospective assay in Fleury® institute database was performed to determine how many subjects had measured the PSA between ages 21 and 39. The Fleury® institute is a private diagnostic center represented by a conglomerate of 33 laboratory units in Sao Paulo, Brazil. We highlight however that this is not a specific center for cancer diagnosis and the tests are usually performed by request of private clinics and companies. Clinical data were not available.

Considering the same PSA ultra - sensitive kit, all samples from 2003 to 2016 were included. In cases of repeated dosages for the same subject, only the first PSA was included in the study. The PSA values > 4.0 ng / mL were analyzed separately for the major association with prostate pathologies such as prostatitis and prostate cancer. Measures of central tendency, variability (mean and standard deviation), median, confidence interval, values range and percentiles were used for quantitative variables. The distribution of data was verified with Shapiro - Wilk test. For PSA age - specific levels comparisons, the Kruskal - Wallis with a Dunn's post - test was applied. The Spearman's test was used for correlation analysis of "PSA" and "Age". Finally, a linear regression analysis considering dependent variable "PSA" and independent variable "Age" was included. Due to the non - normality of the "PSA" variable, the logarithm transformation method was performed.

After this first analysis, we divided the individuals in two groups: "Group 1", with men who performed ≥ 2 dosages and "Group 2" with those who performed dosage only once. Again, only the first PSA was considered and an analysis of mean, median and percentiles was performed for each age in both groups. Additionally, the Chi - Square test was applied to compare the number of men with PSA > 1.0 ng / mL in both groups. The statistical analysis was performed using STATA version 12.0 with a level of significance of 5%.

RESULTS

During the period analyzed (2003 - 2016), 92.995 men performed a PSA between 21 - 39 years (Baseline Group). A total of 128.948 dosages were accounted. There were 32.721 men who repeated the PSA (Group 1) and 60.274 who did not (Group 2).

Considering the "Baseline Group" (Table-1), the most common age was 39 years with 14.525 men (15.62%). The mean PSA value ranged from 0.66 (at age 22) to 0.76 ng / mL (at age 39) and the overall mean was 0.73 ng / mL ± 0.45. Most values remained between 0.3 - 1.0 ng / mL (Figure-1). As for outliers, we found 3783 individuals with a baseline PSA > 1.6 ng / mL (p95) distributed among all ages (Figure-2).

The annual PSA variation was not statistically significant, except for the ages 22 - 23, 34 - 35 and 37 - 38 (Table-2). However, there was a positive correlation between "PSA" and "Age" in the Spearman test (Figure-3). Besides that, the linear regression model showed that in each year there is a PSA increase of 0.0055 ng / mL (β = 0.0055; r^2 = 0.0020; $p < 0.001$). When plotting the "Baseline Group" on graphic model, a plateau between 23 and 32 years and a progressive rise after 33 years could be noted. Thus, the values for the ages 36 - 39 remained above the group mean (0.73 ng / mL) (Figure-4). Concerning percentiles, there were only minimal variations among ages demonstrating thus, a similar kinetics (Figure-5).

When comparing "Group 1" and "Group 2", the number of men with baseline PSA > 1.0 ng / mL was higher in last group (17.6% vs. 17.3%; $p < 0.001$) and graph curves presented a similar trend (Table-3 and Figure-6). In a separate analysis, considering the remainder with PSA > 4.0 ng / mL, we found 596 individuals with a mean age of 33.8 years and mean PSA of 9.0 ng / mL (Figure-7).

DISCUSSION

PSA determination before 40 years seems interesting because it not suffers yet the drawbacks related to more advanced ages. Some other studies have examined PSA levels in young men. Preston et al. (5), for example, based on 1176 samples from a military screening

Table 1 - Descriptive analysis of total PSA according to age.

Age	PSA						
	n (%)	Mean (SD)	Median	Min	Max	Percentiles 25;75	Percentiles 90;95
21	800 (0.86)	0. 683 (0.42)	0.60	0.1	3.8	0.42; 0.82	1.2; 1.4
22	956 (1.03)	0.664 (0.37)	0.57	0.1	3.6	0.41; 0.82	1.1; 1.3
23	1112 (1.21)	0.718 (0.43)	0.62	0.1	4.0	0.44; 0.87	1.2; 1.5
24	1265 (1.36)	0.711 (0.43)	0.62	0.1	4.0	0.44; 0.86	1.2; 1.5
25	1531 (1.65)	0.720 (0.46)	0.61	0.1	3.9	0.43; 0.87	1.2; 1.5
26	1829(1.97)	0.721 (0.45)	0.62	0.1	3.8	0.43; 0.88	1.2; 1.5
27	2099(2.26)	0.717 (0.41)	0.64	0.1	3.6	0.44; 0.89	1.2; 1.5
28	2593 (2.79)	0.713 (0.44)	0.61	0.1	4.0	0.43; 0.86	1.2; 1.5
29	3106 (3.34)	0. 720 (0.44)	0.62	0.1	3.9	0.44; 0.89	1.2; 1.5
30	3106 (4.34)	0. 720 (0.44)	0.62	0.1	4.0	0.43; 0.87	1.2; 1.5
31	4459 (4.79)	0. 712 (0.44)	0.62	0.1	3.9	0.43; 0.87	1.2; 1.5
32	5293 (5.69)	0.711 (0.42)	0.61	0.1	3.9	0.43; 0.88	1.2; 1.5
33	5942 (6.39)	0. 722 (0.43)	0.62	0.1	4.0	0.44; 0.88	1.2; 1.5
34	6796 (7.31)	0. 727 (0.45)	0.62	0.1	4.0	0.44; 0.89	1.2; 1.5
35	7682 (8.26)	0. 734 (0. 44)	0.63	0.1	4.0	0.44; 0.89	1.2; 1.6
36	8579 (9.23)	0. 741 (0. 45)	0.64	0.1	4.0	0.44; 0.91	1.2; 1.5
37	9457 (10.17)	0. 748 (0. 46)	0.64	0.1	4.0	0.45; 0.91	1.3; 1.6
38	10925 (11.75)	0. 768 (0.47)	0.66	0.1	4.0	0.46; 0.94	1.3; 1.6
39	14525 (15.62)	0. 764 (0.46)	0.66	0.1	4.0	0.46; 0.93	1.3; 1.6
Total	92995 (100)	0. 737 (0.45)	0.63	0.1	4.0	0.44; 0.90	1.3; 1.6

Figure 1 - Distribution of PSA values.

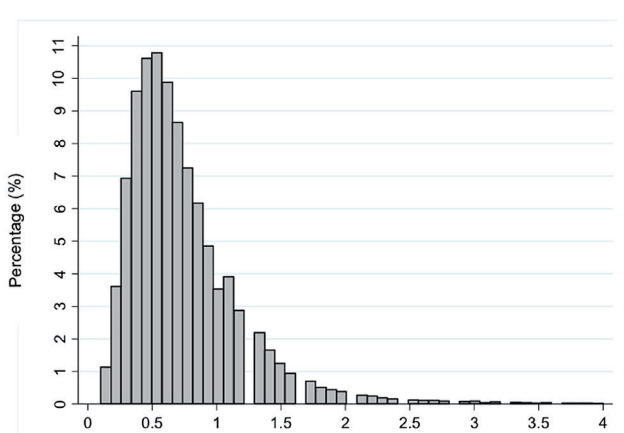


Figure 2 - Box plot of PSA according to age.

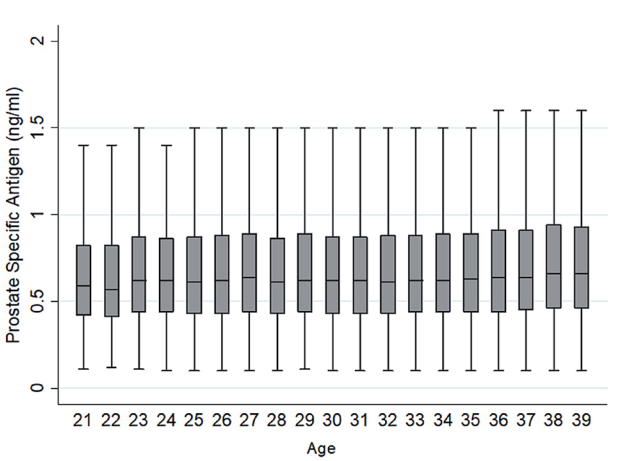
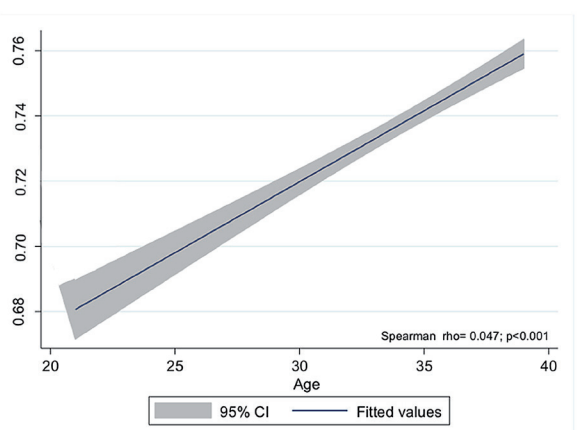


Table 2 - Pos-hoc test of PSA according to age.

Age	p	n
21 vs 22	0.331	1756
22 vs 23	0.004*	2068
23 vs 24	0.427	2377
24 vs 25	0.344	2796
25 vs 26	0.331	3360
26 vs 27	0.171	3928
27 vs 28	0.068	4692
28 vs 29	0.173	5699
29 vs 30	0.410	6212
30 vs 31	0.257	7565
31 vs 32	0.418	9752
32 vs 33	0.072	11235
33 vs 34	0.440	12738
34 vs 35	0.040*	14478
35 vs 36	0.294	16261
36 vs 37	0.090	18036
37 vs 38	0.005*	20382
38 vs 39	0.490	25450

* post-hoc test Dunn test p<0.05.

Figure 3 - Correlation between PSA values and ages.



program, described the median PSA between 20 - 45 years. In black men, the median PSA was 0.38, 0.45, and 0.52 ng / mL at 20 - 29, 30 - 39 and 40 - 45 years respectively. Similarly, white men presented 0.38, 0.45, and 0.40 ng / mL respectively for the same age ranges (5). In addition, Mott (2005) enrolled 845 military officer students and reported a median PSA of 0.66 ng / mL in the 40s (6). In a second study, the same author described the mean PSA as 0.9 ng / mL between 30 - 59 years (7).

These previous studies included only a small number of individuals who also had a wide age range so the results were variable, impairing conclusions. In addition, there wasn't a predetermined age to perform the baseline PSA: in most studies, this refers to the time of the first test. So, these data probably have a limited role, when considering a large population.

We need to consider that several genetic factors may be involved and determine even greater variations when comparing different

Figure 4 - Graphic model of the mean values of PSA according to age.

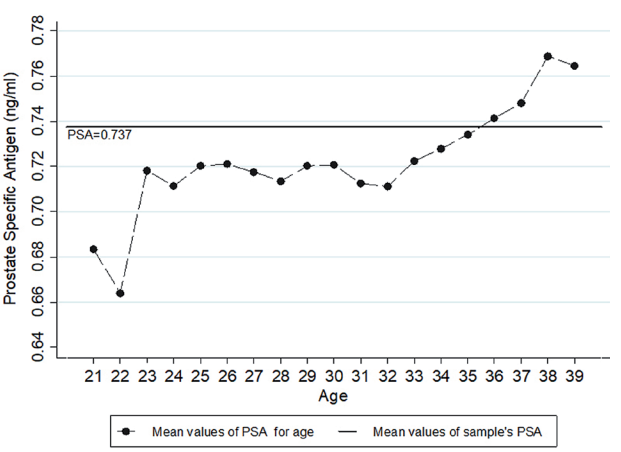


Figure 5 - PSA percentiles values according to age.

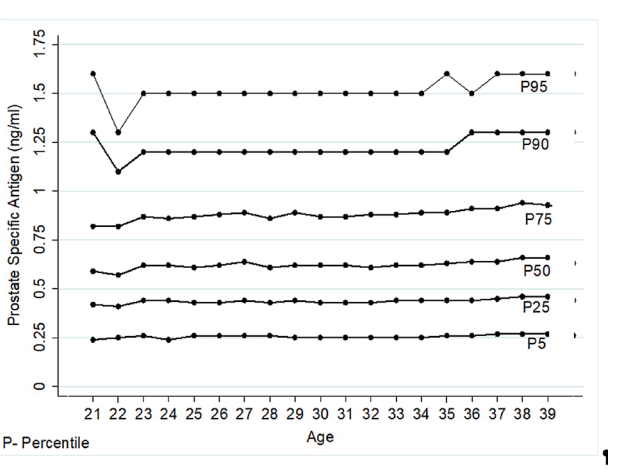


Table 3 - Descriptive analysis of Groups 1 and 2.

Age	n (%)		Mean (SD)		Percentiles 25;75		Percentiles 90;95	
	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
21	143(0.44)	657(1.09)	0.63 (0.54)	0.69 (0.39)	0.32; 0.75	0.44; 0.85	0.99; 1.5	1.2; 1.4
22	200(0.61)	756(1.25)	0.63 (0.38)	0.67 (0.37)	0.39; 0.73	0.42; 0.84	1.4; 2.2	1.1;1.3
23	220(0.67)	902(1.50)	0.72 (0.5)	0.71 (0.41)	0.39; 0.86	0.45; 0.88	1.2; 1.6	1.2; 1.5
24	277(0.85)	988(1.64)	0.66 (0.66)	0.72 (0.44)	0.41; 0.8	0.45; 0.87	1.2; 1.5	1.2; 1.5
25	307(0.94)	1224(2.03)	0.74 (0.51)	0.71 (0.45)	0.43; 0.87	0.42; 0.87	1.4; 1.5	1.2; 1.5
26	407(1.24)	1422(2.36)	0.66 (0.43)	0.73 (0.46)	0.40; 0.81	0.44; 0.90	1.1; 1.4	1.2; 1.5
27	496(1.52)	1603(2.66)	0.7 (0.44)	0.72 (0.4)	0.41; 0.86	0.45; 0.90	1.3; 1.5	1.2; 1.4
28	632(1.93)	1961(3.25)	0.67 (0.41)	0.72 (0.45)	0.41; 0.82	0.44; 0.88	1.1; 1.5	1.2; 1.6
29	832(2.54)	2274(3.77)	0.68 (0.45)	0.73 (0.43)	0.40; 0.82	0.45; 0.91	1.1; 1.5	1.2; 1.5
30	1129(3.45)	2907(4.82)	0.7 (0.45)	0.72 (0.44)	0.42; 0.85	0.44; 0.88	1.2; 1.5	1.3; 1.5
31	1308(4.00)	3151(5.23)	0.7 (0.46)	0.71 (0.43)	0.41; 0.84	0.44; 0.88	1.2; 1.4	1.2; 1.5
32	1638(5.01)	3655(6.06)	0.69 (0.43)	0.71 (0.41)	0.40; 0.86	0.44; 0.88	1.2; 1.5	1.2; 1.4
33	1959(5.99)	3983 (6.61)	0.71 (0.44)	0.72 (0.42)	0.43; 0.87	0.44; 0.89	1.2; 1.5	1.2; 1.5
34	2377(7.26)	4419(7.33)	0.71 (0.46)	0.73 (0.44)	0.41; 0.87	0.45; 0.91	1.2; 1.5	1.3; 1.5
35	2740(8.37)	4942(8.20)	0.72 (0.45)	0.74 (0.43)	0.43; 0.87.	0.45; 0.91	1.2; 1.6	1.3; 1.5
36	3276(10.01)	5303(8.80)	0.73 (0.47)	0.74 (0.45)	0.44; 0.90	0.45; 0.91	1.2; 1.5	1.3; 1.5
37	3839(11.73)	5618(9.32)	0.74 (0.48)	0.75 (0.44)	0.44; 0.90	0.46; 0.91	1.3; 1.6	1.3; 1.6
38	4577(13.99)	6348(10.53)	0.75 (0.48)	0.77 (0.47)	0.45; 0.91	0.46; 0.96	1.3; 1.6	1.3; 1.7
39	6364(19.45)	8161(13.54)	0.76 (0.48)	0.76 (0.45)	0.45; 0.92	0.46; 0.94	1.3; 1.6	1.3; 1.6
Total	32721 (100)	60274 (100)	0.73 (0.47)	0.74 (0.44)	0.43; 0.89	0.45; 0.91	1.3; 1.6	1.3; 1.5

Group 1 = Individuals with one or more PSA measurements; **Group 2 =** Individuals with only one PSA measurement

countries. Some single - nucleotide polymorphisms, for example, were exclusively associated with PSA levels without affecting the risk of prostate cancer (8). Moreover, in some countries, such as Brazil, to analyze the PSA by ethnicity, we should consider the miscegenation of the population (9, 10).

Concerning studies that evaluated the link between baseline PSA and prostate cancer, Angulo et al. studied the Spanish population with 40 - 49 years. They reported that PSA > 1.0 ng / mL and ≥ 1.9 ng / mL were associated with 27.38 - fold and 161.28 - fold risk of developing cancer compared with ≤ 1.0 ng / mL (11). Similarly, based on Vickers et al. (12), the European guidelines advise that men with PSA > 1.0 ng / mL at 40 years are at elevated risk of prostate cancer several decades later (13). Indeed, across distinct populations, higher baseline PSA were associated with an increased prostate cancer risk in later years. Considering our 92.995 subjects, approximately 17% presented a baseline PSA > 1.0 ng / mL. So, it seems that this criterion probably overestimates the true risk population.

Figure 6 - Graphic model comparing Groups 1 and 2 curves.

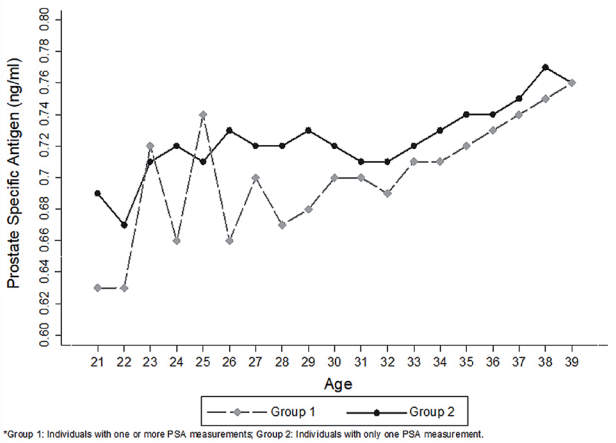
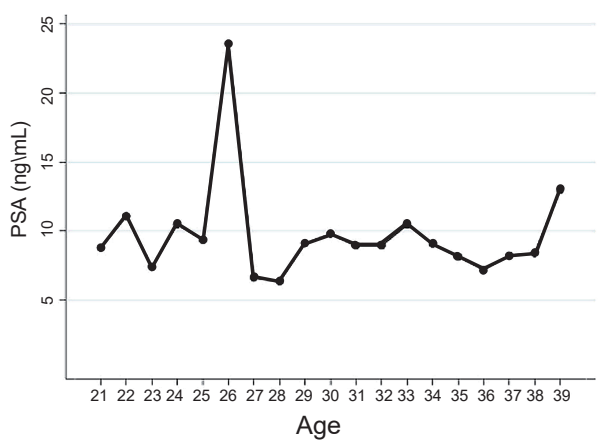


Figure 7 - Separate analysis of those with PSA > 4.0 ng/mL.



Some autopsy - based studies have detected cases of prostate cancer in younger men. In this setting, Yin et al. reported 0.5% incidence of prostate cancer among men under 49 years (14). Similarly, Soos et al. (15) reported 0%, 15% and 26.6% of prostate adenocarcinoma at 18 - 30, 31 - 40 and 41 - 50 years respectively (74% were low grade lesions). Therefore, a baseline PSA obtained before 40 years could also reduce the prostate cancer influence.

Regarding PSA in subjects younger than 40 years, we didn't find studies in the literature that included as many patients as this. Some may think that our data probably refer to patients with a familiar history of prostate cancer or another prostatic pathology to perform the PSA so early. However, most patients did not repeat the dosages (only 35.18% repeated) and among those who did, a high PSA (such as > 1.0 ng / mL) was probably not the reason. Therefore, these tests were apparently collected as health check - ups provided by some companies. Another possibility to explain these so early tests could be the widespread use of finasteride to treat alopecia. Commonly, dermatologists ask PSA before initiate the medication to have a baseline value and sometimes these tests could have been repeated during the treatment.

The novelty of this study was demonstrating the kinetics of PSA before 40 years. Considering that PSA varied only 0.1 ng / mL in 19 years (growth of 0.0055 / year), it can be affirmed that this is a very slow and progressive phenomenon. This annual growth appears to be the same regardless of the assessed percentile. In other words, it seems that even those with higher PSA tend to remain stable during this period. It could be suggested that any PSA between 21 - 39 years represent the baseline value without significant distortions.

Considering the large "n" and the inclusion of all age ranges, the data obtained reflect somewhat general population and may be useful to assess this topic and advise patients. A similar tendency of curves was observed when comparing the two groups ("Group 1" and "Group 2"). This similarity reinforces the representativeness of the data in relation to the PSA kinetics and could be interpreted as a kind of internal validation.

The fact that all dosages have been carried out at one institute with the same kit may represent an advantage considering the marked diversity of PSA assay techniques used by various laboratories (16). For example, Loeb et al. tested two different PSA tests in the same serum sample. The median and mean presented a difference of 17% and 38% respectively. Furthermore, PSA differed by greater than 0.4 ng / mL in 26%, greater than 0.75 ng / mL in 14.5% and greater than 2.0 ng / mL in 4.5% of the studied population (17).

The greatest limitation of this study was the lack of clinical data which could have in-

fluence in PSA (like history of prostatitis or recent urologic procedures). Thus, the reasons for these assays in young patients were not clear, affecting the representativeness of data. Other limitations are the retrospective nature of this study and the different numbers of individuals included per age. However, considering our outcomes, we might consider evaluating all these subjects as a single group. Finally, even after analyzing the values > 4.0 ng / mL separately of our main analysis, we still have several cases of outliners at all ages. Nevertheless, they represented few patients when considered the universe of the study.

CONCLUSIONS

Regarding baseline PSA, this study enrolled the largest number of individuals. It was demonstrated that the kinetics of PSA before 40 years is a very slow and progressive phenomenon, regardless of the assessed percentile. It could be suggested that any PSA performed in this age range could represent the baseline value without significant distortions. Finally, we found about 17% of baseline PSA > 1.0 ng / mL. Thus, although this cutoff correlates with a higher risk of prostate cancer in previous studies, it could overstate the true population at risk.

CONFLICT OF INTEREST

None declared.

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Correspondence address:
Cristiano Linck Pazeto, MD
Departamento de Urologia, Faculdade de Medicina do ABC
Rua: Dr. Henrique Calderazo, nº 321, Paraiso, 2º andar
São Paulo, SP, 09190-615, Brasil
E-mail: cristianolinckpazeto@gmail.com

ORIGINAL ARTICLE

Vol. 44 (6): 1122-1128, November - December, 2018
doi: 10.1590/S1677-5538.IBJU.2018.0181

Adequate rectal preparation reduces hospital admission for urosepsis after transrectal ultrasound – guided prostate biopsy

Yu-Chen Chen [1, 2], Hao-Wei Chen [1, 2], Shu-Pin Huang [2], Hsin-Chin Yeh [3], Ching-Chia Li [2]

[1] Graduate Institute of Clinical Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan; [2] Department of Urology, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan; [3] Department of Urology, Kaohsiung Municipal Ta-Tung Hospital, Kaohsiung, Taiwan

ABSTRACT

Objectives: Previous studies have compared infectious outcomes on the basis of whether rectal preparation was performed; however, they failed to evaluate the quality of each rectal preparation, which may have led to confounding results. This study aimed to compare hospitalizations for urosepsis within 1 month after transrectal ultrasound-guided prostate biopsy between patients with adequate and traditional rectal preparations.
Materials and Methods: Between January 2011 and December 2016, a total of 510 patients who underwent transrectal ultrasound - guided prostate biopsy at our institutions and were orally administered prophylactic antibiotics (levofloxacin) were included. Two rectal preparations were performed: (1) adequate rectal preparation confirmed by digital rectal examination and transrectal ultrasound (Group A, n = 310) and (2) traditional rectal preparation (Group B, n = 200). All patient characteristics were recorded. A logistic regression model was used to assess the effects of the two different rectal preparations on urosepsis, adjusted by patient characteristics.
Results: There were a total of three and nine hospitalizations for urosepsis in Groups A and B, respectively. Differences in the demographic data between the two groups were insignificant. Logistic regression showed that adequate rectal preparation before biopsy significantly decreased the risk for urosepsis after biopsy (adjusted odds ratio: 0.2; 95% confidence interval: 0.05 - 0.78; P = 0.021).
Conclusions: Adequate rectal preparation could significantly reduce hospitalizations for urosepsis within 1 month after transrectal ultrasound-guided prostate biopsy. The quality of rectal preparation should be evaluated before biopsy. If adequate rectal preparation is not achieved, postponing the biopsy and adjusting the rectal preparation regimen are suggested.

ARTICLE INFO

Keywords:
Prostate; Prostatic Neoplasms; Ultrasound, High-Intensity Focused, Transrectal

Int Braz J Urol. 2018; 44: 1122-8

Submitted for publication:
March 13, 2018

Accepted after revision:
July 15, 2018

Published as Ahead of Print:
August 05, 2018

INTRODUCTION

Transrectal ultrasound - guided prostate biopsy (TRUS - Bx) is a standard procedure used to diagnose prostate cancer. Although it is generally considered safe, infectious complications related to TRUS - Bx occur, which include urinary tract infection (UTI; > 6%), prostatitis, and sepsis, requiring hospital admission for intravenous antibiotic treatment (~3%) (1, 2). It is be-

lieved that bacteria in the rectum are seeded in the prostate, bladder, and / or bloodstream by the hollow core biopsy needle traversing the rectum into the prostate during TRUS - Bx and may lead to post - biopsy infection (3). The effect of rectal preparation on reducing post - TRUS - Bx infections has been debated in previous studies and remains controversial in the American Urological Association and European Association of Urology Nurses guidelines (2, 4). Although previous studies have reported on whether rectal preparation was conducted (5, 6) and whether different rectal preparation regimens influenced the post - biopsy infectious complication rates (7), they failed to confirm the quality of rectal preparation, which may have led to confounding results. In fact, an inadequate rectal preparation occurs frequently despite using cleansing enema. Hence, we focused on the quality of rectal preparations, and this study aimed to evaluate whether achieving adequate rectal preparations may be effective in reducing hospitalizations for urosepsis within 1 month after TRUS - Bx.

MATERIALS AND METHODS

Between January 2011 and December 2016, all patients who underwent TRUS - Bx at our medical centers were retrospectively reviewed. Demographic data, such as age, prostate - specific antigen (PSA) levels, prostate volumes, body mass index (BMI), diabetes mellitus (DM), hypertension (HTN), and TRUS - Bx pathological reports, were analyzed. Hospitalizations for urosepsis within 1 month after TRUS - Bx were assessed via a chart review. This study was approved by the institutional review board of Kaohsiung Medical University Hospital (ID: KMUHIRB-E(I)-20170227).

Inclusion / Exclusion Criteria

The indications for biopsy were high serum PSA levels or abnormal findings on digital rectal examination (DRE), TRUS, or magnetic resonance imaging, from which prostate cancer was strongly suspected. Patients who underwent TRUS - Bx between January 2011 and December 2016 and received prophylactic antibiotics (500 mg of levofloxacin) orally once a day starting on the day of the biopsy and lasting for 2 days were included. The exclusion criteria were UTI, acute bacterial prostatitis (NIH classification I), chronic bacterial prostatitis (NIH classification II), or use of quinolone for some other reason within 3 months before the prostate biopsy. Patients who were lost to follow--up and those with unclear medical and procedure reports were also excluded.

Study Protocol

All biopsies within the abovementioned period were scheduled as inpatient procedures. All patients received a phosphate enema before the TRUS - Bx. Two rectal preparations were performed: (1) adequate rectal preparation confirmed by DRE and TRUS (Group A) and (2) traditional rectal preparation (Group B). In Group A, DRE and TRUS were performed immediately before the TRUS - Bx to confirm the quality of the rectal preparation and to ensure that each patient achieved an adequate rectal preparation. We defined an adequate rectal preparation as the achievement of an empty rectal vault, which meant that there was no gross stool on the gloved finger during DRE nor was it visualized in the rectal vault under TRUS. The TRUS - Bx was cancelled if an adequate rectal preparation was not achieved and re - arranged until no stool was found under DRE and TRUS. For those who did not achieve an adequate rectal preparation, an additional bowel movement was required, and / or an intensified rectal preparation regimen was considered depending on the patient's clinical condition. In Group B, the rectal preparation quality was not evaluated, and the TRUS - Bx was performed as scheduled, even if the rectal vault was not completely empty. Thus, we identified Group B as the traditional rectal preparation group.

All patients in both groups received the same prophylactic antibiotics (500 mg of levofloxacin) orally, once a day, starting on the day of the biopsy and lasting for 2 days. After providing adequate information of the procedure and on the potential hazards and obtaining the informed consent from patients, TRUS - Bx was performed in an operating room with local anesthesia. For all patients, the rectal wall

was cleansed using povidone - iodine before the TRUS - Bx. In the lithotomy position, an 18 - gauge Bard® Max - Core® Disposable Core Biopsy needle was used to obtain the biopsy cores. A total of 12 cores were collected from the prostate of all patients, that is, six cores from each side.

The patients were discharged once they achieved smooth micturition after the biopsy. All patients returned to the urology outpatient clinic 1 week after the biopsy to receive their pathology report, and we could ascertain whether any infectious or noninfectious complications had occurred. The patients were also instructed to return to the hospital if they developed any symptoms of infection.

Outcome

The study's end - point was hospitalization for urosepsis within 1 month after the TRUS - Bx. Sepsis was defined as the presence of two or more of the following conditions along with bacterial infection: temperature of > 38.0°C or < 36.0°C, heart rate > 90 bpm, respiratory rate > 20 breaths / min or respiratory alkalosis, and white blood cell count > 12.000 or immature cell form count > 10% in proportion (8). For the patients with urosepsis, both urine and blood samples were collected for culture and fully evaluated.

Statistical analyses

Fisher's exact and Mann - Whitney U--test were used for categorical and continuous variables, respectively. Multivariable logistic regression analysis was used to assess the effects of the two different rectal preparations (adequate rectal preparation versus traditional rectal preparation) on the occurrence of urosepsis within 1 month after TRUS - Bx. Other potential factors considered were the two different prophylactic antibiotic protocols, age, BMI, DM, HTN, prostate volume, and biopsy pathological results as covariates.

All tests were two - sided, and P-values ≤ 0.05 were considered statistically significant. Analyses were performed using IBM SPSS Statistics version 22.0 (IBM® Corporation, NY, USA).

RESULTS

A total of 510 patients who underwent TRUS - Bx in Group A (n = 310) and Group B (n = 200) met the inclusion criteria. Differences in the patients' mean age, BMI, PSA level, prostate volume, incidence of DM and HTN, and biopsy pathological results between the two groups were not significant (Table-1). Multiple logistic regression showed that only patients who achieved an adequate rectal preparation before TRUS - Bx had a decreased risk of developing urosepsis after TRUS - Bx (adjusted odds ratio: 0.2; 95% confidence interval: 0.05 - 0.78, P = 0.021) (Table-2).

There were three and nine cases of urosepsis in Group A and Group B, respectively. Culture data were obtained for all infection - related hospitalizations (Table-3). The cultures revealed presence of Escherichia coli in two and five cases in Group A and Group B, respectively. Fluoroquinolone - resistant organisms were identified in two and four cases in Group A and Group B, respectively. No statistically significant differences were identified in the positive culture findings, rate of Escherichia coli positivity, and rate of quinolone resistance between the two groups.

DISCUSSION

Prostate biopsy is a well - established procedure used to diagnose prostate cancer and can be performed transrectally or transperineally. TRUS - Bx is the most common method utilized because of the need for local anesthesia only; however, it is accompanied by an infectious complication rate of 0.1 - 7.0%, including UTI, prostatitis, epididymitis, orchitis, bacteremia, and urosepsis, requiring hospital admission for treatment with intravenous antibiotics (1, 2, 9). The transperineal approach, which was developed to reduce infection by avoiding the rectum, leads to generally low rates of infectious complications (10, 11). However, this approach is less commonly used because of the need for general anesthesia, greater amount of pain involved, and higher potential risk of perineal hematoma (12). Therefore, it is essential to place a renewed focus on strategies to reduce infectious complications after TRUS - Bx.

Table 1 - Demographic data of the 510 patients stratified in accordance with adequate rectal preparation (Group A) and traditional rectal preparation (Group B).

Variables	Group A (n = 310)	Group B (n = 200)	P-value
Mean age, y (range)	68.75 (46–86)	69.91 (45–86)	0.072
Mean BMI, kg/m^2 (range)	25.11 (16.4–43.6)	24.57 (14.3–33.1)	0.084
DM, n (%)	72 (23)	41 (21)	0.5
HTN, n (%)	143 (46)	95 (48)	0.8
Mean PSA, ng/dL (range)	22.65 (4.1–386)	31.37 (2.15–492)	0.3
Mean prostate volume, mL (range)	52.1 (16.7–197)	49.2 (10.5–181)	0.065
Biopsy pathological result, n (%)			0.6
No prostate cancer	212 (68)	139 (70)	
Gleason score < 7	22 (7)	19 (9)	
Gleason score 7	25 (8)	11 (6)	
Gleason score > 7	51 (17)	31 (16)	

BMI, body mass index; DM, diabetes mellitus; HTN, hypertension; PSA, prostate-specific antigen

The causative bacteria for post - biopsy infection are seeded into the prostate, bladder, and / or bloodstream by the hollow core biopsy needle traversing the rectum into the prostate / bladder during TRUS - Bx (3). Therefore, rectal preparation before TRUS - Bx could theoretically prevent post - biopsy infection by reducing the rectal bacterial load associated with feces and thereby reducing the bacteria brought into the prostate and bladder. However, the effects of rectal preparation on reducing post - TRUS - Bx infections remain controversial. A Cochrane review found that enemas with antibiotics were associated with fewer instances of bacteremia (a reduction from 28% to 4%), but there was no difference in the occurrence of bacteriuria or fever (13). Some reports have suggested that rectal preparations, such as enemas or bisacodyl administration, decrease the rate of infectious complications (5, 7), whereas other studies have suggested otherwise (14). Because of the lack of evidence, rectal preparation has remained controversial in the 2011 European Association of Urology Nurses guidelines and the updated 2016 American Urological Association white paper (2, 4), although almost all patients (79 - 81%) undergo rectal preparation before biopsy in daily practice (15).

Similar to an inadequate bowel preparation reportedly occurring in up to 25% of colonoscopies in the USA (16), we also found that an inadequate rectal preparation occurs frequently (gross stool was often found on DRE or TRUS before TRUS - Bx), especially in those with chronic constipation or those noncompliant to the rectal preparation regimen. Therefore, unlike previous studies (5-7, 13), we aimed to document the quality of rectal preparations, and our results revealed that an adequate rectal preparation could significantly decrease the post - TRUS - Bx infection rate. Moreover, because of different responses in

Table 2 - Logistic regression of potential factors on hospital admissions for urosepsis.

Variables	Hospitalizations for urosepsis	
	Adjusted OR (95% CI)	P-value
Age	0.95 (0.9–1.02)	0.2
BMI	0.99 (0.82–1.2)	0.9
DM	0.77 (0.15–3.98)	0.8
HTN	1.17 (0.34–4.06)	0.8
Prostate volume	1.01 (0.98–1.03)	0.9
PSA	1.01 (0.99–1.01)	0.7
Biopsy pathological results		
No prostate cancer	Ref.	
Prostate cancer Gleason score < 7	2.7 (0.49–15.04)	0.3
Prostate cancer Gleason score 7	2.41 (0.23–25.19)	0.5
Prostate cancer Gleason score > 7	2.47 (0.43–14.03)	0.3
Rectal preparation		
Traditional rectal preparation	Ref.	
Adequate rectal preparation	0.2 (0.05–0.78)	0.021

BMI = body mass index; **CI =** confidence interval; **DM =** diabetes mellitus; **HTN =** hypertension; **OR =** odds ratio; **PSA =** prostate-specific antigen

patients even under the same rectal preparation regimen, the quality of rectal preparation should be evaluated before TRUS - Bx, and an intensified rectal preparation regimen could be considered in patients with a history of an inadequate rectal preparation.

Clinically, hospitalization for urosepsis after TRUS - Bx has been estimated to cost 5.800 US dollars per event (17, 18), and it poses a potentially life-threatening risk to the patients and leads to mistrust in the doctor - patient relationship and subsequent patient transfer, leading to increased medical costs. In our study, the number of patients needing treatment was 28, which indicated that approximately 13.000 New Taiwan dollars of hospitalization with urosepsis could be saved upon 28 adequate rectal preparations.

Antimicrobial prophylaxis is recommended for all patients undergoing TRUS - Bx to defend against bacteria that are inevitably introduced from the rectum via the biopsy needle and reduce the risk of bacteriuria, bacteremia, and clinical infections after prostate biopsy (13). However, the increasing use of fluoroquinolones globally as prophylactic antibiotics has increased the overall resistance to fluoroquinolones, and infection after TRUS - Bx is most commonly caused by fluoroquinolone - resistant Escherichia coli (19, 20). Consistent with the findings of previous studies, Escherichia coli was the most common organism cultured among patients with urosepsis, and quinolone resistance rates were high in both groups in our study.

The limitations of our study are (1) its retrospective, nonrandomized design based on data derived from the medical records of the enrolled patients and the procedure notes of TRUS - Bx; (2) the study outcome which only included hospitalization for urosepsis within 1 month after the TRUS - Bx, but did not include other slightly infectious complications which only needed outpatient care. Further large prospective case - controlled studies are required to confirm the outcomes of the present study.

Table 3 - Culture data of the patients with infection-related hospitalizations in the two groups.

Variables	Group A (n = 310)	Group B (n = 200)	P-value
Infectious number, n	3	9	
Positive culture finding, n (%)	2 (67)	5 (56)	0.7
Escherichia coli rate, %†	100 (2/2)	60 (3/5)	0.3
Quinolone resistance rate, %‡	100 (2/2)	80 (4/5)	0.5

† The *Escherichia coli* rate was defined as the number of positive *Escherichia coli* findings among the positive culture findings.

‡ The quinolone resistance rate was defined as the number of organisms resistant to quinolone among the positive culture findings.

CONCLUSIONS

On the basis of our results, adequate rectal preparation could significantly reduce hospitalizations for urosepsis after TRUS - Bx and avoid increased medical costs. The quality of rectal preparation should be evaluated before TRUS - Bx. We suggest that if adequate rectal preparation is not achieved, postponing the biopsy and adjusting the rectal preparation regimen are suggested.

ACKNOWLEDGEMENTS

Ching-Chia Li takes responsibility for the integrity of data and the accuracy of the data analysis.

CONFLICT OF INTEREST

None declared..

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Correspondence address:
Ching-Chia Li, MD
Department of Urology
Kaohsiung Medical University Hospital
No. 100, Tz-You 1st Road, Kaohsiung 807, Taiwan
Fax: + 88 67 321-1033
E-mail: chanhoward21@hotmail.com

ORIGINAL ARTICLE

Vol. 44 (6): 1129-1138, November - December, 2018
doi: 10.1590/S1677-5538.IBJU.2018.0102

Comparison between multiparametric MRI with and without post – contrast sequences for clinically significant prostate cancer detection

Thais Caldara Mussi [1], Tatiana Martins [1, 2], George Caldas Dantas [1], Rodrigo Gobbo Garcia [3], Renee Zon Filippi [4], Gustavo Caserta Lemos [5], Ronaldo Hueb Baroni [1]

[1] Departamento de Radiologia e Diagnóstico por Imagem, Hospital Israelita Albert Einstein, SP, Brasil; [2] Ecoar Medicina Diagnóstica, Lourdes, Belo Horizonte, MG, Brasil; [3] Departamento de Intervenção Guiada por Imagens, Hospital Israelita Albert Einstein, SP, Brasil; [4] Departamento de Patologia, Hospital Israelita Albert Einstein, SP, Brasil; [5] Departamento de Urologia, Hospital Israelita Albert Einstein, SP, Brasil

ABSTRACT

Background: Dynamic-contrast enhanced (DCE) sequence is used to increase detection of small lesions, based on increased vascularization. However, literature is controversy about the real incremental value of DCE in detection of clinically significant (CS) prostate cancer (PCa), since absence of enhancement does not exclude cancer, and enhancement alone is not definitive for tumor. Purpose: To test the hypothesis that DCE images do not increase CS PCa detection on MRI prior to biopsy, comparing exams without and with contrast sequences. Material and Materials and Methods: All men who come to our institution to perform MRI on a 3T scanner without a prior diagnosis of CS PCa were invited to participate in this study. Reference standard was transrectal prostate US with systematic biopsy and MRI/US fusion biopsy of suspicious areas. Radiologists read the MRI images prospectively and independently (first only sequences without contrast, and subsequently the entire exam) and graded them on 5-points scale of cancer suspicion.

Results: 102 patients were included. Overall detection on biopsy showed CS cancer in 43 patients (42.2%), clinically non-significant cancer in 11 (10.8%) and negative results in 48 patients (47%). Positivities for CS PCa ranged from 8.9% to 9.8% for low suspicion and 75.0% to 88.9% for very high suspicion. There was no statistical difference regarding detection of CS PCa (no statistical difference was found when compared accuracies, sensitivities, specificities, PPV and NPV in both types of exams). Inter-reader agreement was 0.59.

Conclusion: Exams with and without contrast-enhanced sequences were similar for detection of CS PCa on MRI.

ARTICLE INFO

Keywords:
Magnetic Resonance Imaging; Prostatic Neoplasms; Men

Int Braz J Urol. 2018; 44: 1129-38

Submitted for publication:
February 16, 2018

Accepted after revision:
June 17, 2018

Published as Ahead of Print:
September 20, 2018

INTRODUCTION

Prostate cancer (PCa) is a major global health problem, as the most common cancer in men, aside from skin cancer, and the second - leading cause of cancer death in the United States (1). Approximately 30% of men older than 50 years of age have pathologic evidence of PCa; however, only 3% will die from their disease (2, 3).

The diagnosis of PCa increased in the mid 1980's when prostate - specific antigen (PSA) became a screening biomarker. However, PSA screening is a cause of over diagnosis and consequent overtreatment of patients with indolent disease (2). Therefore, the recommendation to use PSA for PCa screening remains controversial (4).

Efforts have been made to better define the clinical behavior of prostate tumors, which can range from indolent and clinically insignificant (CI) tumors to aggressive and metastatic cancer (5, 6).

Benefits of multiparametric magnetic resonance imaging (mpMRI) in patients with clinical suspicion of PCa are already established (7). MpMRI has the ability to improve detection of clinically significant (CS) PCa and decrease the detection of CI tumors prior to biopsy (7-9). Some studies already demonstrated that mpMRI is the best predictor for CS PCa detection (10, 11). Additionally, mpMRI used along with PSA has been shown to increase negative predictive values to rule out PCa, making it an excellent test to avoid unnecessary biopsies in biopsy - naïve patients and men with prior negative biopsies (12-14).

A routine mpMRI should include T1 - weighted (T1W), T2 - weighted (T2W), diffusion - weighted image (DWI), and dynamic contrast - enhanced (DCE) sequences, as recommended by major international guidelines (15). T1W images are used to detect hemorrhages within the prostate and seminal vesicles. T2W images are mostly used to evaluate prostatic anatomy, detect morphological abnormalities, and evaluate extraprostatic extension and seminal vesicle invasion in cases of advanced tumors. DWI is helpful to differentiate CS PCa from benign lesions and predict cancer aggressiveness. It should be used in conjunction with the other sequences. Finally, DCE is used to increase detection of small lesions (13), based on increased vascularization of these lesions. However, the real incremental value of DCE in detection of CS PCa is controversial, since absence of enhancement does not exclude cancer, and enhancement alone is not definitive for tumor (16).

Regardless of its advantages and increased usefulness, mpMRI is expensive and time consuming Gadolinium introduces risk of allergic reaction, potential development of nephrogenic systemic fibrosis and deposition in brain tissue (11, 17-19). However, the clinical effects of deposition of this agent contrast in the brain are not know until nowadays.

The objective of our study is to test the hypothesis that contrast - enhanced images do not increase the detection of CS PCa on mpMRI prior to biopsy, comparing exams with and without contrast in the same patient population.

MATERIALS AND METHODS

Study design

From June 2015 until February 2016, all male patients who came to our institution to perform prostatic mpMRI without a prior diagnosis of CS PCa were invited to participate in this prospective, institutional review board approved study (CAAE number 40942915.7.0000.0071). All male patients included in this study signed informed consent.

Exclusion criteria were: prostate biopsy not performed or performed in another institution, incomplete mpMRI protocol, biopsy performed more than six months after mpMRI, and an exam that was not evaluated by the two radiologists of this study.

A total of 447 patients signed the informed consent to enter the study over a nine month period, and 345 were excluded for the following reasons: prostate biopsy not performed or performed in another institution (n = 339), incomplete mpMRI protocol (n = 2), biopsy performed more than six months after mpMRI (n = 1), and exams not read not by the two study radiologists (n = 3).

Imaging

All patients underwent mpMRI on a 3 - Tesla scanner: Magnetom Prisma (Siemens Medical Solutions, Erlangen, Germany) or Discovery MR 750W (GE Healthcare, Little Chalfont, United Kingdom) with a phased array coil and without an endorectal coil. A routine protocol including triplanar T2W imaging, DWI (b - values = 50, 400, 800 and 1500) and DCE sequences were performed covering the prostate and seminal vesicles. Fifteen post - contrast sequences were acquired with

a temporal resolution of 13 seconds each. Extracellular gadolinium - based contrast media (Magnevist, Bayer, Leverkusen, Germany) was injected at a dose of 0.2 cc / Kg and a rate of 2 cc / sec.

Biopsy protocol

As reference standard, transrectal prostate ultrasound (US) systematic biopsy (14 - cores, 12 from peripheral zone and two from transition zone) and mpMRI / US fusion with additional samples of suspicious areas was adopted. US - guided biopsies were performed using either an Aplio 500 with Smart Fusion (Toshiba Medical System Corporation, Minato, Tokyo, Japan) or a LOGIC E9 with imaging fusion software (GE Healthcare, Little Chalfont, United Kingdom). One out seven radiologists with experience in prostate biopsy with imaging fusion mpMRI / US (minimum of 3 year of experience) performed the prostate biopsy, aware of mpMRI findings.

TOne out four of the pathologists from the hospital performed the histopathologic analysis, with at least 15 years of experience in uropathology. Histological findings were classified for each prostatic region as negative, positive CI tumor (Gleason 3 + 3), or positive CS tumor (Gleason ≥ 3 + 4) (20).

Data analysis

Two fellowship trained radiologists (with 6 and 15 years of experience in prostate mpMRI) read images prospectively and independently (blinded to each other): first they filled in a form classifying the prostate mpMRI in suspicion levels for PCa reading only sequences without contrast. Subsequently, they filled in another form reclassifying the suspicion levels for PCa reading the entire exam including the post - contrast enhancement sequences. Both radiologists were aware of the patient's clinical data. Analysis was performed into eight prostatic regions (apex, mid and base of peripheral zone; transition zone, right and left), and graded on 5 - point scale of cancer suspicion (1: CS PCa is very unlikely; 2: CS is unlikely; 3: presence of CS PCa is equivocal; 4: CS PCa is likely; and 5: CS PCa is very likely). A final consensus analysis was performed to make the final report, which was used to guide the suspicious areas on biopsies. The imaging-pathologic correlation was performed by one of the authors after all the MRI readings were finished (18).

Statistical methods

We performed a histogram analysis to verify the distribution. Because numeric variables were not normally distributed, they were described with median and interquartile range (IQR).

To verify the association between mpMRI categories (1-5) and biopsy results we used generalized estimating equations (21), with permutable correlation structures, using the software R 3.1.3 (R Core Team, 2015). Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy for both readers were calculated using biopsy as reference standard. The level for statistical significance was set at 5%.

Inter - reader agreement was calculated using Cohen's Kappa coefficient of agreement within ordinal weights, and it was defined as: excellent ($k \geq 0.81$), good ($k = 0.61 - 0.80$), moderate ($k = 0.41 - 0.60$), fair ($k = 0.21 - 0.40$), and poor ($k \leq 0.20$).

RESULTS

The final cohort was comprised of 102 patients with a median age of 62.1 years old (range 35.1 - 82.1). Median time between mpMRI and biopsy was 15 days (IQR 14; 16); median PSA level was 4.36 ng / mL (IQR 3.19; 5.83); median number of fragments in the prostate biopsy was 19 (IQR 17; 21); and median number of fragments for each suspicious lesion was 4 (IQR 3; 5). Twenty - five patients (24%) were submitted to prior biopsy, and of those, 19 (76%) had negative results. The remaining 6 patients (24%) were on active surveillance for a CI tumor (up to two fragments of Gleason 3 + 3 on previous biopsy). Previous prostate biopsies were performed with a median time of 21 months (range 2 - 180) prior the mpMRI and those patients had no post - biopsy hemorrhage in the prostate gland during exam analysis.

Overall biopsy results showed CS cancer in 43 (42.2%), CI cancer in 11 (10.8%), and negative result for cancer in 48 (47%) patients. Of the

25 patients who had prior biopsies with negative results or CI tumors, 10 (40%) had new diagnoses of CS tumors and one (4%) maintained CI tumor diagnosis.

Each radiologist evaluated a total of 816 prostatic regions in each phase of the study (eight prostatic regions in 102 patients). Table-1 provides the mpMRI readings on the eight prostatic regions

Table 1 - Positivity results regarding the suspicion level on mpMRI in a sextant pattern.

Radiologist	MRI category	Contrast	Global	Biopsy								
				Negative			Positive clinically non-significant			Positive clinically significant		
			N	n	p (95%CI)	p-value	n	p (95%CI)	p-value	n	p (95%CI)	p-value
1	1	With	10	10	100.0	-	0	0.0	-	0	0.0	-
		Without	10	10	100.0		0	0.0		0	0.0	
	2	With	504	435	86.3 (83.3-89.3)	0.969	21	4.2 (2.4-5.9)	0.868	48	9.5 (7.0-12.1)	0.876
		Without	530	457	86.2 (83.3-89.2)		21	4.0 (2.3-5.6)		52	9.8 (7.3-12.3)	
	3	With	247	202	81.8 (77.0-86.6)	0.813	9	3.6 (1.3-6.0)	0.877	36	14.6 (10.2-19.0)	0.731
		Without	230	190	82.6 (77.7-87.5)		9	3.9 (1.4-6.4)		31	13.5 (9.1-17.9)	
	4	With	36	20	55.6 (39.3-71.8)	0.187	2	5.6 (0.0-13.0)	0.984	14	38.9 (23.0-54.8)	0.185
		Without	28	11	38.9 (20.9-56.9)		2	5.7 (0.0-13.6)		15	55.8 (37.1-74.5)	
	5	With	19	3	15.8 (0.0-32.2)	0.679	0	0.0	-	16	84.2 (67.8-100.0)	0.679
		Without	18	2	11.1 (0.0-25.6)		0	0.0		16	88.9 (74.4-100.0)	
2	1	With	9	9	100.0	-	0	0,0	-	0	0.0	-
		Without	12	9	75.0		0	0,0		3	25.0	
	2	With	482	418	86.7 (83.7-89.8)	0.819	19	3.9 (2.2-5.7)	0.984	45	9.3 (6.7-11.9)	0.799
		Without	485	423	87.2 (84.2-90.2)		19	3.9 (2.2-5.6)		43	8.9 (6.3-11.4)	
	3	With	268	212	79.1 (74.2-84.0)	0.851	10	3.7 (1.5-6.0)	0.924	46	17.2 (12.6-21.7)	0.801
		Without	257	205	79.8 (74.9-84.7)		10	3.9 (1.5-6.3)		42	16.3 (11.8-20.9)	
	4	With	38	27	71.1 (56.6-85.5)	0.673	3	7.9 (0.0-16.5)	0.899	8	21.1 (8.1-34.0)	0.590
		Without	42	28	66.7 (52.4-80.9)		3	7.1 (0.0-14.9)		11	26.2 (12.9-39.5)	
	5	With	19	4	21.1 (2.7-39.4)	0.770	0	0.0	-	15	78.9 (60.6-97.3)	0.770
		Without	20	5	25.0 (6.0-44.0)		0	0.0		15	75.0 (56.0-94.0)	

Mp = multiparametric; **MRI =** magnetic resonance imaging; **N =** number of prostate regions; **95%CI =** 95% confidence intervals; P-value to compare exams without and with contrast.

that had no statistical difference regarding detection of CS PCa in exams with and without contrast for both readers. Positive CS PCa ranged from 8.9% to 9.8% for low suspicion (category 2) and 75.0% to 88.9% for very high suspicion (category 5) on mpMRI categories (Figure-1). The odds of having CS PCa on mpMRI was 2.75 (reader 1) and 2.4 (reader 2). In corroborating these findings, no statistical difference was found when we compared accuracy, sensitivity, specificity, PPV, and NPV in both sets of exams (Table-2). Accuracy was slightly better in exams without contrast for both readers, but without statistical significance.

The best sensitivity and specificity values were obtained when including category 3 (equivocal) as positive on mpMRI studies with and without contrast (Table-3).

Post - contrast sequences changed the overall mpMRI analysis in 11 cases for reader 1 (increasing the category in 10 cases) and in 7 cases for reader 2 (increasing the category in two cases). For reader 1, the post - contrast sequences correlated with biopsy results (positive enhancement in CS tumors or negative enhancement in negative results / CI tumors) in 5 cases (45%) and resulted in misclassification in 6 (55%). For reader 2, the sequences correlated with biopsy results in two cases (29%) and resulted in misclassification in five (71%). Post - contrast sequences identified four regions with CS tumors more than

Figure 1 - Seventy three years old man with PSA level of 3.4 ng/mL and normal DRE. MpMRI shows a 7 mm nodule in the left apical peripheral zone (T2-weighted imaging on A), with marked restricted diffusion seen on ADC map (B) and on b-value of 1500 (C). The lesion was categorized as very high suspicion for CS PCa (category 5) for both readers in both reading sessions despite the small size and DCE sequence. Early enhancement of the lesion is seen on DCE (D). Biopsy confirmed Gleason 4+4.

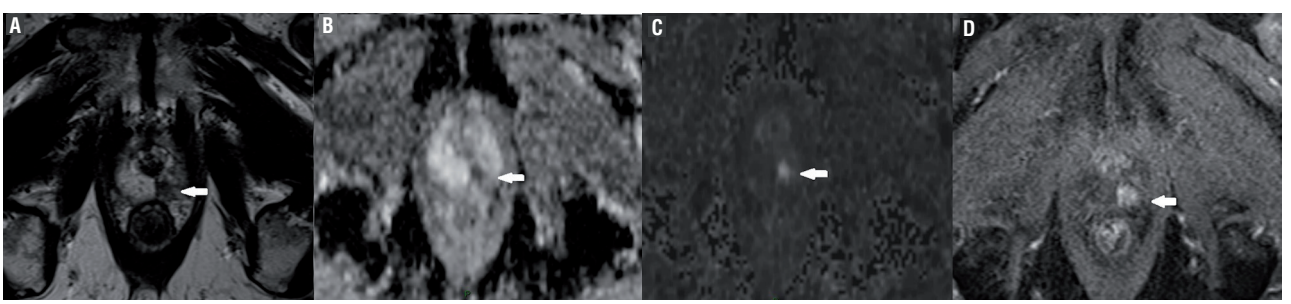

Table 2 - Diagnostic measurements.

Radiologist	Contrast	Accuracy	P-value	Sensitivity	P-value	Specificity	P-value	PPV	P-value	NPV	P-value
1	With	65.2 (61.9-68.5)	0.346	57.9 (48.8-67.0)	0.594	66.4 (62.9-69.9)	0.208	21.9 (17.2-26.5)	0.860	90.7 (88.1-93.2)	0.872
	Without	67.4 (64.2-70.6)		54.4 (45.2-63.5)		69.5 (66.1-72.9)		22.5 (17.5-27.4)		90.4 (87.9-92.9)	
2	With	63.1 (59.8-66.4)	0.837	60.5 (51.6-69.5)	0.892	63.5 (60.0-67.1)	0.781	21.2 (16.8-25.7)	0.979	90.8 (88.3-93.4)	0.961
	Without	63.6 (60.3-66.9)		59.6 (50.6-68.7)		64.2 (60.7-67.8)		21.3 (16.8-25.8)		90.7 (88.2-93.3)	

PPV = Positive Predictive Value; **NPV =** Negative Predictive Value.

Table 3 - Diagnostic measures according to the Likert categories on mpMRI, for both radiologists, for exams read with and without the dynamic post-contrast sequences.

Exam	Reader	Category +	Sensitivity	Specificity
With contrast	1	2	100.0	1.4 (0.5-2.3)
		3	57.9 (48.8-67.0)	66.4 (62.9-69.9)
		4	26.3 (18.2-34.4)	96.4 (95.1-97.8)
		5	14.0 (7.7-20.4)	99.6 (99.1-100.0)
	2	2	100.0	1.3 (0.4-2.1)
		3	60.5 (51.6-69.5)	63.5 (60.0-67.1)
		4	20.2 (12.8-27.5)	95.2 (93.6-96.7)
		5	13.2 (7.0-19.4)	99.4 (98.9-100.0)
Without contrast	1	2	100.0	1.4 (0.5-2.3)
		3	54.4 (45.2-63.5)	69.5 (66.1-72.9)
		4	27.2 (19.0-35.4)	97.9 (96.8-98.9)
		5	14.0 (7.7-20.4)	99.7 (99.3-100.0)
	2	2	97.4 (94.4-100.0)	1.3 (0.4-2.1)
		3	59.6 (50.6-68.7)	64.2 (60.7-67.8)
		4	22.8 (15.1-30.5)	94.9 (93.2-96.5)
		5	13.2 (7.0-19.4)	99.3 (98.7-99.9)

the exam without post - contrast sequences (4 / 114 = 3.5%) for reader 1 and one region more (1 / 114 = 0.9%) for reader 2. On the other hand, the change of the classification for mpMRI positive (categories 3 to 5) with post - contrast sequences had negative results on biopsy in 22 regions (22 / 670 = 3.3%) for reader 1 and in five regions (5 / 670 = 0.7%) for reader 2 (Table-1).

A total of 1632 prostatic regions were evaluated by each readers, and the inter - reader agreement was 0.59 (CI: 0.55 - 0.64), demonstrating good agreement. The inter - reader agreement in the per - patient analysis (a total of 102 patients in each exam phase for each reader) was 0.47 (CI: 0.31 - 0.64) on exams without post - contrast sequences and 0.54 (CI: 0.38 - 0.70) on exams with post - contrast sequences, demonstrating moderate agreement.

DISCUSSION

Due to over - diagnosis and overtreatment of PCa in the PSA era, mpMRI became useful to detect and characterize prostatic lesions in patients with clinical suspicion for cancer prior to biopsy (7-10, 22). The use of MRI to detect CS PCa is already established by many studies performed with complete protocol of mpMRI, including contrast (23-25). Recent studies performed with a complete mpMRI protocol have demonstrated the benefits of MRI over some biomarkers for the detection of PCa (10) and to monitor candidates for active surveillance (26, 27).

As a non - invasive method used for prostatic tumor detection, ideally mpMRI should be as faster and cheaper as possible. It is known that contrast - enhanced mpMRI is more expensive,

time - consuming, and increases the risk of potential allergic reactions, nephrogenic systemic fibrosis, and gadolinium brain tissue deposition (17-19).

In this prospective study we found similar detection rates for CS PCa in exams read with and without contrast - enhanced sequences with no statistical differences for the five levels of suspicion on mpMRI.

Two recent studies showed high accuracy of MRI for the detection of CS PCa, using a Likert scale with only T2W images and DWI (biparametric - MRI) and PSA levels (28, 29). These studies were retrospective, did not categorize the mpMRI suspicion level, and did not compare the results of biparametric - MRI with the gold standard of mpMRI (that includes post - contrast images). In our cohort we included all patients with clinical suspicion of PCa and all mpMRI exams regardless the suspicion level, which probably explains the higher specificity and NPV and lower sensitivity and PPV of our study when compared to their results.

Vargas et al., aiming to evaluate the recommendations in the PI - RADS version 2 and investigate the impact of pathologic tumor volume on PCa detectability on mpMRI, found limited added value of DCE to T2W and DWI sequences (30). Also, few studies showed similar performance for mpMRI with and without contrast media for PCa detection, using both Likert (31, 32) and PIRADS (33) scales. These findings corroborate ours that non - contrast mpMRI can improve PCa detection in the near future.

On the other hand, in a study that included only PI - RADS categories 3 and 4, Druskin et al. showed higher positivity for CS PCa in lesions category 3 with and without enhancement (upgraded to PI - RADS 4); however, both lower compared to PI - RADS 4 (32). This finding shows that a PI - RADS 3 lesion with positive enhancement (which is upgraded to PI - RADS 4) has lower risk of CS PCa than a PI - RADS 4 lesion, as showed in a prospective analysis performed by Mertan et al. (34).

We used a Likert scale to stratify the suspicion level on mpMRI, where the radiologist provided a score based on overall impression instead of a fixed criterion. The grade of diffusion restriction (low, moderate, and high) was the most important criteria to classify risk of CS PCa. When this study was designed PI - RADS version 2 had not been published (13) and PI - RADS version 1 was not in use at our institution. The Likert criteria was already shown to be more accurate when applied by readers with previous experience (35). Our study showed moderate to good rates of inter - reader agreement, similar values of those demonstrated using the PI - RADS classification (36-38).

Our study shows that the use of contrast in mpMRI does not increase the detection rate of CS PCa, and has similar accuracy, sensitivity, specificity, PPV, and NPV as compared to a non - contrast protocol. In this prospective study, we included all patients with no prior diagnosis of CS PCa. The diagnostic results yielded consistently high NPV to rule - out CS PCa (> 90%), which could help avoid unnecessary biopsies in patients with low suspicion on mpMRI (categories 1 and 2). Accuracy and specificity were slightly better for non - contrast exams for both readers, but without statistical significance.

This study had several limitations. First, since our institution is an open hospital, a high number of patients (345) did not perform biopsy at our institution and were excluded. Second, our population study included all patients without diagnostic of CS PCa (biopsy naïve, with negative previous biopsy and in active surveillance) and we did not perform a subgroup analysis. Also, we did not use the PIRADS classification; however, previous studies showed good performance of mpMRI using a Likert classification. We used biopsy as a reference standard instead of prostatectomy specimen, what could introduce an imaging - pathology correlation bias; however, many studies were published using this same methodology, with consistent results (39-41). We did not separate peripheral zone and transitional zone tumors. Our temporal resolution for DCE sequences was 13 seconds instead of 10 seconds recommended nowadays. Finally, the short time between the readings could introduce an interpretation bias, but such bias would favor the reading of images that in-

cluded the contrast - enhanced series, which was performed at the end of the reading session.

In conclusion, our study shows similar performances of mpMRI with and without DCE for CS PCa detection. Further studies should be performed to confirm these results and confirm that a limited, faster, and cheaper mpMRI protocol can be used as standard technique.

ACKNOWLEDGEMENT

We thank Ana Carolina Cintra Nunes Mafra for helping with the statistical analyses.

Ethical Approval

IRB approved. For this type of study formal consent is not required.

CONFLICT OF INTEREST

None declared.

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Correspondence address:
Thais Caldara Mussi, MD
Departamento de Radiologia e Diagnóstico por Imagem
Hospital Israelita Albert Einstein
Av. Albert Einstein, nº 627
São Paulo, SP, 05652-900, Brasil
Telephone: + 55 11 2151-4271
E-mail: thaiscaldara@gmail.com

ORIGINAL ARTICLE

Vol. 44 (6): 1139-1146, November - December, 2018
doi: 10.1590/S1677-5538.IBJU.2017.0569

Prospective Evaluation of Chondroitin Sulfate, Heparan Sulfate and Hyaluronic Acid in Prostate Cancer

Matheus Neves Ribeiro da Silva [1, 2], Aline Mendes [1], João Roberto Maciel Martins [2], Marcos Tobias Machado [2], Maria Aparecida da Silva Pinhal [1]

[1] Departamento de Bioquímica, Universidade Federal de São Paulo (UNIFESP), São Paulo, SP, Brasil; [2] Departmento de Urologia Faculdade de Medicina do ABC, Santo André, SP, Brasil

ABSTRACT

Purpose: The present study evaluates chondroitin sulfate (CS) and heparan sulfate (HS) in the urine and hyaluronic acid (HA) in the plasma of patients with prostate cancer before and after treatment compared to a control group.
Materials and Methods: Plasma samples were used for HA dosage and urine for quantification of CS and HS from forty-four cancer patients and fourteen controls. Clinical, laboratory and radiological information were correlated with glycosaminoglycan quantification by statistical analysis.
Results: Serum HA was significantly increased in cancer patients (39.68 ± 30.00 ng/mL) compared to control group (15.04 ± 7.11 ng/mL; p=0.004) and was further increased in high-risk prostate cancer patients when compared to lower risk patients (p = 0.0214). Also, surgically treated individuals had a significant decrease in seric levels of heparan sulfate after surgical treatment, 31.05 ± 21.01 µg/mL (before surgery) and 23.14 ± 11.1 µg/mL (after surgery; p=0.029). There was no difference in the urinary CS and HS between prostate cancer patients and control group. Urinary CS in cancer patients was 27.32 ± 25.99 µg/mg creatinine while in the men unaffected by cancer it was 31.37 ± 28.37 µg/mg creatinine (p=0.4768). Urinary HS was 39.58 ± 32.81 µg/mg creatinine and 35.29 ± 28.11 µg/mg creatinine, respectively, in cancer patients and control group (p=0.6252).
Conclusions: Serum HA may be a useful biomarker for the diagnosis and prognosis of prostate cancer. However, urinary CS and HS did not altered in the present evaluation. Further studies are necessary to confirm these preliminary findings.

ARTICLE INFO

Keywords:
Biomarkers; Glycosaminoglycans; Prostatic Neoplasms

Int Braz J Urol. 2018; 44: 1139-46

Submitted for publication:
October 24, 2017

Accepted after revision:
June 11, 2018

Published as Ahead of Print:
September 10, 2018

INTRODUCTION

Prostate cancer has a highly variable and unpredictable course. Currently, prostate cancer is diagnosed and its aggressiveness is classified by tumor stage, Gleason score, the extent of tumor at biopsy, and serum levels of prostate-specific antigen (sPSA). When relying solely on that information, patients are potentially unnecessarily over-diagnosed and thus sometimes over-treated, which has resulted in the 2012 U.S. Preventive Services Task Force recommendation of ''D'' (i.e. discouraged) for sPSA as a routine screening test. In 2017 the recommendation was updated and for men between 55 to 69 years the new recommendation is C, meaning that the decision

about whether to be screened for prostate cancer should be an individual one. This discussion has increased the efforts to identify molecules that are expressed in prostate cancer and that can be associated with invasion and metastasis to improve the current prognostic capabilities and management of prostate cancer (1).

Changes in the levels and structure of glycosaminoglycan (GAG) side chains of proteoglycans have been associated with the development and progression of malignancies in various tissues (2-5). Likewise, prostate cancer has been shown to express GAGs, quantitatively and qualitatively, differently from normal and hyperplastic prostatic tissues. Moreover, the magnitude of this difference may even be of prognostic significance (6-13).

Heparan sulfate (HS) plays an important role in cell–cell and cell–matrix communication and cellular signaling, being an essential part of the cell microenvironment. It is extremely important in both development and cancer progression due to its regulation of cellular processes such as angiogenesis, tumor growth, proliferation, tumor invasion and metastasis. HS controls a variety of biological functions by modulating growth factor signaling pathways, such as FGF, VEGF and TGF-β (14).

Heparan sulfate expression in prostate tumors is unlike normal human prostate tissue mainly due to decreased HS content in tissue stroma and heterogeneous HS expression in different tissue compartments (13). Overexpression of syndecan, a heparan sulfate proteoglycan, in prostate cancer was significantly associated with established features indicative of worse prognosis such as higher preoperative PSA, higher Gleason score, positive surgical margins, an extraprostatic extension of disease and biochemical disease progression. Also, metastatic prostate cancer tends to exhibit higher levels of both syndecan and perlecan, another heparan sulfate proteoglycan present at basement membrane (12).

The concentration of chondroitin sulfate (CS) is greatly increased over normal tissue levels in several different malignancies (4, 15), including prostate cancer. Indeed, elevated levels of sulfated chondroitin in the prostate peritumoral stroma are associated with higher incidence of PSA failure in radical prostatectomy (16). Moreover, chondroitin sulfate levels in advanced (cT4) prostate cancer tissues are very similar to the levels present in those early-stage prostate tumors that ultimately progressed (17).

Many tumor types have hyaluronan (HA) as a major part of the extracellular matrix (18). HA is a GAG important for cell division, cell migration and angiogenesis during embryogenesis, inflammation and wound healing (19). HA favors tumor cell invasion, epithelial to mesenchymal transition, cell proliferation, angiogenesis, lymphangiogenesis, and it recruits bone marrow-derived inflammatory and progenitor cells to tumors (18). In prostate cancer, accumulation of HA in tumor stroma and altered hyaluronic acid synthase and hyaluronidase in tumor epithelial cells are associated with increased cell proliferation, invasion, metastasis and poor outcome in men who have undergone radical prostatectomy (6, 7, 20-23).

The aforementioned studies found altered GAG expression in prostate tissue, mainly in the retrospective analysis of patients previously treated for the cancer, when the decision has already been made. However, the main objective of novel biomarkers is to help in the challenging decision--making of how to manage a patient recently diagnosed with prostate cancer. This study evaluates chondroitin sulfate and heparan sulfate in the urine and hyaluronan in the plasma of prostate cancer patients before and after treatment and correlated with known prognostic parameters. A comparative study was also performed with a control group, men that are unaffected by cancer.

MATERIAL AND METHODS

We prospectively collected urine and blood from 44 men newly diagnosed with prostate cancer and 14 controls not eligible for prostate biopsy, considered low risk of harboring prostate cancer (PSA <1.5; non-suspicious digital rectal examination), according to the public academic urology service, during the year of 2009. Cancer patients were evaluated for serum PSA, Gleason

grading performed according to the new modified system based on the 2005 consensus conference (24), D'Amico's clinical risk stratification, pelvic computed tomography and bone scan evaluations for distant metastasis. Cancer patients were treated with open or laparoscopic radical prostatectomy (32 patients), external beam radiotherapy (7 patients), and palliative hormone therapy for advanced disease (5 patients). Urine and blood samples were also collected after treatments, at 3, 6 and 12 months after. All work was performed with the institution-approved protocol with patient consent (ethical committee approval number CEP 019/2009).

Urinalysis and antibiogram were performed, as well as clinical evaluation of other urinary or systemic diseases such as urinary tract infection, functional bladder diseases, mucoviscidosis, diabetic renal disease and amyloidosis. If any of these diseases were present, the patient was excluded.

Urinary Sulfated Glycosaminoglycan Quantification

Urine was collected from patients with prostate cancer and healthy subjects (about 50 mL). Urine samples were placed at 60°C for 1 hour for complete solubilization of proteins. Each sample was filtered using a paper filter at 4°C and the filtrate were centrifuged at 2,500 x g for 20 min. The supernatant (around 10 mL) was concentrated on Millipore filter with a 5,000 Da exclusion limit by centrifugation at 2,500 x g until achieving a total volume of 250 µL. The material (250 µL) was totally dried using vacuum and resuspended in distilled water to a final volume of 5 µL and subjected to agarose gel electrophoresis. Sulfated glycosaminoglycans (HS, DS and CS) were identified and quantified by agarose gel electrophoresis in 0.05 M 1,3-diaminopropane-acetate buffer (PDA), pH 9.0. After electrophoresis, for 1 h, at 100 V, the glycosaminoglycans were precipitated in agarose gel using 0.1% cetyltrimethylammonium bromide (CETAVLON), (Sigma-Aldrich, Saint Louis, MO) for 2 hours at room temperature. The gel was dried and stained with toluidine blue (0.1% in acetic acid: ethanol: water; 0.1 : 5 : 4.9, v:v:v). GAG quantification was carried out by densitometry at 530 nm. The extinction coefficients of the GAGs were calculated using standards of chondroitin 4-sulfate from whale cartilage (Seikagaku Kogyo Co., Tokyo, Japan), dermatan sulfate (from pig skin) and heparan sulfate (from bovine pancreas). The agarose gel electrophoresis method error was on the order of 5%.

Quantification of a non-sulphated glycosaminoglycan hyaluronic acid

HA was measured by a previously described fluorescence-based assay (25). Briefly, standard concentrations (0-500 mg of link protein) of HA obtained from human umbilical cord and urine samples from patients, diluted 1:4 in blocking buffer (100 µL of urine plus 300 µL of blocking buffer) were used. One hundred microliters from each solution was added, in triplicate, into the plates coated with hyaluronic acid-binding protein (HABP). The plates were incubated at 25°C, for 18 hours, washed three times with washing buffer and 100 mL of biotinylated HABP (1 mg/mL), diluted in blocking buffer (1:5000) added to each well. The incubation was performed for 120 min. at 25°C under shaking. The plates were washed six times with washing buffer, and 100 µL of europium-labeled streptavidin (1:5000 in blocking buffer) were added. Incubation was carried out for 30 min. at 25°C, and washed six times to remove unbound streptavidin. Finally, 200 µL of enhancement solution (Perkin-Elmer Life Sciences-Wallac Oy, Turku, Finland) was added to release the europium bound to streptavidin and the plates were shaken for 10 min. A time-resolved fluorometer (Victor 2 from Perkin-Elmer, Life Sciences-Wallac Oy, Turku, Finland) was used to measure free europium and the fluorescence (counts/s). The values were processed automatically in the MultiCalc software program (Perkin-Elmer Life Sciences-Wallac Oy, Turku, Finland). This technique measures HA in concentration as low as 0.2 µg/L.

Statistical analysis

The variables in the study were considered parametric or not based on the Kolmogorov-Smir-

nov test. Student t-test and ANOVA with Tukey's auxiliary test or Kruskal-Wallis and Mann-Whitney tests were used to compare parametric and non-parametric data, respectively. A significance level of 0.05 was adopted in all analysis. Statistical analysis was performed using SPSS version 23.0 (SPSS Inc., Illinois, USA).

RESULTS

Table-1 summarizes clinical features such as serum PSA, Gleason score, D'Amico's clinical risk group and other clinical data from the patients as well as unaffected individuals.

Serum hyaluronic acid (HA) was significantly increased in cancer patients (39.68 ± 30.00 ng/mL) compared to the control group (15.04 ± 7.11 ng/mL); (p=0.004; Mann-Whitney test), as shown in Figure-1. Interestingly, HA was further increased in the group of patients that presented high-risk prostate cancer compared to intermediate risk patients (p = 0.0214; Mann-Whitney test), as shown in Figure-2. Patients with metastatic disease, positive bone scans or TC disclosing increased lymph nodes, have higher levels of hyaluronic acid (45.19 ± 7.32 ng/mL) compared to non-metastatic patients (15.16 ± 10.76 ng/mL), whereas significance was not achieved probably due to the small number of metastatic individuals (n=4; p=0.31; Mann-Whitney test).

There was no statistical difference in the urinary sulfated glycosaminoglycans (CS and HS) between prostate cancer patients and control group. In prostate cancer patients, the urinary CS was 27.32 ± 25.99 µg/mg creatinine and for unaffected individuals 31.37 ± 28.37 µg/mg creatinine (p=0.4768; Mann-Whitney test). There was also no significant difference in the amount

Table 1 - Clinical characteristics of cancer patients and control group.

	Cancer Patients	Control Group
AGE	68 years (46-77)	62 years (49-72)
PSA	27.48 ng/dL (1.4 -150)	1.75 ng/dL (0.35 – 3.2)
DRE	Normal: n=18	Normal: n=14
	Nodule: n=23	
	diffusely endured prostate: n=3	
Gleason (biopsy)	4: n=1	
	6: n=17	
	7: n=15	
	8: n=5	
	9: n=6	
Computed tomography	Normal: n=41	
	Enlarged pelvic LN: n=3	
Bone Scan	Negative : n=43	
	Positive: n=1	
D'Amico's clinical risk	Low: n=15	
	Intermediate: n=8	
	High: n=21	

The numbers in parenthesis represents the average; **DRE =** Digital Rectum Examination; **n =** number of patients; **LN =** Lymph nodes

Figure 1 - Profile of hyaluronic acid in cancer patients (Case) and individuals non affected by prostate cancer (Control). Hyaluronic acid was quantified in the plasma samples as previously described in Methods, using an ELISA-like assay. P = 0.004, Student-T Test.

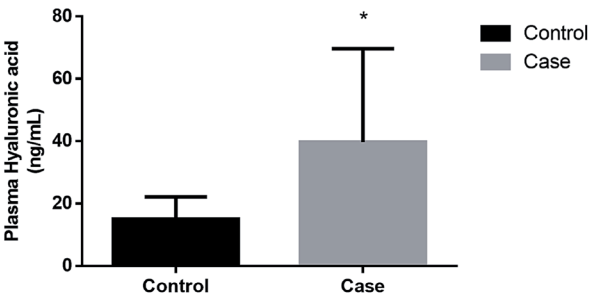
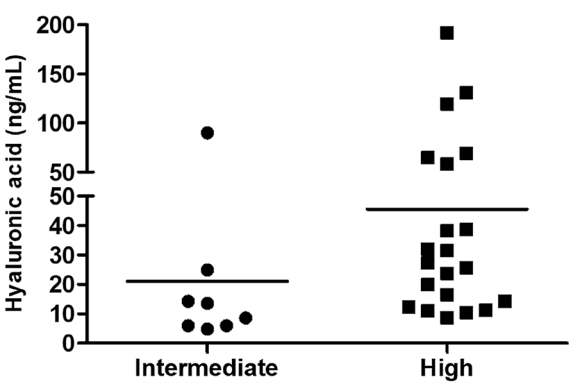


Figure 2 - Hyaluronic acid in prostate cancer patients according to D'Amico's risk classification (Intermediate and High). There was a significant difference in the amount of plasma hyaluronic acid comparing the group of intermediate and high D'Amico's risk. p = 0.0214, Student-t Test.



of urinary heparan sulfate between patient and control groups, 39.58 ± 32.81 µg/mg creatinine and 35.29 ± 28.11 µg/mg creatinine, respectively (p=0.6252; Mann-Whitney test). Unlike hyaluronic acid urinary, chondroitin sulfate and heparan sulfate were not different for D'Amico's risk groups (p=0.471 and p=0.811 respectively; Kruskal Wallis test).

A significant increase in urinary chondroitin sulfate, was detected after hormone ablation compared to the data obtained before the treatment (41.01 ± 24.14 µg/mg creatinine and 24.14 ± 22.64 µg/mg creatinine, respectively) (Table-2). Therefore, patients with prostate cancer that had been submitted to hormone therapy presented higher levels of chondroitin sulfate compared to pre-treatment values. Conversely, surgical treatment promoted a significant decrease in the urinary level of heparan sulfate, 23.14 ± 11.1 µg/mg creatinine, compared to the result obtained before surgery, 31.05 ± 21.01 µg/mg creatinine, as shown in Table-2.

When analyzing others laboratorial parameters, we observed some finds not considered in the initial hypothesis. Using the Spearman's rank correlation coefficient, there was an association between testosterone levels with chondroitin sulfate and heparan sulfate values secreted in the urine of patients with prostate cancer. The results show the higher the level of total testosterone, the higher the amount of the urinary chondroitin sulfate (p = 0.013) and heparan sulfate (p = 0.023). However, higher levels of free testosterone only revealed an increased amount in chondroitin sulfate (p = 0.019), with no significant alteration in heparan sulfate (p = 0.076), as demonstrated in Table-3.

DISCUSSION

Urological associations worldwide still recommend prostate cancer screening, though in a narrower population group, reflecting influence from the US Preventive Task Force recommendation in 2012, and its draft update in 2017. This lack of agreement reflects in part the difficulty to accurately identify the patient who should undergo a biopsy for suspicious significant prostate cancer. Diagnosis and treatment of indolent disease have led to unnecessary morbidity and mortality. Efforts have been made to identify molecular markers that function in promoting invasion/metastasis and could be added as adjuncts to the current diagnostic tools. To date, this study is the first to evaluate a possible role of urinary GAG and plasmatic HA in aiding prostate cancer diagnostic and prognostic evaluation.

Our results demonstrated a significant increase in serum hyaluronic acid in prostate cancer patients, and this increase is even higher in those with high-risk disease. Moreover, this increase in HA tends to be even higher in me-

Table 2 - Chondroitin sulfate and heparan sulfate quantified before and after treatment.

	Chondroitin Sulfate (µg/mg creatinine)			Heparan Sulfate (µg/mg creatinine)		
	Before	After treatment	P	Before	After treatment	P
Surgery	24.48 ± 21.25	21.98 ± 16.67	0.062	31.05 ± 21.01	23.14 ± 11.1	0.021
Hormone Therapy	24.14 ± 22.64	41.01 ± 24.14	0.042	39.12 ± 33.97	41.73 ± 21.60	0.71

p values based on Student-t test

Table 3 - Evaluation of Gleason, PSA and the level of testosterone with the amount of sulfated glycosaminoglycans.

	Chondroitin Sulfate (µg/mg creatinine)	Heparan Sulfate (µg/mg creatinine)
Gleason	p = 0.842	p = 0.675
Total PSA	p = 0.821	p = 0.993
Total Testosterone	p = 0.013	p = 0.023
Free Testosterone	p = 0.019	p = 0.076

The results were obtained in the group of patients before surgery and that were not submitted to hormone therapy; p values based on Spearman's rank correlation coefficient.

tastatic patients. Gomez et al. showed that HA expression in the stroma of prostate cancer and surrounding tissue is higher the higher the PSA, Gleason score and clinical grading (6). In his study, it could predict biochemical recurrence after radical prostatectomy.

It has also been demonstrated in other previous studies of prostate cancer tissue analysis for HA. When we compared plasmatic HA concentration in the different clinical risk groups, the high risk groups had the higher measured plasmatic HA concentrations, which is consistent with previous immunohistochemical studies, where more aggressive cancer demonstrates higher HA expression in the prostate tissue (26, 27). If serum HA concentration is significantly increased in prostate cancer patients compared to non-cancer counterparts as demonstrated herein, it could be a useful tool to help identify patients at risk of harboring prostate cancer, either by adding PSA to routine screening or helping to select patients for re-biopsy. For instance, further study may indicate, in screening patients with low plasmatic HA levels, the likelihood of these patients developing prostate cancer. Likewise, in patients with rising PSA after a negative biopsy, if plasmatic levels of HA could help to determine which patient is at higher risk and therefore should undergo a new biopsy.

The small number of cases observed for metastasis contributes to the difficulty in making a strong association between these parameters and plasmatic HA. A larger series may help to confirm if metastatic patients do indeed have higher HA levels. An extended period of patient follow-up with data acquisition will further determine whether HA could also be an indicator of prostate cancer progression.

In our study, urinary measurement of CS and HS was similar in patients with and without prostate cancer, in contrast to a previous tissue study where both were increased in prostate cancer patients and also showed prognostic significance (8, 9, 16, 28, 29). In most studies, the main difference in tumoral CS concentration was structural, usually in the sulfation status, which could explain why it does not increase urinary CS. Likewise, the main studies where HS was increased

in prostate cancer tissues was in its proteoglycan form and, therefore, possibly not increase HS urinary concentration (12, 30). However, we found a significant decrease in urinary HS after surgical extirpation of disease (and a decreasing trend in urinary CS), suggesting the possibility that a meaningful difference in sulfated urinary GAG concentration between cancer and non-cancer patients may be a matter of sample size.

Hormonal therapy resulted in an increase in urinary GAG concentrations, albeit in a small group. Also, there was a positive association between serum testosterone levels and urinary GAG concentration, pointing to a possible interference of steroid hormones in GAG synthesis.

As a preliminary study, the sample size is a major limitation to the strength of our conclusions, whereas for HA the difference between the groups was so striking that statistic tests provided strong results. Undoubtedly, validation of our finds in larger studies is essential to confirm HA as a useful biomarker and possibly encounter a role for the others studied GAGs, as suggested by others authors.

CONCLUSIONS

In conclusion, we have shown a significant increase in serum hyaluronic acid in prostatic adenocarcinoma patients compared with controls, and such augment seems to be greater with higher grade of D'Amico's risk and metastatic patients. The results suggest that HA may be useful as a biomarker and predictive of disease aggressiveness in prostate cancer patients. However, a larger study is necessary to confirm these results, in order to define whether plasmatic HA measurement could be used to identify and help determine prognosis for prostate cancer patients, what would be particularly interesting since it is a non-invasive method.

ABREVIATIONS

sPSA = serum levels of prostate-specific antigen
GAG = glycosaminoglycan
HS = Heparan sulfate
CS = chondroitin sulfate
HA = hyaluronan or hyaluronic acid

CONFLICT OF INTEREST

None declared.

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Correspondence address:
Matheus Neves Ribeiro da Silva, MD
Departamento de Bioquímica
Universidade Federal de São Paulo (UNIFESP
Rua Três de Maio, 100
São Paulo, SP, 04044-020, Brasil
E-mail: ribeiromed@hotmail.com
Telephone: + 55 11 5576-4438

ORIGINAL ARTICLE

Vol. 44 (6): 1147-1155, November - December, 2018
doi: 10.1590/S1677-5538.IBJU.2017.0629

Prognostic significance of body mass index in patients with localized renal cell carcinoma

Chengtao Wang [1], Zebin Chen [2], Jun Dong [1], Bixiu Wen [1], Yong Fang [2]

[1] Department of Radiation Oncology, First Affiliated Hospital of Sun Yat - Sen University, Guangzhou, China; [2] Department of Urology, First Affiliated Hospital of Sun Yat - Sen University, Guangzhou, China

ABSTRACT

Objective: To investigate the relationship between the pretreatment body mass index (BMI) and the clinical outcomes in patients with localized stage I - III renal cell carcinoma (RCC) surgically treated.

Materials and Methods: From January 2000 to December 2012, 798 patients with stage I - III RCC were recruited from First Affiliated Hospital and Cancer Center of Sun Yat - Sen University. Patients were divided into two groups of BMI < 25 kg / m2 or BMI ≥ 25 kg / m2 according to the World Health Organization classifications for Asian populations. The differences in the long-term survival of these two BMI groups were analyzed.

Results: The 5 - year failure - free survival rates for BMI < 25 kg / m2 and BMI ≥ 25 kg / m2 groups were 81.3% and 93.3%, respectively (P = 0.002), and the 5 - year overall survival rates were 82.5% and 93.8%, respectively (P = 0.003). BMI was a favored prognostic factor of overall survival and failure - free survival in a Cox regression model.

Conclusions: Pretreatment body mass index was an independent prognostic factor for Chinese patients surgically treated, localized stage I - III RCC.

ARTICLE INFO

Keywords:
Body Mass Index; Carcinoma, Renal Cell; Prognosis

Int Braz J Urol. 2018; 44: 1147-55

Submitted for publication:
December 09, 2017

Accepted after revision:
June 11, 2018

Published as Ahead of Print:
September 15, 2018

INTRODUCTION

Renal cell carcinoma (RCC) is one of the most common urologic malignancies, and its incidence has steadily increased in recent decades. Several risk factors for developing of RCC have been reported, including smoking, hypertension and obesity (1).

Stratification of the patients with RCC into categories with different risk of local recurrence, progression and survival would improve the standard of preoperative patient's counseling and treatment planning. Several anatomical, clinical, histological and molecular variables can predict the probabilities of recurrence, progression, and both overall and cancer - specific survival of the patients with RCC (2).

The relationship between Body Mass Index (BMI) and the prognosis of cancer is not consistent. According to some published studies, a high BMI was associated with a favorable prognosis for various tumor types, including head and neck cancer (3), esophageal cancer (4), colon cancer (5) and endometrial cancer (6). However, the results

of some studies showed that patients with a higher BMI had a worse prognosis for breast cancer (7), and prostate cancer (8). In renal cell carcinoma, obesity seems to increase the risk of developing RCC (1).

Whereas, more recent studies conducted in Western countries indicate that obese patients treated with surgery for RCC may have a more favorable prognosis than patients with normal BMI (9-12). It is unknown whether the same associations are seen in Chinese patients, who have a different body composition from White and Black populations. As we all known, different ethnic groups may show clinically significant increases in fat composition or glucose at lower BMI than those predicted in established BMI cut points.

Therefore, the aim of this study was to assess the influence of BMI, using the World Health Organization (WHO) categories recommended for Asians (13), on treatment results in Chinese patients with surgically treated, localized stage I - III RCC.

MATERIALS AND METHODS

Patients

By using the departmental surgical database of our two institutions (First Affiliated Hospital and Cancer Center of Sun Yat - Sen University), we identified 798 patients aged 18 years old who were treated with radical nephrectomy for unilateral, sporadic localized stage I - III RCC between 2000 and 2012. Data collected from each patient included age at diagnosis, gender, pretreatment BMI, pretreatment hemoglobin (Hb), pretreatment alkaline phosphatase (ALP), pretreatment platelets (PLT), TNM stage, histological subtype and survival time. BMI was calculated as the patient's weight on day 1 of admission (in kilograms) divided by the patient's height squared (in meters).

Tumors were classified in accordance with the 2002 TNM staging system. Histological subtypes were stratified in accordance with the 2002 AJCC / UICC classification, and only tumors of clear - cell, chromophobe, and papillary histology were included. The BMI was categorized based on WHO recommendations for Asians.

Statistical analysis

All events were measured from the date of surgery, and statistical tests were performed using SPSS V17.0 (SPSS Inc., Chicago, IL). The actuarial rates were calculated with the Kaplan - Meier method, and the differences were compared with the log - rank test. The time to the first defining event was assessed for the following endpoints: failure free survival (FFS - disease failure at any site), and overall survival (OS - all cause mortality). The survival rates were calculated using the Kaplan - Meier method and compared with the log - rank test. A 2 - tailed P value of less than 05 was considered statistically significant.

The entire cohort was analyzed using the Cox proportional hazards model for OS and FFS. Potentially important prognostic factors considered in the modeling process were patient gender (male vs. female), age (≥ 50 years vs < 50 years), symptoms at presentation (yes vs. no), histology (chromophobe vs. papillary vs clear cell), pTNM stage (III vs. II vs. I), Hb (non - anemia vs. anemia), PLT (> 300 vs. ≤ 300), ALP (> 70 vs. ≤ 70), tumor necrosis (yes vs. no) and BMI (≥ 25 kg / m^2 vs. < 25 kg / m^2).

The last follow-up visit was in June 2015, with a median follow-up period of 46 months.

RESULTS

Table-1 summarizes the clinical and pathologic characteristics of 798 patients according to the WHO BMI subgroups. The mean age was 51 years (range: 19 - 84 years) and the mean BMI was 23.8 kg / m^2 (range: 14.4 - 41.7 kg / m^2) for the entire group. Three hundred and thirty - seven (42.2%) patients had a BMI less than 25 kg / m^2, and 461 (51.2%) had a BMI equal or greater than 25 kg / m^2. When comparing risk parameters between BMI categories, the two BMI groups showed similar demographics, such as in the age, histology, pTNM stage, ALP and tumor necrosis. Aside from these factors, gender, symptoms at presentation, pretreatment Hb and PLT were significantly different. There were more female patients and patients with symptoms at presentation in BMI

Table 1 - Baseline Characteristics by BMI Group.

Characteristics	Total (%)	BMI Group		P value
		<25 (%)	≥ 25 (%)	
Case (Percentage)	798(100)	337(42.2)	461(57.8)	
Mean BMI [mean(range)]	23.8 (14.4-41.7)	20.7 (14.4-22.9)	26.0 (23-41.7)	
Age(y) [mean(range)]	51 (19-84)	51(19-81)	52 (19-84)	
<50 year	383 (48.0)	168(49.9)	215(46.6)	0.369
≥ 50 year	415 (52.0)	169(50.1)	246(53.4)	
Sex				<0.001
Male	545 (68.3)	206 (61.1)	339 (73.5)	
Female	253 (31.7)	131 (38.9)	122 (26.5)	
Symptoms at presentation				0.008
Yes	296 (37.1)	143 (42.4)	153 (33.2)	
No	502 (62.9)	194 (57.6)	308 (66.8)	
Histology				0.406
Clear cell renal carcinoma	720 (90.2)	302(89.6)	418(90.7)	
Papillary renal cell carcinoma	43 (5.4)	22(6.5)	21(4.6)	
Chromophobe renal carcinoma	35 (4.4)	13(3.9)	22(4.8)	
pTNM stage				0.070
I	596 (74.7)	238(70.6)	358(77.7)	
II	171 (21.4)	85(25.2)	86(18.7)	
III	31 (3.9)	14(4.2)	17(3.7)	
Hb				0.003
Male: Hb<120; Female: Hb<110	106 (13.3)	59(17.5)	47(10.2)	
Male: Hb ≥ 120; Female: Hb ≥ 110	692 (86.7)	278(82.5)	414(89.8)	
PLT				0.017
≤ 300	690 (85.5)	280(83.1)	410(88.9)	
>300	108 (13.5)	57(16.9)	51(11.1)	
ALP				0.894
≤ 70	445 (55.8)	187(55.5)	258 (56.0)	
>70	353 (44.2)	150(44.5)	203 (44.0)	
Tumor necrosis				0.660
Yes	111 (13.9)	288 (85.5)	399 (86.6)	
No	687 (86.1)	49 (14.5)	62 (13.4)	

BMI = body mass index; Hb = hemoglobin; **PLT =** platelets; **ALP =** alkaline phosphatase

less than 25 group. Patients with a BMI less than 25 were significantly more likely to have lower pretreatment Hb and higher pretreatment PLT (P = 0.003, P = 0.017, respectively; Table-1). Laparoscopic partial nephrectomy was performed in 39 (4.9%) patients, radical nephroureterectomy was performed in 435 (54.5%) patients, open partial nephrectomy completed in 183 patients (22.9%), and 141 of them had a laparoscopic radical nephrectomy (17.7%). The distribution of surgery modality was balanced in both BMI groups.

Surgery complications were as follow: hemorrhage and hematoma occurred in 21 patients; peritoneal injury in 36 patients; abdominal organ injury in 5 patients; vascular injury in 13 patients; urinary fistula in 7 patients; pleura injury in 3 patients; wound infection in 8 patients and severe hypercapnia in 2 patients. At a median follow-up period of 46.0 months, 11.2% (89 / 798) patients developed tumor progression (31 with tumor recurrence, 58 with distant metastasis) and10.5% (84 / 798) patient were dead. The 5 - year failure - free survival rates for BMI < 25 kg / m^2 and BMI ≥ 25 kg / m^2 groups were 81.3% and 93.3%, respectively (P = 0.002) and the 5 - year overall survival rates were 82.5% and 93.8%, respectively (P = 0.003). Lower BMI was associated significantly with poor prognosis. Patients with preoperative BMI less than 25 kg / m^2 had a significantly reduced rate of survival than those BMI equal or greater than 25 kg / m^2 with regard to FFS and OS (Figures 1 and 2).

Univariable analyses of the factors influencing FFS and OS are shown in Table-2. Univariable analysis demonstrated that the presence of symptoms at presentation, age, pTNM stage, tumor necrosis, non - anemia, ALP, BMI and thrombocytosis were significant predictors of FFS and OS (Table-2).

Stepwise multivariable analysis showed that BMI (HR, 0.54; P = 0.029) was an independent predictor of OS, along with the presence of symptoms at presentation (HR, 1.68; P = 0.031), pTNM stage (HR, 2.30; P < 001), age (HR, 1.72; P = 0.023) and non-anemia (HR, 0.55; P = 0.025) (Table-3). Stepwise multivariable analysis showed that BMI (HR, 0.53; P = 0.022) was an independent predictor of FFS, along with the presence of symptoms at presentation (HR, 1.60; P = 0.043), pTNM stage (HR, 2.59; P < 001), age (HR, 1.75; P = 0.016) and non - anemia (HR, 0.60; P = 0.045) (Table-3).

Figure 1 - Overall survival by BMI group among Chinese patients with RCC.

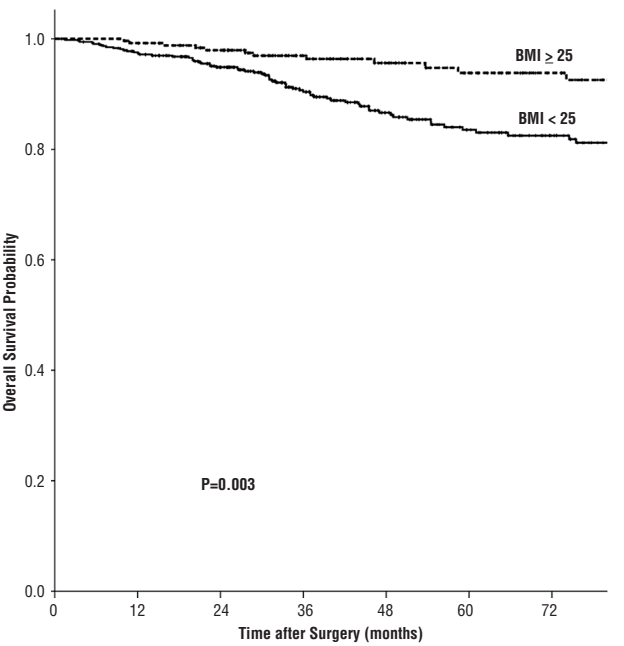

Figure 2 - Failure Free Survival by BMI group among Chinese patients with RCC.

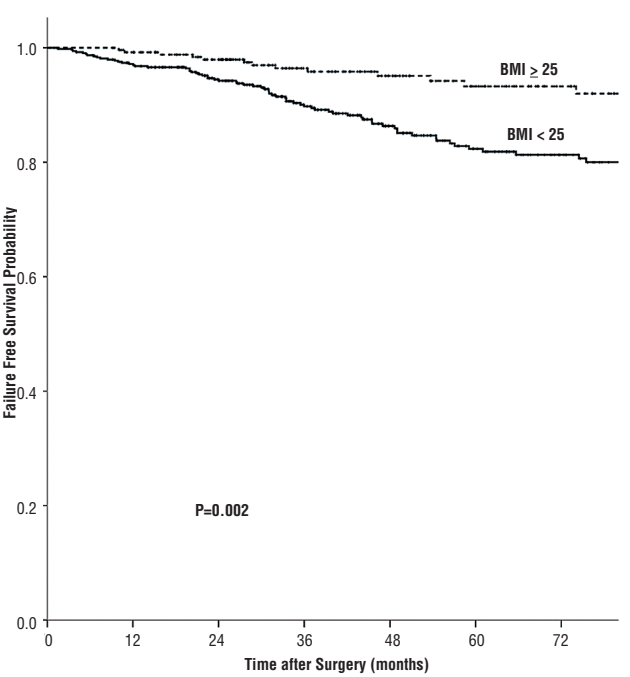

Table 2 - Pretreatment BMI Effect on Different Endpoint: univariate Analysis in Cox Regression Model.

Characteristics	FFS			OS		
	HR	95% CI	p value	HR	95% CI	p value
Gender (male vs female)	1.50	0.92-2.45	0.102	1.38	0.84-2.26	0.201
Age (≥ 50 years vs<50 years)	1.99	1.27-3.10	0.002	2.03	1.28-3.21	0.003
Symptoms at presentation (yes vs no)	2.81	1.83-4.32	0.000	2.94	1.88-4.58	0.000
Histology(Chromophobe vs Papillary vs Clear cell)	1.56	0.75-3.25	0.233	1.63	0.77-3.45	0.207
pTNM stage(III vsIIvsI)	3.36	2.53-4.47	0.000	3.12	2.34-4.18	0.000
Hb (non-anemia vs anemia)	0.30	0.19-0.48	0.000	0.27	0.17-0.42	0.000
PLT (>300 vs ≤ 300)	2.68	1.69-4.25	0.000	2.89	1.81-4.61	0.000
ALP (>70 vs ≤ 70)	5.89	2.84-12.2	0.000	3.65	2.22-5.99	0.000
Tumor necrosis (yes vs no)	3.08	1.98-4.78	0.000	3.22	2.05-5.05	0.000
BMI Group (≥ 25 kg/m^2 vs <25 kg/m^2)	0.45	0.27-0.77	0.003	0.45	0.26-0.78	0.004

BMI = body mass index; **Hb =** hemoglobin; **PLT =** platelets; **ALP =** alkaline phosphatase

Table 3 - Pretreatment BMI Effect on Different Endpoint: Multivariate Analysis in Cox Regression Model.

Characteristics	FFS			OS		
	HR	95% CI	p value	HR	95% CI	p value
Age (≥50 years vs<50 years)	1.75	1.11-2.75	0.016	1.72	1.08-2.76	0.023
Symptoms at presentation (yes vs no)	1.60	1.02-2.52	0.043	1.68	1.05-2.69	0.031
pTNM stage(III vs II vs I)	2.59	1.83-3.64	0.000	2.30	1.62-3.28	0.000
Hb (non-anemia vs anemia)	0.60	0.36-0.99	0.045	0.55	0.33-0.93	0.025
PLT (>300 vs ≤ 300)	1.22	0.72-2.08	0.456	1.33	0.77-2.27	0.306
ALP (>70 vs ≤ 70)	2.04	0.91-4.57	0.083	1.94	0.86-4.37	0.111
Tumor necrosis (yes vs no)	1.29	0.78-2.11	0.321	1.38	0.83-2.30	0.210
BMI Group (≥ 25 kg/m^2 vs < 25 kg/m^2)	0.53	0.31-0.91	0.022	0.54	0.31-0.94	0.029

BMI = body mass index; **Hb =** hemoglobin; **PLT =** platelets; **ALP =** alkaline phosphatase

DISCUSSION

To our knowledge, several epidemiological studies have suggested that obesity is a risk factor for the development of RCC (1, 11, 14). Due to the high rate of comorbidities, obesity is frequently considered to represent a major risk factor for complications after surgery (15). Previous reports have identified postoperative complications correlating with a high BMI (16-18). However, there are conflicting data relating obesity as a risk factor affecting overall or progression - free survival. Since 1991 when Yu et al. found a paradoxical positive association between obesity and overall and disease - free survival, there have been no prospective studies further evaluating this finding

(19). A more contemporary retrospective review of 400 patients undergoing nephrectomy for RCC by Kamat et al. appears to confirm a more favorable prognosis and disease specific survival in overweight and obese patients when compared to normal weight patients (20). With regard to urologic neoplasms, it was shown that a high BMI does not affect oncologic outcomes after surgery. All these studies were conducted in Western countries. The body composition profile of Asian populations differs from that of white and black populations (13). Some studies conducted in Japan and Korea also demonstrated this phenomenon (12, 21). The present study is the first to investigate the influence of obesity on RCC prognosis in a Chinese population. In this study, we examined the association between BMI and other clinical / pathological characteristics, and evaluated the prognostic association of BMI with FFS and OS in Chinese patients with RCC who underwent radical or partial nephrectomy. We found pretreatment BMI was a favorable prognostic factor for Chinese patients with stage I - III RCC. Although obesity predisposed to an increased risk for developing RCC, the prognosis for obese patients treated with surgery was no worse and possibly better than normal weight subjects.

In this retrospective study, clinical / pathological factors significantly impacting FFS and OS for the study population were similar to previously published factors including age older than 50 years at surgery, symptoms at presentation, pTNM stage. Obese patients were more likely to have favorable clinical and pathologic conditions at diagnosis, including younger, less symptoms at presentation, lower stage, lower PLT and lower anemia when compared with under - to - normal weight patients. We therefore carefully adjusted for age, pTNM stage, symptom presence, baseline Hb, ALP and PLT, which may be related to patient survival. Although adjustment for other important risk factors associated with survival weakened the association for both OS and FFS, the association between obesity and RCC prognosis remained strong and highly significant. Being obese at the time of surgery might have a positive prognostic effect in patients. This result is in accordance with the retrospective studies. Parker et al. evaluated 970 patients with RCC and were unable to identify obesity (BMI ≥ 30 kg / m^2) as a prognostic factor (HR 0.90, 0.65 - 1.23, P = 0.488) for CSS in their multivariate analysis, which also included the prognostic factors: Mayo Clinic Stage, size, TNM stage groups, nuclear grade and tumor necrosis. They concluded that BMI offers little additional prognostic information beyond the accepted prognostic features (10, 22). Being obese at the time of surgery might have a positive prognostic effect in patients.

The mechanism by which preoperative obesity may improve RCC survival is not well understood, although mechanisms linking obesity with RCC incidence have long been studied (23). RCC is a heterogeneous and complex disease (24), and the histologic subtypes of RCC differ with respect to genetic, pathologic, and clinical parameters (25-27). On the basis of this evidence, the relationship between obesity and RCC prognosis might be subtype specific. Furthermore, recent studies have shown that the association between obesity and the risk of developing RCC is subtype specific (28-30). Nevertheless, in the previous studies assessing the association between obesity and RCC survival, histologic subtype has been considered as a simple variable that is divided into two groups, cRCC and non - cRCC (31-34), or only patients with cRCC were included (9, 35). The relationship between obesity and RCC prognosis might be subtype specific in our study was not significant.

Some protein factors and signals in adipose tissue that suppress RCC progression have been reported (36). For example, adipose tissue synthesize leptin and the circulating levels of leptin are strongly related to obesity. Leptin has also been shown to play an important role in stimulating pro - inflammatory T helper 1 immune responses (37). In contrast, a change in the predominant immunologic response from T helper 1 to T helper 2 has been reported to correlate with increasing RCC stage (38). Therefore, as proposed by Rasmuson et al., leptin might play a pivotal role in delaying RCC progression (39). Another study showed an association between preoperative nutritional deficiency and poor OS and disease - free survival in RCC patients who

underwent renal surgery (40). Patients with higher BMI, who generally have large appetites and high lipid concentrations, may adequately preserve their fat and muscle mass, thus allowing better nutritional status and potential survival advantage (41, 42). It may be plausible that obesity indicates favorable general health condition rather than it being responsible for improved outcomes.

The present study had some weaknesses. First, the study was retrospective, and the median follow-up time of 46.0 months for patients still alive was short. Second, our study lacked a central review of pathology, although most of the large multicenter studies did. Instead, urologic pathologists reviewed all specimens at each institution. Third, we could not assess potential prognostic factors, such as smoking, molecular markers, and sarcomatoid features in all patients. These factors may allow the identification of patients at high risk and affect the prognosis. However, our study includes the most widely accepted independent prognostic factors of nonmetastatic RCC, including age, pTNM stage, and tumor necrosis. Last, all of our study subjects were Chinese, and the distribution of BMI or cut - off value for Asian populations may be different than those for Western populations. Therefore, our results may not be directly applicable to Western populations. Taken together, a multi - institutional prospective study with a large number of patients would be required to confirm the present finding. Furthermore, basic biologic research would be needed to explain the contradictory effects of BMI on the risk and prognosis of RCC.

In conclusion, these findings suggest that pretreatment high BMI prior to renal surgery is associated with improved OS, FFS when compared with low BMI in Chinese population. This evidence may provide new insight into the effects of preoperative high BMI on improvements in RCC survival, and this could help physicians in predicting overall prognosis. Further research is needed to explain the biological mechanisms responsible for the benefit of high BMI on improved RCC survival, and to determine whether other modifiable lifestyle factors contribute to RCC survival.

CONFLICT OF INTEREST

None declared.

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Correspondence address:
Yong Fang, MD, PhD
Department of Urology
First Affiliated Hospital of Sun Yat - Sen University
Guangzhou 510080, China
Telephone: + 86 20 8775 5766 R 8227
E-mail: fangyongsysu@sina.com

ORIGINAL ARTICLE

Vol. 44 (6): 1156-1165, November - December, 2018
doi: 10.1590/S1677-5538.IBJU.2017.0505

Surgical techniques for facilitating laparoscopic intracorporeal orthotopic neobladder: initial experience

Lianchao Jin [1,2], Mingshuai Wang [1], Feiya Yang [1], Yinong Niu [1], Nianzeng Xing [1]

[1] Department of Urology, Beijing Chao-yang Hospital, Capital Medical University, Beijing, China; [2] Department of Urology, Peking University Shougang Hospital, Beijing, China

ABSTRACT

Purpose: To describe our technique and outcomes for laparoscopic intracorporeal ileal neobladder (ICNB) reconstruction.
Materials and Methods: From April 2014 to November 2016, 21 patients underwent laparoscopic ICNB at our tertiary referral centre. ICNB with bilateral isoperistaltic afferent limbs and several technique improvements were introduced. Demographics, clinical, and pathological data were collected. Perioperative, 1-year oncologic, 1-year Quality of life and 1-year functional outcomes were reported.
Results: ICNB was successfully performed in all 21 patients without open conversion and transfusion. Mean operative time was 345.6±66.9 min, including 106±22 min for LRC and PLND and 204±46.4 min for ICNB, respectively. Mean established blood loss was 192±146 mL. The overall incidence of 90-d complication was 33.3%, while major complication occurred in 4.8%. One-year daytime and night-time continence rates were 85.7% and 57.1%, respectively. One patient died from myocardial infarction six months postoperatively, and two patients had lung metastasis five months and six months respectively.
Conclusions: We described our experience of 3D LRC with a novel intracorporeal orthotopic ileal neobladder, and the technique improvements facilitate the procedure. However, further studies are required to evaluate long-term outcomes of the intracorporeal neobladder with bilateral isoperistaltic afferent limbs.

ARTICLE INFO

Keywords:
Laparoscopy; Quality of Life; Cystectomy

Int Braz J Urol. 2018; 44: 1156-65

Submitted for publication:
September 15, 2017

Accepted after revision:
March 14, 2018

Published as Ahead of Print:
April 07, 2018

INTRODUCTION

Radical cystectomy (RC) with pelvic lymph node dissection (PLND) has become the standard treatment option for muscle-invasive and high-risk superficial bladder cancer. Laparoscopic or robotic-assisted radical cystectomy is an alternative to open radical cystectomy with comparable oncological outcomes in multiple centers (1, 2). Following minimally invasive radical cystectomy, urinary diversion may be performed through an open approach or entirely within the abdomen (3, 4). For technical difficulties and relatively longer operative time, urologists were not optimistic about the intracorporeal neobladder (ICNB) formation at first (5). With the development of minimally invasive technique and device, ICNB was reconsidered in large medical centers in recent years (4, 6). To data, most of the intracorporeal urinary diversion was performed in robotic-assisted approach (7).

After Gill et al. firstly reported laparoscopic radical cystectomy (LRC) and ICNB in 2002 (3), laparoscopic intracorporeal U shape orthotopic neobladder was proposed in many centers (8-10). They simplified the procedure of ICNB construction, however, the function of the U shape neobladder is controversial as it is not in a global shape, which may result in relatively small neobladder capacity and higher neobladder pressure.

Hence, we describe a time efficient method of ICNB reconstruction and a series of technique improvements to overcome challenges during the procedure.

MATERIALS AND METHODS

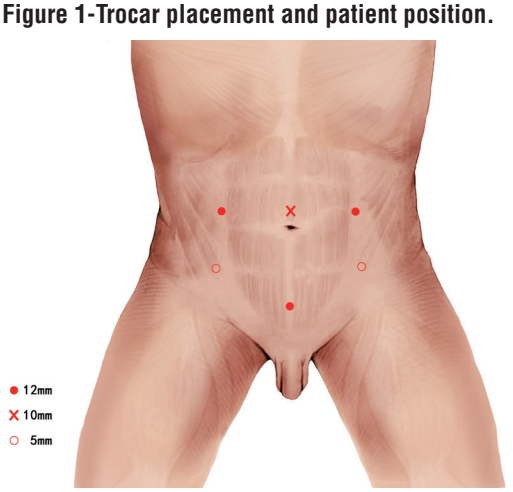
We reviewed medical records of patients who underwent LRC with ICNB formation in our tertiary center from April 2014 to November 2016. The study was approved by the Institutional Review Board of Beijing Chao-yang Hospital, Capital Medical University (Protocol number 2014-R-141), and all patients provided written informed consent. All surgeries were performed by one experienced surgeon (N.X.), who has over 200 cases experience of LRC with orthotopic ileal neobladder. Indications for LRC were muscle-invasive bladder cancer (T2-3, N0-x, M0), high risk and recurrent non-muscle-invasive cancer, T1G3, extensive non-muscle-invasive bladder cancer that could not be controlled by transurethral resection and intravesical therapy. Our exclusion criteria were tumor in the urethra, urinary incontinence, history of recurrent urethral strictures, abnormal abdominal straining, local radiotherapy and severe comorbidities and decompensated renal function.

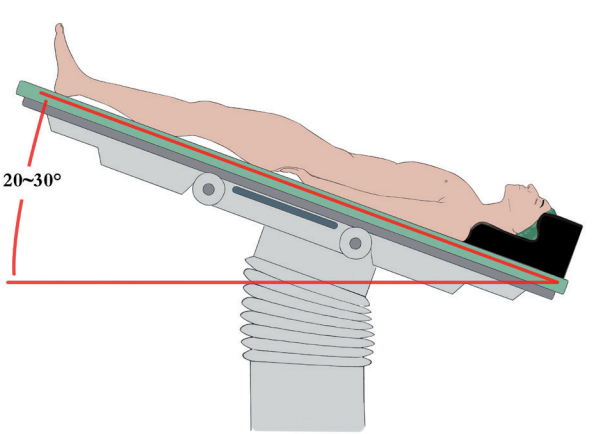
Perioperative variables including patient's characteristics, surgical outcomes, postoperative complications, oncologic outcomes and Quality-of-life data were analyzed. Postoperative complications were analyzed according to the Clavien-Dindo classification (11). After discharge, patients were followed up at 2 weeks, 3, 6, and 12 months, and then yearly. Neobladder function was assessed at 6 months postoperatively. Daytime or night-time continence referred to the requirement of <1 pad during daytime or night-time, respectively. Incontinence was defined as the need for more than 1 pad per day or night. Patients were asked to complete the European Organization for Research and Treatment of Cancer (EORTC) generic (QLQ-C30) and bladder cancer specific instruments (QLQ-BLM30) questionnaires for Quality of Life assessment every year.

Surgical Techniques

All procedures were performed using Olympus 3-D laparoscopic system. The patient was placed in a dorsal supine with a 30° Trendelenburg position. Six laparoscopic ports were utilized as shown in Figure-1. The first 10 mm port for the camera was placed 1 cm cephalad to the umbilicus in the midline. Two 12 mm ports were symmetrically placed at the level of the umbilicus

Figure 1-Trocar placement and patient position.





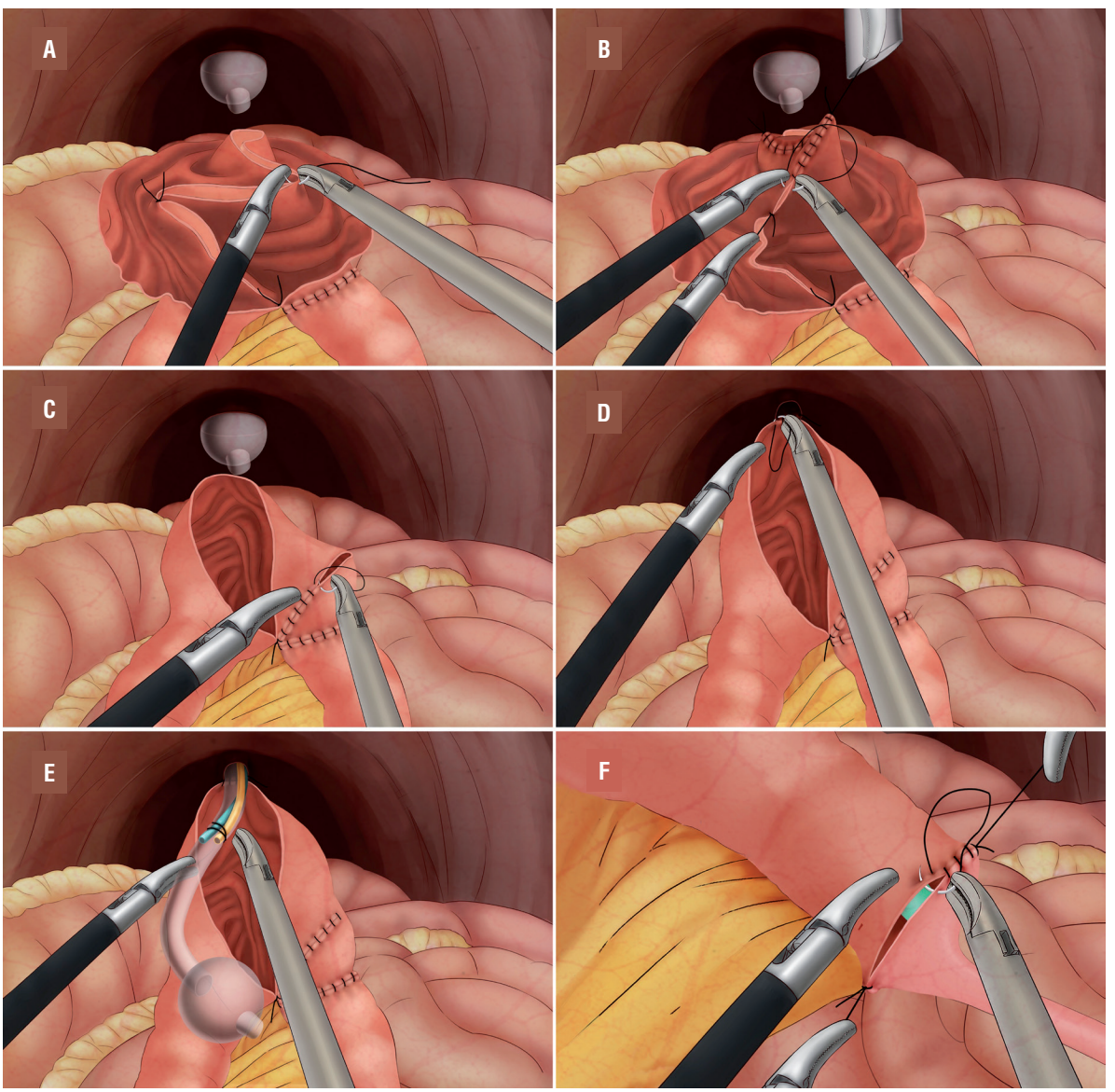
on the left and right lateral to the rectus abdominis. Two 5 mm ports were placed 2-3 cm superior and medial to the anterior superior iliac spines on each side. The sixth 12 mm port was placed 1 cm cephalad to the pubic symphysis in the midline after finishing the LRC and PLND for Endo-GIA.

After the peritoneum was incised and the sigmoid colon was mobilized, the ureters were mobilized down to the bladder without injuring the periureteral vessels. Then, the umbilical arteries were dissected and divided between Hem--o-lok clips. The peritoneum was incised along the vas deferens in the direction of the pouch of Douglas, and the seminal vesicles were completely dissected. The posterior layer of the Denonvilliers fascia was then incised and the pre-rectal fatty tissue could be visualized. The dissection was continued in a blunt and sharp fashion as far as possible towards the apex of the prostate. The bilateral bladder pedicles were divided between Hem-o-lok clips. Then, the Retzius space was dissected and the incision was continued in the lateral direction up to the external iliac vessels and the endopelvic fascia. After cleaning fatty tissue on the prostate, the endopelvic fascia was incised on both sides. Then, both puboprostatic ligaments were fully dissected, and the dorsal vein complex was ligated with 2-0 V-Loc suture. In nerve-sparing procedures, the bilateral prostatic fascia was dissected and the prostatic pedicles were clipped and cut with cold scissors step by step to detach neurovascular bundles from the prostatic capsule. In non-nerve-sparing procedures, LigaSure and ultrasonic scalpel were used to directly divide the prostatic pedicles towards the apex of the prostate. Then, the bilateral ureters were divided, and the urethra was clearly dissected and cut after clipped by Hem-o-lok. The cystoprostatectomy specimen was placed into an EndoCatch bag.

Extended PLND involved removal of nodal tissue cranially up to the inferior mesenteric artery, and including the internal iliac, presacral, obturator fossa and external iliac nodes was performed after the LRC. The lymph nodes were marked and put into the EndoCatch bag. Then, the bag was put in the abdomen and taken out after urinary diversion.

For intracorporeal orthotopic ileal neobladder construction, a 60 cm segment of ileum, 15-20 cm from the ileocecal junction, was isolated using laparoscopic Endo-GIA with a 60 mm staplers. The continuity of the small bowel was restored using the Endo-GIA with a 60 mm stapler, positioning the distal and proximal end of the ileum side to side with the antimesentery parts facing each other, and then the open end was closed with transverse firing of the Endo-GIA stapler. The proximal 10 cm segment of the isolated ileal segment was transected with ultrasonic scalpel and was manually anastomosed with the distal end in end-to-end manner. The middle 40 cm of the ileal segment was detubularised with scissors along its antimesenteric line, and the remaining 10 cm each side for reservoir limbs. The posterior wall of the reservoir was closed using 3-0 V-Loc suture in a simple continuous full thickness fashion. During the procedure, the posterior wall was firstly interrupted sutured using 2-0 Vicryl suture, and two assistants were pulling the interrupted knots to tension ileum to facilitate suturing ("pulling technique") (Figures 2A and 2B). The distal half of the anterior wall of the reservoir was sutured using the 3-0 V-Loc suture (Figure--2C). The proximal half of the back wall of the reservoir was anastomosed with the urethra back wall, and the anterior wall of the reservoir was left open (Figure-2D). Then, the catheter and two single J stents were introduced from the opening through the urethra (Figure-2E). The opening was then anastomosed with the urethra and closed with 3-0 V-Loc suture using pulling technique. After the placement of a single J stent in the ureter and renal pelvis on each side, the ureters were spatulated for 2-3 cm and were end-to-end anastomosed with ipsilateral limb in a continuous manner using 3-0 Vicryl sutures, respectively (Figure-2F). The neobladder was then filled with 50 cc of saline to check for leakage. A drain was introduced and placed in the pelvis. The EndoCatch bag was retraced through an enlarged sixth port incision (usually 5 cm) in the midline of the abdominal wall for male, and the specimen was removed from vagina for female. The single J stents were removed two weeks after surgery, and the catheter was also removed two weeks after surgery after confirmed by cystography the absence of leakage.

Figure 2-A) Interrupted suturing of the posterior wall of the reservoir using 2-0 Vicryl suture. B) Pulling the interrupted knots to tension ileum to facilitate suturing ("pulling technique"). C) Closure of the distal half of the anterior wall of the reservoir. D) The proximal half of the back wall of the reservoir was anastomosed with the urethra back wall. E) The catheter and two single J stents were introduced through urethra. F) The ureters were spatulated for 2-3 cm and were end-to-end anastomosed with ipsilateral limb in a continuous manner, using 3-0 Vicryl sutures, respectively.



STATISTICAL METHODS

Mean values with standard deviations were computed and reported for continuous data. Median, range were used to report categorical data. Continuous and categorical variables were compared with Student t test and the X2 test, respectively. All p values <0.05 were considered statistically significant. Statistical analysis was performed using STATA v12 (StataCorp LLC, College Station, Texas, USA).

RESULTS

LRC with ICNB was successfully accomplished in all 21 patients without open conversion.

Tables 1 and 2 show the patient's characteristics and surgical outcomes. The mean age was 60 years old with a mean BMI 24.5 kg/m2. Of 21 patients, four patients received neoadjuvant chemotherapy and fourteen patients had history of transurethral resection of bladder tumor. The median Charlson comorbidity index score was 4, and ASA score was 2.

Table 1 - Patient's Characteristics.

VARIABLES	RESULTS
Patients, n	21
Age (mean±SD), year	60±10.1
Male, n (%)	19 (90.5%)
BMI (mean ± SD), kg/m2	24.5±3.9
Neoadjuvant chemotherapy, n (%)	4 (19.0%)
Charlson comorbidity index (median [range])	4 (2-6)
ASA score, n	
1-2	21
3-4	0
Smoking history, n (%)	9 (42.9%)
Abdominal surgery history, n (%)	5 (23.8%)

BMI = body mass index; **ASA** = American Society of Anesthesiologists

The mean operative time was 345.6±66.9 min, including 106±22 min for LRC and PLND and 204±46.4 min for neobladder reconstruction. Figure-3 shows the learning curve of the procedure. The curve of LRC and PLND time was relatively stable, while the ICNB time gradually decreased. A significant decrease of mean ICNB times was observed in the first ten cases compared with the last ten cases (237.3 min vs. 169.0 min, P<0.001) (Table-3). The mean established blood loss was 192 mL without intraoperative transfusion, and no patient was sent to ICU after surgery. The median time of ambulation was POD 1 (range: 1-4). The median time of intake of liquid diet was 4 days (range: 3-12). The ureteric stents were removed on POD 14, and the catheter was removed on POD 16 after cystography confirmed no urine leaked from the bladder. The median length of hospital stay was 14 days (range: 8-22).

Overall incidence of complications of any grade at 90-d was 33.3%, and major complications (Clavien grade ≥3) occurred in one patients (4.8%). One patient had prolonged ileus and urinary tract infection at one week postoperatively, who was treated with antibiotics (Clavien grade 2). One patient had ileus at 2 weeks postoperatively, which was resolved by supportive treatment (Clavien grade 1). One patient had urinary tract infection and was treated with antibiotics (Clavien grade 2). Three patients suffered urinary tract infection at 30-90 d and were treated with antibiotics (Clavien grade 2), one of them was readmitted for treatment of urinary tract infection. One patient had incision hernia at 8 weeks postoperatively and received hernia repair (Clavien grade 3b).

Pathologic results showed TisN0M0 for two patients, T1N0M0 for eight patients, T1N1M0 for one patient, T2N0M0 for six patients, T2N3M0 for one patient, T3N0M0 for one patient, T3N2M0

Table 2 - Surgical Outcomes.

VARIABLES	RESULTS
Total operative time (mean±SD), min	345±66.9
Time of LRC and PLND / (mean±SD), min	106±22.0
Time of ICNB (mean±SD), min	204±46.4
EBL (mean±SD), mL	192±146
Transfusion, n (%)	0
ICU after surgery, n (%)	0
POD 1 VAS score (median [range])	1 (0-4)
Time of intake of liquid diet / (median [range]), day	4 (3-12)
Time of ambulation (median [range]), day	1 (1-4)
Length of hospital stay / (median [range]), day	14 (8-22)
30-day complication rates, n (%)	
Minor (I-II)	4 (19.0%)
Major (III-V)	0
30-90-day complication rates, n (%)	
Minor (I-II)	3 (14.3%)
Major (III-V)	1 (4.8%)
30-day readmission, n (%)	1 (4.8%)
Lymph node yield (mean±SD), n	18±9.2
Positive surgical margin, n (%)	0
Incidental prostate adenocarcinoma, n (%)	2 (10.5%)
Lymph node positive patients, n (%)	3 (14.3%)

LRC=laparoscopic radical cystectomy; **PLND**=pelvic lymph node dissection; **ICNB**=intracorporeal neobladder; **EBL**=estimated blood loss; **POD**=postoperative; **VAS**=Visual analogue scale

Table 3 - Comparison of the first and last ten cases.

	Case (1-10)	Case (12-21)	P
Total operative time (mean±SD), min	393±47.6	297±50.0	<0.001
LRC and PLND time (mean±SD), min	114±25.9	97.7±15.8	0.11
ICNB time (mean±SD), min	237±33.7	169±31.3	<0.001
EBL (mean±SD), mL	155±95.6	240±182.3	0.21

LRC=laparoscopic radical cystectomy; **PLND**=pelvic lymph node dissection; **ICNB**=intracorporeal neobladder; **EBL**=estimated blood loss

for one patient and small cell cancer for one patient. The median lymph node yield was 18 (range: 2-38), and 3 patients had positive lymph nodes (1/18, 2/24, 18/38). All surgical margins were negative. Incidental prostate cancer was detected in 10.5% (2/19) of the patients.

The median follow-up time was 15 (3-30) months. No patient suffered hydronephrosis access by ultrasound or abdominal CT. The continence and neobladder function outcomes are shown in Table-4. The complete daytime continence rate (pad-free) at 6 months and 12 months were

Figure 3-Learning Curve of the laparoscopic radical cystectomy with intracorporeal orthotopic neobladder.

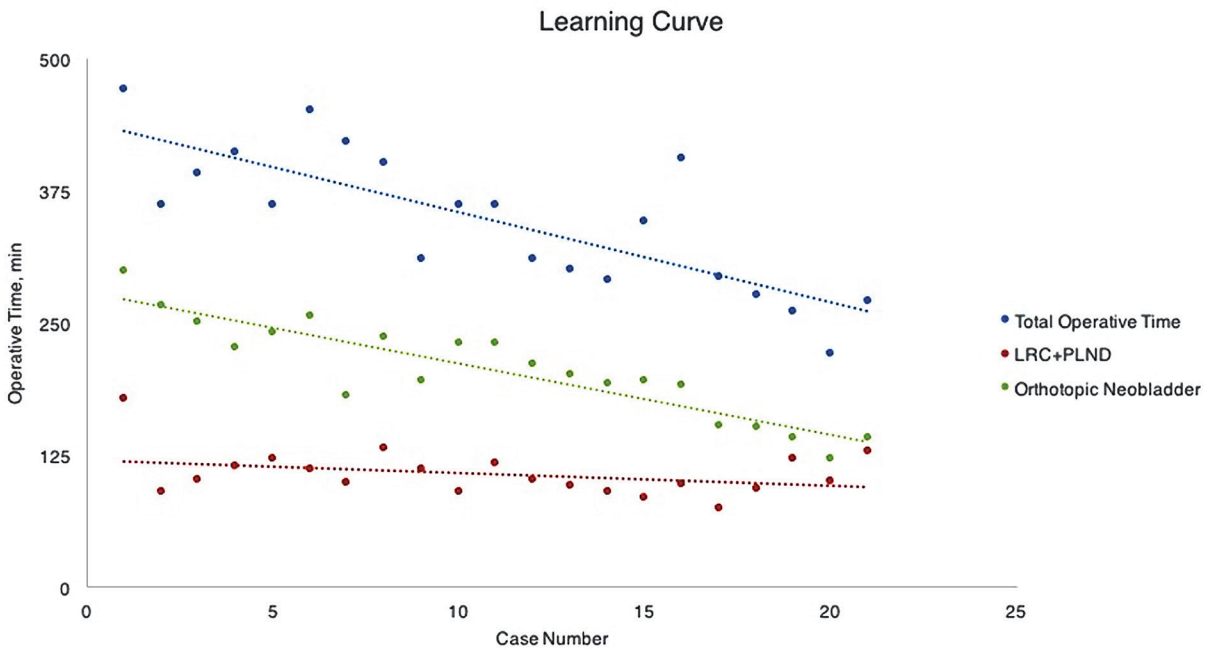


Table 4 - Continence and Neobladder function Outcomes.

VARIABLES	6 MONTHS	12 MONTHS
Urinary Continence		
Day time continence 0-1 pad/d	15/17 (88.2%)	12/14 (85.7%)
Night time continence 0-1 pad/d	6/14 (42.9%)	8/14 (57.1%)
Neobladder function		
Neobladder Capacity (Median [range]), mL	214 (170-330)	375 (310-495)
Residual volume (Median [range]), mL	27 (0-135)	38 (20-160)
Max flow rate (Median [range]), mL/sec	17 (9-22)	19 (8-21)
Clean intermittent catheterization	0	1

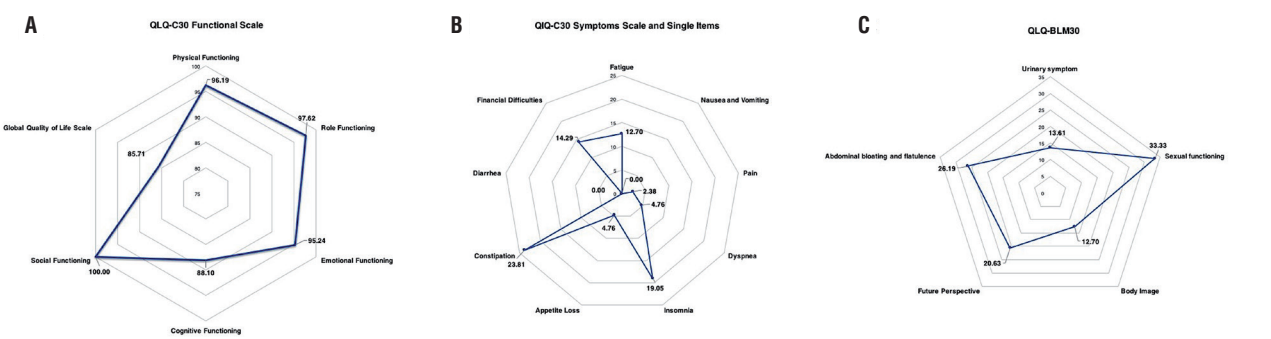
88.2% (15/17) and 85.7% (12/14), respectively. However, the complete night continence rate at 6 months and 12 months were 42.9% (6/14) and 57.1% (8/14), respectively. Four patients need one pad per night at 12 months. The neobladder capacity was 214 mL (170-330) and 375 mL (310-495) at 6 months and 12 months measured by ultrasound, respectively. One patient needed clean intermittent catheterization at 12 months follow-up. Only two patients received urodynamic study at 12 months, and the neobladder pressures were 12 cmH2O and 17cmH2O, respectively.

During follow-up period, no patient experienced recurrence. One patient died from myocardial infarction six months postoperatively, and two patients suffered lung metastasis at five months and six months postoperatively, whose TNM stage were T3N2M0 and T2N3M0, respectively.

Of 21 patients, only seven patients were followed up for more than one year and completed

the QLQ-C30 and QLQ-BLM questionnaires. Mean questionnaire scores (QLQ-C30, QLQ-BLM30) are shown in the Figure-4. The lowest level of functioning in QLQ-C30 was "global quality of life" domain, and the highest level in QLQ-C30 was "social functioning" domain. The lowest level of QLQ-C30 symptoms scale was found in the "nausea and vomiting" and "diarrhea" domain and the highest level in the "constipation" domain. The QLQ-BLM30 questionnaire revealed the lowest level of symptomatology in the "abdominal bloating and flatulence" domain and the highest level in the "sexual functioning" domain.

Figure 4-A) EORTC QLQ-C30 Functional Scale: a high scale score represents a high/healthy level of functioning (0-100). For the global quality of life scale, a high scale score represents a low level of functioning. B) EORTC QLQ-C30 Symptoms scale and single items: a high scale score represents a high level of symptomatology/problems (0-100). C) EORTC QLQ-BLM30: a high scale score represents a high level of symptomatology/problems (0-100).



DISCUSSION

Laparoscopic and robotic techniques have been increasingly advocated for the potential advantages, such as decreased blood loss, decreased analgesic requirements and quicker recovery (12, 13). Some studies demonstrated that complete ICNB reconstruction may lead to decreased bowel exposure, reduction of insensitive fluid losses, and shorter time to oral intake (14). Recently, more and more studies reported robot-assisted ICNB reconstruction with acceptable operative time, complication rates and oncologic outcomes (7, 15, 16). However, the experience of laparoscopic ICNB reported in the literature is limited because of the complex and time consuming procedure (3, 8, 10). Compared to laparoscopic technique, robotic systems simplified suturing procedure which lead to a decrease in ICNB reconstruction time because of 3-dimensional vision and robotic arms. Although 3-dimensional laparoscopic technique could cover the shortage of vision, many surgical improvements are still required for traditional laparoscopic ICNB reconstruction.

In this study, we demonstrated a time efficient neobladder reconstruction and described many surgical improvements which could facilitate the procedure of ICNB without compromising complications. The time efficient neobladder has bilateral isoperistaltic afferent limbs which was reported by us before in the open approach (17). Having bilateral isoperistaltic afferent limbs, the left ureter could be anastomosed with left limb in situ avoiding being exceedingly mobilized. Additional mobilization of the left ureter for being brought to the right side could worsen the blood supply and thus lead to the development of ureteroileal stricture because of chronic ischemia (18). The surgical improvements are: (1) the division and continuity of the ileum was made using laparoscopic staplers; (2) "pulling technique" was applied during neobladder wall suture, which could facilitate suture and decrease ICNB time; (3) the ureters were end--to-end anastomosed with ipsilateral limb directly. In our experience, no patient developed ureteroileal stricture after the end-to-end anastomosis of ureter and ileal limb, and unidirectional peristalsis

of the ureters and the afferent tubular ileal segment sufficiently protected the upper urinary tracts combined with low pressure neobladder. After applying the surgical improvements, the ICNB reconstruction time decreased from average 237 min for first ten cases to 169 min. for the last ten cases.

In many institutional centers worldwide, orthotopic neobladder has now replaced the ileal conduit as the standard form of reconstruction. Several types of neobladder have been described, but all should have the following features: high capacity, low pressure, absence of reflux, and complete voiding by abdominal straining and perineal relaxation (19). In our study, the neobladder capacity was about 375 mL, which was smaller than mean volumes reported for open neobladder construction of 450-524 cm3 (20 ,21). However, no patient has hydronephrosis or ureterectasis one year after operation. Two patient's neobladder pressure was low (12 cm H2O and 17 cm H2O). We will ask more patients to receive urodynamic study to evaluate the function of the neobladder. The most common postoperative complication is urinary tract infection, which can be well controlled by antibiotics.

The EORTC QLQ-30 and the QLQ-BLM30 module are the most commonly used generic and disease-specific instruments. Comparing to a study by Ciro Imbimbo et al., mean values obtained from the physical functioning, role functioning, emotional functioning, social functioning and global quality of life scale were higher in the present study (22). Cognitive functioning value was lower in the present study because one patient suffered memory difficult at one year after operation. The symptom scales of fatigue, nausea and vomiting, pain, dyspnea, appetite loss, diarrhea and financial difficulties were better than the results reported in the study by Ciro Imbimbo et al. (22). Our results have demonstrated lower mean values for the urinary symptom, body image and future perspective and higher mean values of sexual functioning and abdominal bloating and flatulence compared with published data. Our data showed that sexual functioning was worse because only one of the seven patients underwent nerve-sparing surgery.

This study has several limitations. Firstly, this is a retrospective study with small sample size and short length of follow-up. Secondly, the orthotopic neobladder is based on our experience and practice, and more urodynamic and longtime follow-up data are required to evaluate its function.

CONCLUSIONS

We described our experience of 3D LRC with a novel intracorporeal orthotopic ileal neobladder, and many technique improvements facilitate the procedure. However, further studies are required to evaluate long-term outcomes of the intracorporeal neobladder with bilateral isoperistaltic afferent limbs.

ACKNOWLEDGMENTS

This work was supported by Beijing Municipal Administration of Hospital's Ascent Plan. NO. DFL20150301

ABBREVIATIONS

RC=Radical cystectomy
PLND=Pelvic lymph node dissection
ICNB=Intracorporeal neobladder
LRC=Laparoscopic radical cystectomy

CONFLICT OF INTEREST

None declared.

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Correspondence address:
Nianzeng Xing, MD, PhD
Department of Urology
Beijing Chao-Yang Hospital
Gongti South Street, No. 8,
Chao-yang District, Beijing, 100020, China
Fax: +86 010 859-35241
E-mail: xingnianzeng@hotmail.com

ORIGINAL ARTICLE

Vol. 44 (6): 1166-1173, November - December, 2018
doi: 10.1590/S1677-5538.IBJU.2017.0460

Retroperitoneoscopic resection of retroperitoneal nonadrenal ganglioneuromas: our technique and clinical outcomes

Changjin Shi [1], Feng Li [1], Yanchao Wang [1], Long Pei [1], Tao Wang [2]

[1] *Department of Urology, the Fourth Affiliated Hospital of Hebei Medical University, Shijiazhuang, China;*
[2] *Department of Nephrology, Hebei Provincial General Hospital, Shijiazhuang, China*

ABSTRACT

Objective: To report our experience of retroperitoneoscopic technique in semi-lateral decubitus position for the retroperitoneal nonadrenal ganglioneuromas in 18 patients, and to evaluate its clinical outcomes.
Materials and Methods: From January 2012 to May 2016, 18 patients with retroperitoneal nonadrenal ganglioneuromas underwent retroperitoneoscopic resection. With the patients in semi-lateral decubitus position, a 4-port retroperitoneal approach was used. Data were collected on the tumor size, tumor location, perioperative outcomes, pathology, and last-known disease status. We reviewed the operative videos to identify surgical tips and tricks.
Results: All procedures were carried out successfully without converting to open surgery. The tumors had an average size of 5.2cm. The mean operative time was 86.5 min, with a mean estimated blood loss of 85.4mL. There were three patients suffering from intraoperative complications. Postoperatively, all patients achieved an uneventful recovery; the mean postoperative hospital stay was 5.5 days. The postoperative pathology revealed to be retroperitoneal ganglioneuromas. With a mean follow-up of 39.5 months, all patients were recurrence free. The review of the operative videos revealed several tips and tricks, including keeping peritoneum and posterior Gerota fascia intact to provide a favorable operative exposure of tumors, and placing the harmonic scalpel through different ports during tumor dissection.
Conclusions: With the patient in semi-lateral decubitus position and a 4-port retroperitoneal approach, retroperitoneoscopic resection of retroperitoneal nonadrenal ganglioneuroma is a feasible, effective, and safe procedure. This approach has distinct advantages including direct access to the tumor, optimal exposure of tumor and less intraperitoneal interference.

ARTICLE INFO

Keywords:
Retroperitoneal Neoplasms; Ganglioneuroma; Pathology

Int Braz J Urol. 2018; 44: 1166-73

Submitted for publication:
August 10, 2017

Accepted after revision:
January 10, 2018

Published as Ahead of Print:
February 28, 2018

INTRODUCTION

Ganglioneuromas are rare, benign neurogenic tumors that originate from the neural crest. Arising along the sympathetic chain, ganglioneuromas are commonly localized in the posterior mediastinum followed by retroperitoneum, cervical region and adrenal gland (1). Retroperitoneal ganglioneuromas are usually nonfunctioning and asymptomatic until they reach large sizes, in which case they cause symptoms due to local expansion and pressure on adjacent structures (2). Surgical resection represents the only choice for both diagnosis and treatment (3).

Retroperitoneal tumors have traditionally been excised using a standard open technique (4-7). Recently, due to advances in laparoscopic instruments and surgical techniques, indications for the laparoscopic approach have been broadened to the retroperitoneal tumors (8-10). However, surgical access to the retroperitoneal space is generally achieved by abdominal transperitoneal approach. Reports on the retroperitoneal laparoscopic approach to nonadrenal retroperitoneal tumors are limited (11). In comparison with transperitoneal laparoscopic surgery, the main advantages of retroperitoneal approach include a faster access to the tumor, requiring little dissection without violating the peritoneal cavity. Herein, we report our experience of retroperitoneal laparoscopic resection of nonadrenal retroperitoneal ganglioneuroma in 18 patients and analyze the feasibility and safety of our technique.

MATERIALS AND METHODS

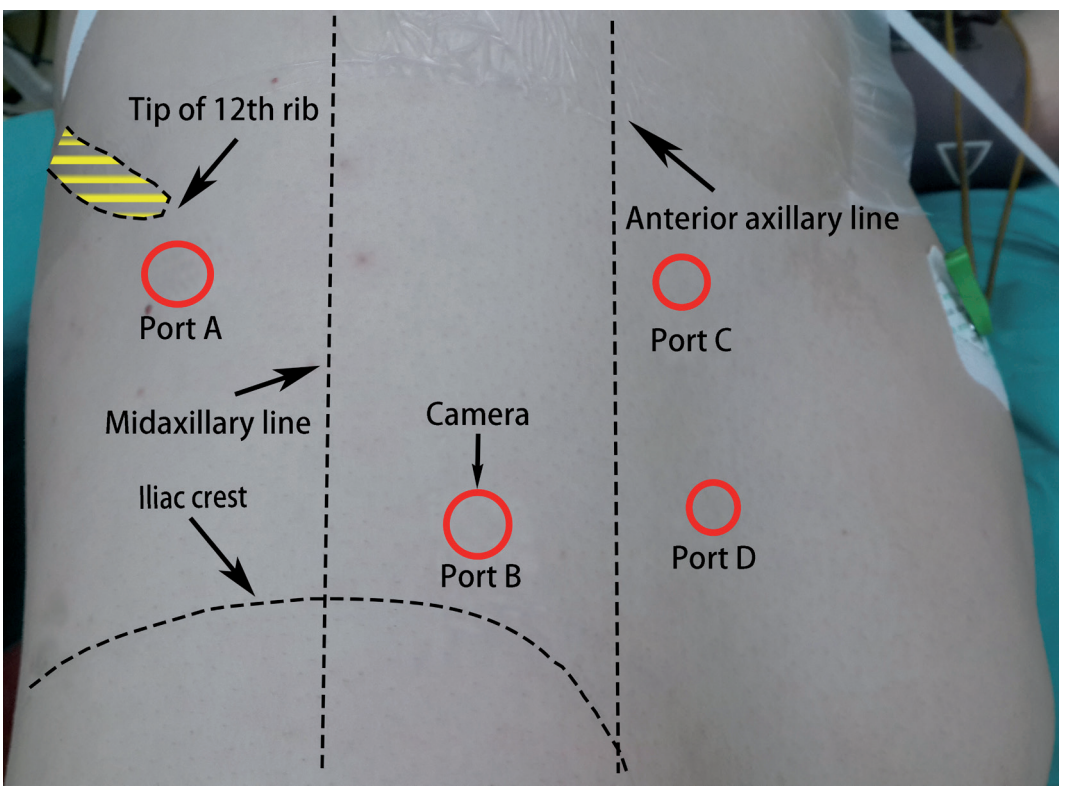
From January 2012 to May 2016, 18 patients (6 males and 12 females) underwent retroperitoneoscopic resection of nonadrenal retroperitoneal ganglioneuromas. The average age of the patients was 40.6 years, ranging from 21 to 65 years. The therapy modality was approved by the hospital ethics committee and written informed consent from patients was obtained prior to surgery. Preoperative assessment of each patient's general condition was carefully performed, including routine blood laboratory investigation, coagulation profile, urinalysis, hepato-renal function. Laboratory data and the tumor markers (neuron-specific enolase, NSE, serum carcinoembryonic antigen, CEA and carbohydrate antigen 199, CA-199) were all within normal limits. The patient's catecholamine levels in 24-hour urine samples were measured to exclude paragangliomas. All patients were evaluated preoperatively with abdominal computed tomography (CT) or magnetic resonance imaging (MRI). The mean tumor size was 5.8cm the tumor location was suprahilar in 4, and infrahilar in 14 cases. The patient's characteristics and operative data are summarized in Table-1. Abdominal CT was performed 3 and 6 months postoperatively. Thereafter, follow-up was then continued at 6-month intervals.

After induction of general anesthesia, the patient was placed in the semi-lateral decubitus position with the side of the lesion elevated at 60. A 2cm skin incision was made below the tip of 12th rib (point A) (Figure-1). The retroperitoneal space was entered using sharp and blunt dissection through the flank abdominal muscles and lumbodorsal fascia, and then an index finger was inserted for a simple dissection to develop an initial retroperitoneal pocket. A homemade latex balloon dissector was placed into the retroperitoneal space, and 800-1000mL air was infused to maintain the balloon dilation for 3-5 minutes. The air was then evacuated, and the balloon dissector was removed. Under the guidance of the index finger extending into the retroperitoneal space through the incision, a 10-mm trocar was inserted 2cm above the superior border of the iliac crest and medial to the midaxillary line (point B), the other two 5-mm trocars were placed along the anterior axillary line and moved 2-3cm toward the midline (point C and D). A 10mm trocar was inserted at point A, and the skin incision was closed around the port using a mattress suture to avoid gas leakage. The laparoscope was placed through the trocar at point B, which was connected to the carbon dioxide insufflator to achieve the pneumoretroperitoneum (pressure range, 13-15mm Hg). The retroperitoneal fat was partially freed to reveal the lateral conal fascia, which was then incised longitudinally. Dissection proceeded over the quadratus lumborum and then to the psoas muscle. Tumor was easily identified in the retroperitoneal space adjacent to the medial of the psoas muscle, and it was dissected and mobilized from adjacent structures. In order to facilitate the manipulation of the tumor, the harmonic scalpel was placed through ports C for dissection of the upper pole of the tumor, port D was used to retract. For dissection of the lower pole of the tumor, the harmonic scalpel was switched to port D, port C was used to retract. Hemostasis was checked carefully after lowering the pressure of the pneumoretroperitoneum. A closed suction drain was placed through the port B into the space. Carbon dioxide was evacuated, and the port sites were closed. The closed suction drain was subsequently removed if the drainage output had not increased and was less than 10mL in 24 hours.

Table 1 - Patients' characteristics and surgical outcomes.

Patient No./Sex/ Age (y)	Symptom	Tumor location	Tumor size (cm)	Operative time (min)	Blood loss (mL)	Intraop/ postop complications	Postop stay (days)	Follow-up time (months)
1/F/46	No	Left/ suprahilar	5.8	121	77	No	6	64
2/M/40	No	Left/ infrahilar	4.8	82	80	No	4	58
3/F/34	No	Right/ infrahilar	3.2	113	86	peritoneum breach	5	54
4/M/33	Abdominal pain	Left/ infrahilar	6.4	97	90	No	5	52
5/F/55	No	Right/ infrahilar	7.1	62	60	No	6	52
6/F38	Abdominal pain	Right/ infrahilar	6.6	72	98	No	7	50
7/F/65	No	Right/ suprahilar	4.7	105	70	peritoneum breach	5	46
8/M/46	No	Right/ infrahilar	5.1	68	50	No	4	45
9/F/52	Left flank pain	Left/ suprahilar	6.2	84	75	No	5	42
10/M/29	No	Right/ infrahilar	7.4	102	260	lumbar vein injury	7	39
11/M/62	Abdominal pain	Right/ infrahilar	5.8	75	60	No	6	38
12/F/27	No	Right/ infrahilar	3.6	69	50	No	4	35
13/F/34	No	Right/ infrahilar	4.8	78	58	No	5	33
14/F/21	No	Right/ infrahilar	7.8	89	90	Chylous leakage	9	29
15/F/54	Right flank pain	Right/ infrahilar	6.3	93	65	No	6	24
16/M/39	No	Left/ infrahilar	4.8	69	80	No	4	20
17/F/25	No	Left/ infrahilar	6.3	72	78	No	5	18
18/F/31	Abdominal pain	Right/ suprahilar	8.2	106	110	No	6	12

Figure 1 - Patient position and distribution of trocars. The patient was placed in the semilateral decubitus position with the side of the lesion elevated at 60°. Trocar A, below the tip of 12th rib. Trocar B, 2-cm above the superior border of the iliac crest and medial to the midaxillary line. The other two trocars (C and D), along the anterior axillary line and 2–3 cm towards the midline.

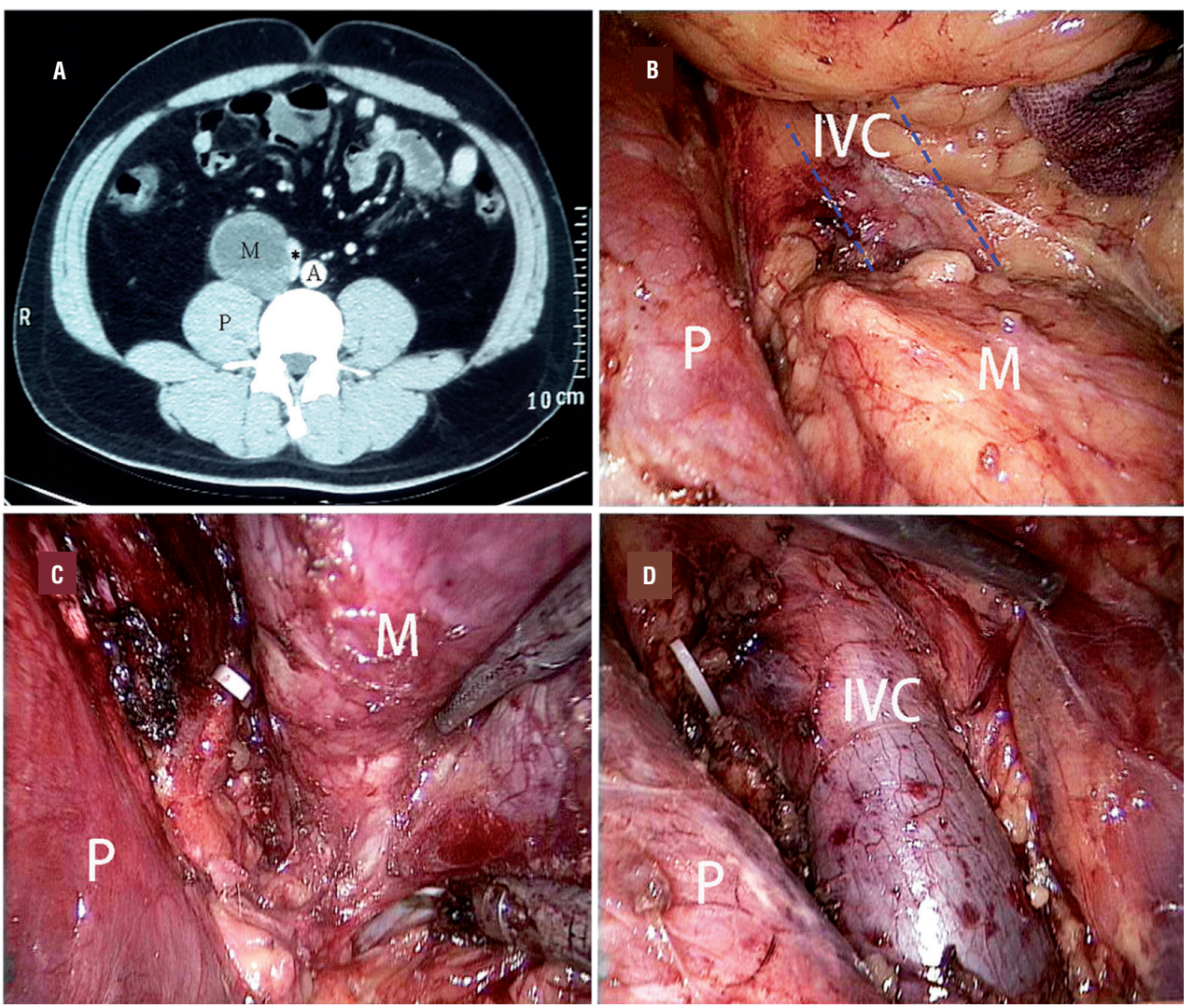


RESULTS

The detailed characteristics and perioperative data of the patients are summarized in Table-1. Of the 18 treated patients, 12 patients were asymptomatic and detected incidentally during health screening. All operations were completed laparoscopically without conversion to open surgery. Intraoperatively, the tumors appeared to be well-encapsulated and were mostly dissected free from adjacent structures easily (Figure-2). Surgical time ranged from 62 to 121 minutes, with an average of 86.5 minutes. The mean blood loss during the operation was 85.4mL (range, 50-260mL), and none of the patients required blood transfusion. There were three patients suffering from intraoperative complications, one with the lumbar vein injury and other two with the peritoneum breach. During the operations, there were no instances of ureter and renal pedicle injury in these cases. Regarding the postoperative complications, chylous leakage was observed in one patient and was managed conservatively. All other patients achieved an uneventful recovery. Oral intake was resumed after a delay of 2 days (range, 1-3 days) after surgery. The mean postoperative hospital stay was 5.5 days (range, 4-9 days). Postoperatively, histopathologic examination results revealed ganglioneuroma in all the patients. All resected specimens showed a negative incisional margin. At a mean follow-up of 39.5 months (range, 12-64 months), abdominal computed tomography showed no recurrence in all patients.

We reviewed our surgical videos and clarified several technical tips and tricks. Keeping peritoneum and posterior Gerota fascia intact were helpful to obtain a favorable operative view. In 2 cases with the peritoneum breach, a Veress needle was placed in the umbilicus, but it didn't fully resolve the impingement of the retroperitoneal working space. In the cases with tumors located above the renal hilum, rotating the kidney was helpful to approach

Figure 2 - Preoperative CT and intraoperative findings of patient 6. A) Preoperative CT scan image showing a large tumor located between the IVC (asterisk) and the psoas muscle. The IVC was compressed medially. B) The tumor was identified after entering the retroperitoneal space. It was located medially to the psoas muscle. The IVC was compressed medially to the tumor. C) Laparoscopic view during dissection. The IVC had been released from the media side of the tumor. D) Laparoscopic view after resecting the tumor. CT indicates computed tomography; A = aorta; IVC = inferior vena cava; M = mass; P = psoas muscle.



the tumors. It was helpful for facilitating the tumor manipulation to place the harmonic scalpel through different ports alternately during tumor dissection, especially in the cases with tumors located below the renal hilum.

DISCUSSION

Ganglioneuromas are tumors of the sympathetic nervous system that arise from the neural crest cells (12). They are accepted as slow-growing benign tumors constituted by mature sympathetic ganglion cells (13). Whereas ganglioneuromas can be found everywhere along the sympathetic chain, the posterior mediastinum, retroperitoneal area, and adrenal glands are the most common locations. Ganglioneuromas primarily affect the pediatric age group, two-thirds of patients are under the age of 20 years, and ganglioneuromas are rarely observed over the age of 60 years (14). They

are mostly sporadic but there are a few reports of ganglioneuromas associated with neurofibromatosis type II and multiple endocrine neoplasia type II B (15). They are common in young females and usually asymptomatic until they reach a large size when they compress and displace adjacent structures (6, 16). Ganglioneuromas rarely produce vasoactive intestinal polypeptide and catecholamines. These tumors may cause some symptoms like diarrhea, sweating and hypertension related to those peptides (17). In our cases, the patients presented with a retroperitoneal mass that did not have secretory activity.

The current advanced imaging techniques may be useful for evaluating the extent of the ganglioneuromas and differential diagnosis. CT most commonly reveals a homogenous and well-encapsulated tumor with non-enhancement or slight enhancement in arterial phase and progressive mild enhancement in delayed phase. Circumscribed or spotted calcification may be observed in 20% of the patients (18). On MRI, T1-weighted images show a low-signal intensity, whereas T2-weighted images show a heterogeneous high-signal intensity (14, 19). Fine-needle aspiration (FNA) can be used preoperatively, but it usually leads to inconclusive diagnosis. In the largest series with ganglioneuromas of presacral location, the diagnosis could not be achieved in 60% of cases with FNA (20). In particular, although a catecholaminergic crisis has never been described subsequent to FNA, this theoretical possibility exists (21). Without intention to perform FNA due to inconclusive results and the possibility of catecholaminergic crisis, we considered a benign neurogenic tumor as the presumed diagnosis according to CT and MRI features and lack of enhancement of the lesion. CT and MR imaging can demonstrate important characteristics of these tumors and help narrow the differential diagnosis; however, there is a substantial overlap of imaging findings among different tumors. We have 4 cases of misdiagnosis in our experience. Based on the CT characteristics, they were diagnosed as ganglioneuromas before surgical resection, whereas the postoperative pathology revealed schwannomas which were not included in this article. Here, we only selected patients whose postoperative histopathologic examination revealed ganglioneuromas.

Retroperitoneal tumors were excised traditionally by laparotomy (4-7). However, in recent decades, with advances in laparoscopic technique and the associated equipment, laparoscopic excision for some retroperitoneal tumors is the ideal approach nowadays (8-10). The laparoscopic approach has been associated with fewer postoperative complications including less blood loss, minor postoperative adhesion formation, and shorter hospital stay than laparotomy. Laparoscopic retroperitoneal tumor excision can be performed through the retroperitoneal or transperitoneal approach. However, surgical access to the retroperitoneal space is generally achieved by abdominal transperitoneal approach. Reports on the retroperitoneal laparoscopic approach to nonadrenal retroperitoneal tumors are limited. In comparison with transperitoneal laparoscopic surgery, the main advantages of retroperitoneal approach include a faster accessing to the tumor, requiring little dissection without violating the peritoneal cavity. Walz et al. previously reported their experiences of laparoscopic or retroperitoneoscopic surgery for 27 paragangliomas. They used the prone position combined with a gas pressure of 20-24mmHg in retroperitoneoscopic surgery (22). Zhang S et al. reported their retroperitoneoscopic technique in supine position for the primary tumors located below the level of renal pedicle (23). In our surgical technique, we also preferred the retroperitoneal approach on the basis of our extensive experiences. But, we used semi-lateral decubitus position.

The incidence of retroperitoneal tumors is too infrequent for most surgeons to gain sufficient experience in laparoscopic excision. In our cases, we selected the tumors located below or above the level of the renal pedicle. Therefore, for an experienced surgeon who is adroit at retroperitoneoscopic adrenalectomies, nephrectomies and others, retroperitoneoscopic resection of a retroperitoneal tumor below or above the level of the renal pedicle can be performed easily. Since the tumor was partially sheltered from the psoas muscle, we modified the patient position as used in nephrectomy. We performed the procedure with the patient in a semi-lateral decubitus position in order to get an optimal exposure of the tumor. We

slightly modified port positioning which we used in retroperitoneoscopic nephrectomy. Briefly, all four ports were moved 2-3cm toward the midline in order to facilitate the exposure and manipulation of the tumor.

In our experience, dissecting along the surface of the psoas muscle was sufficient to expose the tumor. The posterior Gerota fascia and perinephric fat should be left intact to keep its adherence to the peritoneum, which can play a role of "self-retraction" to avoid dropping of the fascia, otherwise may prevent the surgeon's ability to maneuver. Maintaining the integrity of the peritoneum is a key factor during the retroperitoneal performance. However, the peritoneum could be damaged and opened inadvertently, losing the surgical field exposure advantage provided by the pneumoretroperitoneum. There were two cases suffered from the peritoneal breach in our study. They occurred during the trocar placement. Extended and more careful finger dissection to separate the adherent peritoneum from the abdominal wall may reduce this complication. Furthermore, incising the lateral conal fascia longitudinally along the quadratus lumborum, which is far away from lateral peritoneal reflection, may further contribute to preventing peritoneal injury.

Arising along the sympathetic chain, retroperitoneal ganglioneuromas are commonly located in a deep, narrow space and adjacent to major vessels, so it is difficult to perform a laparoscopic resection of the tumors especially when tumors are adherent to adjacent major vessels. However, retroperitoneal approach affords rapid and direct access to the tumors, with the retraction of psoas muscle and Gerota fascia, the laparoscopic magnification provides an excellent exposure. In our cases, all operations were completed laparoscopically without conversion to open surgery. When a tumor adhered to important adjacent vessels, as showed in our patient 6, the tumor adhered to the inferior vena cava, meticulous dissection was necessary. During the tumor dissection, port C and D could offer different operative direction around the tumor. As a surgical tip, we found it was helpful for facilitating the tumor manipulation to place the harmonic scalpel through ports D and C, alternately.

Because of the benign nature of ganglioneuromas, adjuvant systemic chemotherapy or local radiotherapy are not indicated after surgical resection. As ganglioneuromas have a tendency to remain silent for a long time, and are often associated with a long-term disease-free survival (6), regular follow-up is necessary to assess local recurrence. In our patients, recurrence has not been observed at a mean follow-up of 39.5 months.

We present a small retrospective study, more cases and further follow-up are still needed to establish that retroperitoneoscopic resection does not have a deleterious effect on the long-term outcome. Secondly, our study could not answer the question of whether laparoscopic surgery is a viable option for malignant retroperitoneal tumors. However, we consider that our study further supports the feasibility of retroperitoneal laparoscopic resection of retroperitoneal ganglioneuromas in experienced hands, and we offer several surgical tips and tricks.

ABBREVIATIONS

NSE = neuron-specific enolase
CEA = serum carcinoembryonic antigen
CA-199 = carbohydrate antigen 199
CT = computed tomography
MRI = magnetic resonance imaging
FNA = fine-needle aspiration

CONFLICT OF INTEREST

None declared.

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Correspondence address:
Changjin Shi, MD
Department of Urology
The Fourth Affiliated Hospital of Hebei Medical University
2 Jian Kang Road, Shijiazhuang
People's Republic of China
Shijiazhuang 050011, China
Telephone: + 86 139 3213-5986
E-mail: chjshi@aliyun.com

ORIGINAL ARTICLE

Vol. 44 (6): 1174-1181, November - December, 2018
doi: 10.1590/S1677-5538.IBJU.2017.0326

Retroperitoneal laparoscopic nephroureterectomy with distal and intramural ureter resection for a tuberculous non – functional kidney

Canqiang Li [1], Yi Yang [1], Le Xu [1], Minjie Qiu [1]

[1] *Department of Urology, The Affiliated Hexian Memorial Hospital of Southern Medical University, Guangzhou, Guangdong, China*

ABSTRACT

Objective: To evaluate the safety and feasibility of total retroperitoneal laparoscopic nephroureterectomy with urinary-bladder junction resection for a tuberculous non-functional kidney.
Materials and Methods: A total of 27 individuals diagnosed with unilateral non-functional kidney secondary to tuberculosis were treated between June 2011 and June 2015. All patients had normal renal function on the contralateral side and underwent the standard four-drug anti-tuberculosis treatment for at least four weeks before surgery. Total retroperitoneal laparoscopic nephroureterectomy was performed in all patients, and the urinary-bladder junction of distal ureter was managed using different auto-suture techniques.
Results: Nineteen male and 8 female patients with an average age of 47.3 years (range, 36-64 years) underwent surgery. All the operations were successfully performed without conversion. The median operative time was 109.3 min (range, 75-138 min), the median blood loss was 157.5 mL (range, 70-250 mL), and the median hospitalization time was 3.7 days (range, 3-6 days). No serious perioperative complications occurred. Anti-tuberculosis chemotherapy was prescribed to all patients, with the entire course of treatment lasting six months. No recurrence of tuberculosis of the bladder or the contralateral kidney was observed during the median follow-up period of 26.7 months (range, 6-54 months).
Conclusion: Retroperitoneal laparoscopic nephroureterectomy with urinary-bladder junction resection is a safe and feasible approach for the treatment of tuberculous non-functional kidneys.

ARTICLE INFO

Keywords:
Tuberculosis, Renal;
Nephroureterectomy;
Nephrectomy

Int Braz J Urol. 2018; 44: 1174-81

Submitted for publication:
June 01, 2017

Accepted after revision:
March 06, 2018

Published as Ahead of Print:
April 03, 2018

INTRODUCTION

Tuberculosis (TB) remains a major challenge to human health worldwide. In 2013, 9 million people fell ill with TB, and 1.5 million died from the disease (1). The kidneys are a common site of extrapulmonary TB, and the incidence of renal TB is estimated to be up to 73% in TB patients from regions with an extremely high prevalence of pulmonary TB (2). Because of its slow progress and nonspecific manifestations, the diagnosis of renal TB is often delayed, by which point one of the kidneys may have stopped functioning. The condition usually results from extensive calcification of the renal parenchyma and/or multiple infundibular stenosis or ureteric strictures. Therefore, the

surgical management of nephrectomy, whether using a traditional open approach or a retroperitoneoscopic approach, becomes inevitable for those patients with a non-functional kidney. However, it remains controversial whether total resection of the ipsilateral ureter is safe and feasible during the performance of retroperitoneal laparoscopic nephrectomy in tuberculous patients. Although total resection of an affected ureter is rarely indicated for the treatment of a tuberculous non-functional kidney, the removal of the more tuberculous tissues associated with an affected ureter could theoretically prevent the occurrence of ureteral stump syndrome. This syndrome is clinically interpreted as febrile urinary tract infections, hematuria, and lower quadrant pain that may occasionally occur when a ureteral stump, a segment of the ureter, is left in place after nephrectomy. However, to the best of our knowledge, few studies have reported on the feasibility and outcome of retroperitoneal laparoscopic nephroureterectomy for the treatment of tuberculous non-functional kidneys (3, 4).

In this present study, we report our experience with retroperitoneal laparoscopic nephroureterectomy for the treatment of tuberculous non-functional kidneys, with the aim of evaluating the feasibility and safety of this approach.

PATIENTS AND METHODS

Institutional review board approval was obtained from the ethics committees prior to the initiation of the study. Information from a database of prospectively collected data that included the hospital chart data and complications of all patients treated with total retroperitoneal laparoscopic nephroureterectomy was retrospectively reviewed. The diagnosis of a tuberculous non-functional kidney was established based on clinical manifestations, urinalysis, real-time polymerase chain reaction (PCR) for Mycobacterium tuberculosis, the erythrocyte sedimentation rate (ESR), intravenous pyelography, enhanced computed tomography and a nephrogram. In certain patients, cystoscopy with a bladder biopsy was performed when the clinical diagnosis of TB was not confirmed. The glomerular filtration rate of the kidney was also evaluated via a renal nuclear scan. A unilateral non-functional kidney was defined by a glomerular filtration rate of the diseased kidney of less than 15 mL/min/1.73 m^2, associated with a cortical thickness of less than 5 mm, whereas the glomerular filtration rate of the contralateral side was normal or more than 60 mL/min/1.73 m^2. Patients were excluded from the study if they had a history of retroperitoneal surgery on the ipsilateral side of the diseased kidney or if assessment of the presence of renal TB was not performed. Patients in the active phase of TB were also excluded.

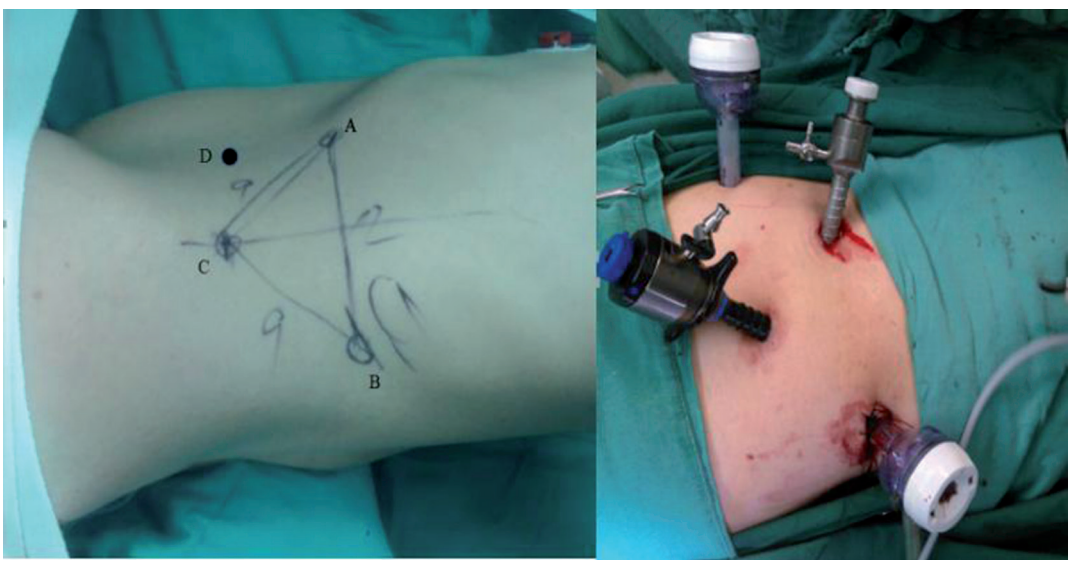
Study population

In all, 27 patients with a unilateral non-functional kidney due to TB who were admitted to our hospital between June 2011 and June 2015 were included in this study. Information regarding the advantages and risks of the laparoscopic surgery was provided for formal informed consent, and permission was obtained from the patients before surgery. The group included 19 males and 8 females, and the tuberculous lesion occurred on the right side in 16 patients and the left side in 11 patients. The mean age was 47.3 years (range, 36-64 years). The most common clinical manifestation was irritative voiding symptoms (19 cases), followed by recurrent urinary tract infection (5 cases), gross hematuria (2 cases) and ipsilateral flank pain (1 case). Standard 4-drug anti-TB chemotherapy, including isoniazid 10 mg/kg, rifampicin 10 mg/kg, pyrazinamide 20 mg/kg and ethambutol 15 mg/kg, was prescribed to every patient for simultaneous intake once daily for at least 4 weeks before the operation.

Operative technique

All the operations were performed by a surgeon who had mastered the technique of retroperitoneoscopic radical nephrectomy. After general anesthesia induction, the patients were placed in the lateral flank position with elevation of the waist on the surgical side. Three laparoscopic working channels were first established to perform the nephrectomy approach and the dissection of the upper ureter (Figure-1). A 1.5 cm skin incision was made 2 cm above the intersection point of the axillary line and the iliac crest (port C). The muscle layer and lumbodorsal fas-

Figure 1 - Distribution of the 4 port sites (Port D was established after nephrectomy).



cia were bluntly penetrated and distracted with forceps. Dilation of the retroperitoneal space was performed using a homemade balloon (by tying the finger of a glove over an F10 red rubber catheter) inflated with air to a volume of up to 500-700 mL (5). A 10 mm trocar was placed at port C to form the laparoscope working channel, and pneumo-retroperitoneum was created by carbon dioxide insufflation, with its pressure maintained at 15 mm Hg. A 10 mm trocar and a 12 mm trocar were inserted under the monitoring laparoscope at the anterior axillary line around the 11th rib tip (port A) and the posterior axillary line around the 12th rib (port B), respectively. Gerota's fascia was incised, and the renal hilum was explored first by separating the space between Gerota's fascia and the psoas fascia. The renal pedicle was sought from bottom to top along the plane behind the ureter on the left side or in front of the inferior vena cava on the right side. After the vessels in the renal hilum were clearly identified, three Hem-O-Lock clips (Teleflex Medical, Research Triangle Park, NC, USA) were utilized to ligate the renal artery and vein. That procedure had to be accomplished before dissecting the diseased kidney from the surrounding tissue to reduce bleeding. The remaining dissection of the kidney was performed in the space between Gerota's fascia and the fatty capsule of the kidney. Adhesions and scarring were usually present during the isolation of the ventral side of the tuberculous kidney, but it was not too difficult to separate. The main reason for dissection within Gerota's fascia was to reduce the prospect of peritoneal injury. The adrenals were left in situ during the isolation of the upper renal pole in all patients. The proximal ureter was identified during the dissection of the lower pole of the kidney and ligated using one Hem-O-Lock clip in case tuberculous urine leaked from the kidney collecting system. The ureter was separated distally from the bifurcation of the common iliac artery.

Port D was established in the midclavicular line under the monitoring laparoscope and formed an isosceles triangle together with ports A and C. The position of the patient was then changed through adjustment of the operating Table to attain a head-down posture. The surgeon also changed his standing position from the back side of the patient to the ventral side. The laparoscope was inserted in port A, and ports B and D were the main working channels for dissection of the lower ureter. In premenopausal female patients, the uterine artery was preserved during isolation of the pelvic segment of the ureter. A grasper was inserted through port C to stretch the ureter toward the head side. The distal ureter was dissected toward

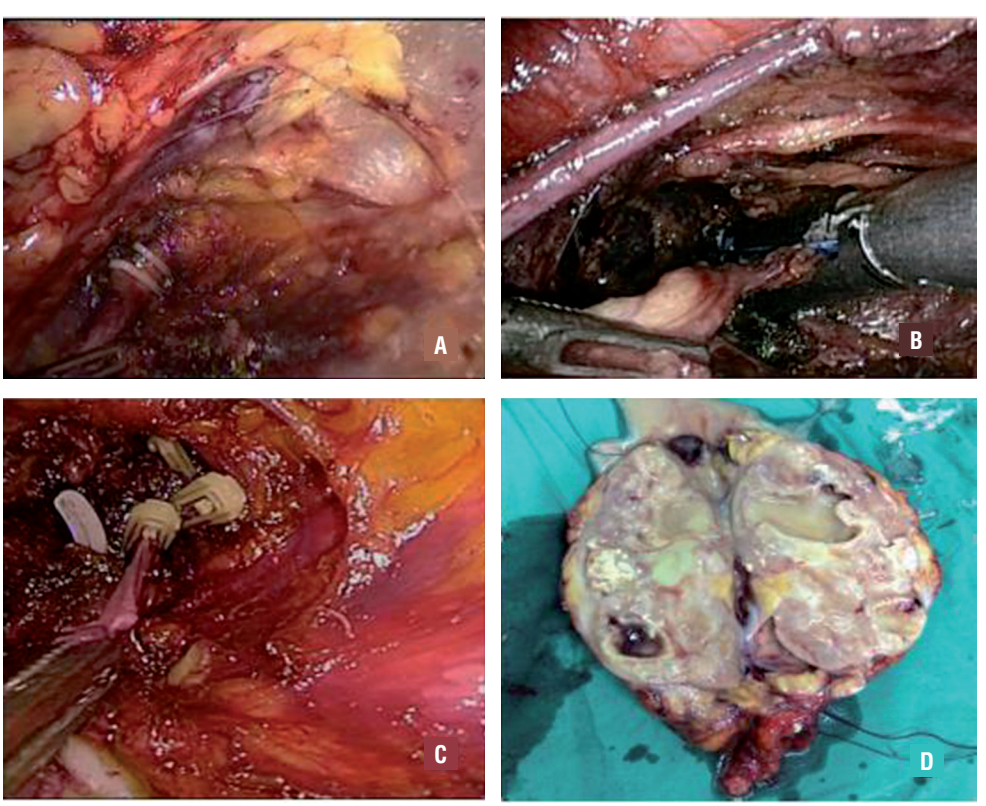
the bladder until the enlargement of the intramural ureter appeared. The sectioning of the ureteral enlargement was performed using a Hem-O-Lock clip, a 30 mm Endo-GIA (Tyco Healthcare Group LP, Glover Avenue, CT, USA) or an absorbable 12 mm Lapro-Clip (Covidien IIC, Hampshire Street, MA, USA) (Figure-2). An extending incision was then made for port A, through which the tuberculous kidney and the entire ureter were placed in a surgical bag and removed together.

Finally, drainage tubes were placed through ports C and D in the retroperitoneal space near the renal pedicle and the bladder wound, after which the trocar incisions were closed.

RESULTS

All operations were successfully performed without conversion to open surgery. The median operation time was 109.3 min (range, 75-138 min), and the median blood loss was 157.5 mL (range, 70-250 mL) without intraoperative transfusion of blood cells. The terminal ureters of 5 patients and 3 patients were ligated with Hem-O-Lock clips and Endo-GIA, respectively. Ligation of the terminal ureter in the remaining 19 patients was accomplished with an absorbable Lapro-Clip. The median weight of the tuberculous kidneys was 141.8 g (range, 25-527 g). The perioperative complications are shown in Table-1. No injury to the inferior vena cava or abdominal aorta occurred, and there was no injury to adjacent abdominal organs. Moreover, acute renal failure did not occur in any patient after surgery. There were 2 patients and 1 patient who experienced injury to the renal vein and lumbar vein, respectively, mainly resulting from adhesions around the renal hilum. Rupture of the distal ureter occurred in 1 patient during

Figure 2 - The different methods of mutilation of the distal ureter.



(A: the Hem-O-Lock clip; B: Endo-GIA; C: The absorbable Lapro-Clip and D; the renal specimen).

Table 1 - Details of perioperative complications.

Complications	Number	Percentage
Intraoperative	5	18.5%
Peritoneum injury	1	3.7%
Renal vein injury	2	7.4%
Lumbar vein injury	1	3.7%
Rupture of distal ureter	1	3.7%
Postoperative (Clavien Grade)	**8**	**29.6%**
Fever (Grade I)	3	11.1%
Pneumoscrotum (Grade I)	4	14.8%
Incision infection (Grade II)	1	3.7%

dissection of the lower ureter, probably because of the increased fragility of the diseased ureter and traction from the grasper. No spillage of pus occurred in that patient because the upper ureter had been ligated with a Hem-O-Lock clip in advance. One patient experienced the complication of incision infection, and the infection wound healed after dressing and the application of antibiotic. Four patients experienced pneumoscrotum that generally disappeared completely without any intervention within three days after surgery. Three patients experienced postoperative pyrexia. Their maximum body temperature was not over 39°C, and they were all cured with conservative therapy. In all, the median hospitalization time was 3.7 days (range, 3-6 days). Pathological examination confirmed the preoperative diagnosis of renal TB in all patients, and the pathological characteristics of TB were found in the diseased kidney and ureter. Anti-TB chemotherapy was continually performed in all patients after surgery, with a total treatment period of 6 months. During the median follow-up period of 26.7 months (range, 6-54 months), the signs of irritation of the bladder disappeared, and routine urine tests and tests for acid-fast bacilli in the urine became negative. All patients were scheduled to undergo ultrasonography of the urinary tract at 3 months, 6 months and 12 months after the operation. Cystoscopic examination was not carried out routinely after surgery. Two patients who complained of notably frequent micturition and urgency underwent cystoscopy. Bladder tumors and stones did not occur, and the symptoms were relieved by α_1-blocker treatment. No recurrence of TB of the bladder or the contralateral kidney was observed during the follow-up period.

DISCUSSION

TB is still a major challenge to public health in developing countries (6). China has the second greatest number of cases of TB in the world, with 1.3 million new cases arising per year (7). The urogenital system is the second most common site of extrapulmonary TB after lymphatic TB, occurring in 4-73% of adult TB patients (2). Because of its insidious onset and nonspecific symptoms, which mostly manifest as irritation of the bladder, late diagnosis of urinary system TB is quite common, especially in developing countries. A non-functional kidney due to destruction of the renal collecting system by Mycobacterium tuberculosis is usually present in patients with a late diagnosis. Anti-TB drug therapy has been available for tuberculous patients since the 1950s and remains the major first-line therapeutic regimen. However, this treatment is not able to cure patients with a late diagnosis. Nephrectomy intervention is recommended when there is a non-functional kidney, whether the diseased kidney is associated with calcification or is extensively destroyed, accompanied by hypertension or ureteropelvic junction obstruction (8). Figueiredo et al. reported that the rate of nephrectomy among 8961 cases of

urogenital TB was 27.6% and that the difference in the nephrectomy rate between developed and developing countries was not significant (27.9% vs. 26.0%) (9). Multiple studies have shown that the retroperitoneal laparoscopic procedure is an optimal alternative to open surgery for treating patients with a non-functional kidney resulting from TB (5, 10, 11). This procedure not only reduces interference with the abdominal organs but also avoids the possible spread of TB bacteria into the abdominal cavity. However, this approach remains a challenge for surgeons in certain cases because of perinephric adhesion and poor anatomic landmarks.

The main reasons for the conversion of retroperitoneoscopic resection of the tuberculous kidney to open surgery are major bleeding and difficulty in separating perinephric adhesions (5, 12). In the present study, the renal pedicle was controlled early, before dissecting the tuberculous kidney, to reduce intraoperative bleeding. Perinephric adhesions were another major challenge during the operation because the normal anatomic structure had usually been destroyed by the infection. The closer that adhesions were to the surface of the tuberculous kidney, the more severe the adhesions were. Duarte et al. reported that transperitoneal laparoscopic dissection outside of Gerota's fascia achieved a success rate of 72% in the management of patients with inflamed kidneys (13). Those authors preferred the transperitoneal approach to the retroperitoneal approach to avoid adhesions and fibrous tissue, mainly because the former allowed more work space and clear anatomic landmarks. However, the selection of the transperitoneal vs. retroperitoneal approach depends mainly on surgeon preference. In the present study, we endeavored to perform renal dissection within Gerota's fascia to reduce the risk of peritoneal injury because carbon dioxide gas would enter the abdominal cavity if injury to the peritoneum occurred. The retroperitoneal space would then be reduced to such an extent that it would impact the operation, especially during dissection of the lower ureter. Although the normal anatomic structure had been destroyed in certain severe cases, we found that the infective adhesion between Gerota's fascia and the fatty capsule was not difficult to separate.

Laparoscopic nephroureterectomy has been shown to be feasible and safe for upper-tract urothelial carcinoma (14, 15), but the practicability of this procedure has rarely been assessed in the management of patients with tuberculous non-functional kidneys. Tuberculous lesions can affect the entire collection system, including the kidney, ureter and bladder. The more tuberculous that lesions resected from a diseased ureter are, the less likely postoperative ureteral stump syndrome is to occur (3, 16). In addition, if tuberculous lesions of the distal ureter persist, contracture of the bladder and delayed healing may result. In the present study, the lower ureter was resected as distally as possible. The complete excision of bladder cuff can be achieved via transperitoneal laparoscopic surgery (17, 18); however, this is difficult to achieve using the retroperitoneal laparoscopic technique because of the limited retroperitoneal space. Furthermore, the resection of bladder cuff would reduce the bladder capacity of tuberculous patients to some extent and may have adverse effects on the recovery of postoperative bladder function.

Three devices were utilized to ligate the terminal ureter, and these exhibited different characteristics. Endo-GIA can be used to effectively close the terminal ureter, but this device is too expensive to apply in patients from developing countries, who usually do not have medical insurance. Moreover, both Endo-GIA and large Hem-O-Lock clips are non-absorbable and may move into the bladder, resulting in stone formation and urinary infection (19-21). Therefore, absorbable Lapro-Clips have been used to ligate the distal ureter at our center since November 2012 to prevent the possibility of stone formation in the long term.

In the present study, the median operation time and blood loss were 109.3 min and 157.5 mL, respectively. Chibber et al. reported that they detached and ligated the distal ureter below the level of bifurcation of the common iliac artery and that the corresponding indicators were 208.5 min and 326.25 mL in the tuberculosis TB group (3). Tian X et al. described the use of a Gibson incision to dissect the distal ureter of patients with tuberculous non-functional kidneys, with a median operation

time and blood loss of 123.0 min and 134.0 mL, respectively (4). Compared with these studies, the present study showed a shorter operation time and less blood loss. With respect to perioperative complications, no serious complications above grade III of the modified Clavien classification occurred in our study or the two cited studies, except for a retroperitoneal hematoma after surgery that required re-operation in the study by Tian X et al. (4). To prevent the potential contamination of the surgical field by tuberculous tissue, it is essential to utilize a surgical bag to completely remove the tuberculous specimen.

The present study is limited by its retrospective design, its focus on a single center and the relatively short period of follow-up. However, to the best of our knowledge, there have been no other studies to date that have included a larger sample size of patients with tuberculous non-functional kidneys who underwent total retroperitoneal laparoscopic nephroureterectomy.

CONCLUSIONS

Total retroperitoneal laparoscopic nephroureterectomy can be a safe and feasible approach for the management of patients with tuberculous non-functional kidneys. However, further studies are necessary to confirm our results.

CONFLICT OF INTEREST

None declared.

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Correspondence address:
Canqiang Li, MD
Department of Urology
The Affiliated Hexian Memorial Hospital of Southern Medical University
2 Qinghe Road, Panyu district, Guangzhou 511400, P.R. China
Fax: + 86 020 8463-4164
E-mail: doctorlicanqiang@sina.com

ORIGINAL ARTICLE

Vol. 44 (6): 1182-1193, November - December, 2018
doi: 10.1590/S1677-5538.IBJU.2018.0127

Oxidative stress in the bladder of men with LUTS undergoing open prostatectomy: a pilot study

Marcio Augusto Averbeck [1, 2, 3], Nelson Gianni de Lima [4], Gabriela Almeida Motta [1], Lauro Beltrão [2], Nury Jafar Abboud Filho [2], Clarice Pereira Rigotti [2], William Nascimento dos Santos [2], Steven Kitzberger Jaeger dos Santos [2], Luis Fernando Batista da Silva [2], Ernani Luis Rhoden [2, 3, 5]

[1] Departamento Pós-graduação em Ciências da Saúde da Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA), Porto Alegre, RS, Brasil; [2] Serviço de Urologia, Complexo Hospitalar Santa Casa, Porto Alegre, RS, Brasil; [3] Serviço de Urologia, Hospital Moinhos de Vento, Porto Alegre, RS, Brasil; [4] Serviço de Urologia, Hospital de Nossa Senhora dos Navegantes, Torres, RS, Brasil; [5] Disciplina de Urologia, Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA), Porto Alegre, RS, Brasil

ABSTRACT

Purpose: This study aims to evaluate the link between preoperative parameters and oxidative stress (OS) markers in the bladder wall of men undergoing open prostatectomy.
Materials and Methods: From July 2014 to August 2016, men aged ≥ 50 years and presenting with LUTS were prospectively enrolled. Preoperative assessment included validated questionnaires (IPSS and OAB - V8), lower urinary tract ultrasound and urodynamics. Bladder biopsies were taken during open prostatectomy for determination of OS markers. Increased OS was defined by increased concentration of malondialdehyde (MDA) and / or decreased concentration of antioxidant enzymes (superoxide dismutase and / or catalase). P<0.05 was regarded as statistically significant.
Results: Thirty - eight consecutive patients were included. Mean age was 66.36 ± 6.44 years, mean prostate volume was 77.7 ± 20.63 cm3, and mean IPSS was 11.05 ± 8.72 points. MDA concentration was increased in men with severe bladder outlet obstruction (BOO grade V - VI according to the Schaefer's nomogram) in comparison with BOO grade III - IV (p = 0.022). Patients with severe LUTS also had higher MDA concentration when compared to those with mild LUTS (p = 0.031). There was a statistically significant association between increased post - void residual urine (cut off ≥ 50 mL) and not only higher levels of MDA, but also reduced activity of SOD and catalase (p < 0.05).
Conclusions: This pilot study showed that severity of LUTS and BOO were associated with increased MDA concentration in the bladder wall of men undergoing open prostatectomy. Further studies are still needed to assess the role of non - invasive biomarkers of OS in predicting bladder dysfunction in men with LUTS.

ARTICLE INFO

Keywords:
Urinary Bladder; Lower Urinary Tract Symptoms; Urinary Bladder Neck Obstruction; Oxidative Stress

Int Braz J Urol. 2018; 44: 1182-93

Submitted for publication:
February 25, 2018

Accepted after revision:
August 12, 2018

Published as Ahead of Print:
September 20, 2018

INTRODUCTION

Current evidence suggests that bladder response to chronic obstruction occurs adaptively (1). Bladder functional changes caused by obstruction may be urodynamically classified in three main groups: (a) detrusor overactivity with or without reduced bladder compliance; (b) detrusor underactivity (DU) with impaired voiding; and (c) mixed pattern (2).

Unfortunately, to date there is no reliable marker to predict which patients with bladder outlet obstruction (BOO) will inexorably present deterioration of bladder contractility, which, by itself, has been associated with poorer surgical outcomes in men with benign prostatic hyperplasia (BPH). Thomas et al. demonstrated the lack of long - term symptomatic or urodynamic gains from transurethral resection of the prostate (TURP) in men with both BPH and detrusor underactivity (3). On the other hand, persistent detrusor overactivity is also clinically relevant in patients undergoing prostate surgery, as it may impose increased risk of urgency urinary incontinence (4).

According to animal models, oxidative stress (OS) and bladder dysfunction (BD) may be related to ischemia - reperfusion process and BOO (5, 6). Reactive oxygen species (ROS), including hydroxyl radicals, superoxide anions, and hydrogen peroxide, are normally produced in low levels during univalent reduction of oxygen to water and are important for diverse biological processes, including apoptosis, immunity, and cell defense against microorganisms (7). Increased formation of ROS and / or decreased antioxidant defense can be defined as OS, which may cause cell damage. Endogenous antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) are key to prevent damage from ROS. OS induces lipid peroxidation, which is expressed by formation of malondialdehyde (MDA) (8). Sezginer et al. has recently investigated the effects of different degrees of obstruction on bladder function in rats, showing that MDA levels were increased in severe partial BOO (9). Nevertheless, this association has not been properly assessed in humans so far.

Our study aims to evaluate the link between preoperative parameters (clinical, ultrasound and urodynamic findings) and OS markers in the bladder wall of men undergoing open prostate surgery. We hypothesized that severe bladder outlet obstruction was associated with increased OS in the bladder wall of men with lower urinary tract symptoms (LUTS).

MATERIALS AND METHODS

This was a pilot study, approved by the local Ethics Committee (approval number: 660.810). From July 2014 to August 2016, men presenting with LUTS, aged ≥ 50 years, prostate volume ≥ 40 mL, undergoing prostate surgery in a single university hospital were invited to take part in the study. The target population consisted of patients with BPH or organ - confined prostate cancer with concomitant LUTS in the perioperative period of open prostatectomy.

Our primary endpoint was the association between OS markers and severity of BOO. Exploratory endpoints included the link between OS markers various preoperative characteristics, such as obesity, severity of LUTS, overactive bladder symptoms, ultrasound and urodynamic parameters.

Exclusion Criteria: Patients without complaint of one or more voiding LUTS over the last 3 months, previous pelvic surgery, neurological disease with secondary neurogenic lower urinary tract dysfunction, established cardiovascular disease (including prior stroke, myocardial ischemia and / or peripheral vascular disease) and patients relying on clean intermittent catheterization or taking drugs with potential effects on bladder function (e.g. anticholinergics and 5 - alpha - reductase inhibitors).

Patients who met the criteria for inclusion were invited to participate and received comprehensive information on further evaluations, which included lower urinary tract ultrasound, urodynamics and a bladder wall biopsy. Only those who were able to understand the risk - benefit profile of the assessments and provided written informed consent were included in the study. This study was carried out in accordance with the ethical standards of the responsible institutional committee and with the Helsinki Declaration.

Clinical, laboratory and anthropometric assessments

We collected clinical, and anthropometric data such as age, comorbidities, weight, height, body mass index (BMI), fasting glucose, and blood pressure. A single examiner performed the anthropometric measurements, in a standardized way (average of two or more measurements). Weight (kg) was acquired using a precision balance and height was measured using a wall - mounted

stadiometer. BMI was calculated as the ratio between weight (kg) and height squared (m^2), and defined the following reference values: normal (18.5 - 24.9 kg / m^2), overweight (25.0 - 29.9 kg / m^2), and obesity (30 kg / m^2 or higher).

LUTS assessment

LUTS were assessed using the International Prostate Symptom Score (IPSS) (10). LUTS severity was classified as follows: mild (IPSS ≤ 7 points), moderate (IPSS ≥ 8 and ≤ 19 points), and severe (IPSS ≥ 20 points). The OAB - V8 questionnaire (Overactive Bladder - Validated 8 - question Screener) was also used to estimate the prevalence of overactive bladder symptoms, which were defined by a score ≥ 8 points (11).

Ultrasound assessment

Ultrasound examination of the lower urinary tract was performed with the device Siemens Sonoline G50® (Siemens AG, Munich, Germany). Total thickness of the bladder wall was measured by a mean of two sagittal measurements of the anterior bladder wall, with 250 mL of bladder filling (12, 13). Bladder wall thickness (BWT) was defined by the distance between the mucosa and the adventitia, both with hyperechogenic characteristics (14). Parameters such as prostate gland volume and intravesical prostatic protrusion (IPP) were also evaluated, according to the technique described by Yuen et al. (15). All measurements were performed transabdominally, by a single trained researcher using a high frequency transducer (7.5 MHz). All ultrasound assessments were performed in the urodynamics unit, which allowed measurements with standardized bladder filling (250 mL).

Urodynamic assessment

Urodynamic studies were performed 2 to 3 weeks before open prostatectomy, using the Laborie Dorado KT® device (Laborie Medical, Ontario, Canada). All assessments were performed by a single trained researcher, in compliance with the International Continence Society (ICS) Good Urodynamic Practices (16). Post void residual (PVR) was defined as the volume of urine inside the bladder at the end of micturition (17). In our study, PVR was measured by catheterization after free uroflowmetry (before starting the filling cystometry). Increased PVR was arbitrarily defined as a volume ≥ 50 mL (18).

Bladder outlet obstruction (BOO) was defined by the formula: detrusor pressure at maximum flow - (2x maximum flow). A value greater than 40 was regarded as BOO, less than 20 as no obstruction, and between 20 and 40 as undetermined (19). The Schaefer's nomogram was used to assess BOO severity (19). Severe BOO was defined as zone V or VI on the nomogram. Detrusor underactivity was defined by the bladder contractility index (BCI), calculated by the formula: detrusor pressure at maximum flow + (5 x maximum flow). Values under 100 were regarded as detrusor underactivity (19).

Bladder biopsies

A full - thickness fragment of the bladder wall measuring 1.0 cm^2 was obtained from the anterior bladder wall during prostatectomy for determination of OS markers, including catalase, SOD and MDA. In order to aim at the detrusor muscle, each fragment had the mucosa and the perivesical fat removed, and then was frozen in liquid nitrogen at - 70°C in order to preserve the material for later analysis.

Oxidative Stress Analysis

Prior to performing OS analyzes, the bladder fragments were manually homogenized with 1.15% KPi buffer (pH = 7.4) containing protease inhibitors, at a ratio of 5 mL buffer (1.15% KCl) for each gram of tissue, and then the total protein concentrations of the bladder tissue were measured by the Bradford method (20) in a spectrophotometer at 535 nm.

Thiobarbituric acid reactive substances test was performed by spectrophotometry at 535 nm to assess the concentration of MDA, which is a biomarker of peroxidative damage to lipids (21). Analysis of the activity of antioxidant enzyme SOD was performed by the pyrogallol autoxidation method (22), using spectrophotometry at 420 nm. Determination of catalase activity, which is an antioxidant enzyme, was carried out by the

rate of hydrogen peroxide (H_2O_2) decomposition (spectrophotometry at 240 nm) (23).

Definition of increased oxidative stress

Increased OS was defined by either of the following criteria: increased concentration of MDA and / or decreased concentration of antioxidant enzymes (SOD and / or catalase).

Statistical analysis

Data were expressed as mean ± standard deviation. Results were compared using the Student t test, and controlled for the use of alpha - blockers. Nominal variables were analyzed using the Fisher exact test. Bonferroni adjustment has been used for multiple testing correction. For specific parameters, such as prostate volume and BWT, distinct quartiles were taken to compare OS levels and define cutoffs. Statistical analyses were performed using SPSS® version 22.0 for Windows (SPSS Inc., Chicago, IL, USA) and an alpha error inferior to 5% ($p < 0.05$) was considered statistically significant.

RESULTS

Thirty - eight consecutive patients were included. Mean age was 66.36 ± 6.44 years. Mean body mass index (BMI) was 26.36 ± 2.98 kg / m^2. The most common comorbidities were systemic arterial hypertension (50%) and diabetes mellitus type 2 (DM2) (29%). Regarding LUTS severity, 14 patients (36.8%) presented mild symptoms, 18 (47.3%) moderate and 6 (15.7%) severe symptoms. Sixteen patients were taking alpha - blockers regularly in the last 6 months before surgery. Prevalence of overactive bladder symptoms (OAB - V8 score ≥ 8 points) was 36.8% (n = 14). Baseline characteristics are described in Table-1.

Mean preoperative PSA was 8.17 ± 3.55 vs. 4.48 ± 3.14 ng / mL in patients undergoing open radical prostatectomy (n = 34) and open retropubic prostatectomy (n = 4), respectively (p = 0.16). There were no statistically significant differences in baseline characteristics between the two groups ($p > 0.05$). All 4 patients who underwent open simple suprapubic prostatectomy had pathological examination confirming BPH. All patients who underwent open radical prostatectomy had low - grade (Gleason 3 + 3 or 3 + 4) organ - confined prostate cancer and concomitant BPH in the pathological examination.

Mean prostate volume estimated by transabdominal ultrasound was 77.7 ± 20.63 cm^3. Mean bladder wall thickness (BWT) was 3.99 ± 1.39 mm. Seventeen patients (44.7%) presented with intravesical protrusion of the median prostatic lobe, with a mean of 1.54 ± 0.64 cm.

In regards to urodynamics, 13 patients (34.2%) had increased bladder sensation and 7 patients (18.4%) presented reduced bladder sensation.

Table 1 - Baseline characteristics of men aged ≥ 50 years with LUTS and undergoing open prostate surgery*.

	Mean ± SD
Clinical parameters	
Age (years)	66.36 ± 6.44
Weight (kilograms)	77.71 ± 8.07
BMI (kg/m^2)	26.36 ± 2.98
IPSS score	11.05 ± 8.72
OAB-V8 score	7.69 ± 8.44
Ultrasound parameters	
Prostate volume	77.7 ± 20.63
IPP¥	1.54 ± 0.64
BWT	3.99 ± 1.39
Urodynamic parameters	
First desire (mL)	195.62 ± 78.57
Maximum cistometric capacity (mL)	360.11 ± 82.05
Compliance (mL/cmH_2O)	79.70 ± 171.72
BOOI	63.59 ± 31.76
BCI	108.64 ± 27.33

* **n =** 38, ¥ **n =** 17

BMI = Body mass index; **IPSS =** International Prostate Symptom Score; **OAB-V8 =** Overactive Bladder-Validated 8-question Screener Questionnaire; **IPP =** Intravesical prostate protrusion; **BWT =** Bladder wall thickness; **BOOI =** Bladder outlet obstruction index; **BCI =** Bladder contractility index

Twelve patients (31.6%) had reduced bladder compliance and 7 patients (18.4%) had reduced cystometric capacity. Detrusor overactivity was demonstrated in 11 patients (28.9%) and urgency urinary incontinence in 5 (13.2%). In the flow - pressure study, reduced peak urinary flow (< 15 mL / sec) was observed in 35 patients (92.1%). Increased post - void residual urine (> 50 mL) was observed in 22 patients (57.9%). BOO was observed in 29 patients (76.3%). Detrusor underactivity was present in 17 patients (44.7%).

Primary endpoint

Severe BOO (zones V and VI on Schaefer's nomogram) was associated with increased MDA concentration in the bladder wall (242.74 ± 220.20 vs. 114.90 ± 54.08 pmoL / mg; p = 0.022) (Figure-1).

Exploratory endpoints

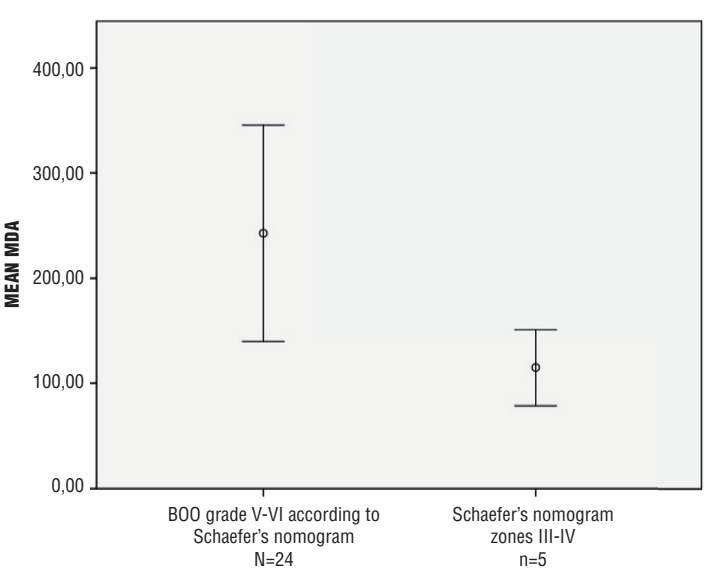
Concentration of OS markers (MDA, SOD, catalase) in the bladder wall according to preoperative parameters (clinical, ultrasound and urodynamic findings) are listed in Table-2.

Obesity was associated with reduced activity of the antioxidant enzyme catalase (170.88 ± 27.46 vs. 317.11 ± 268.98 pmoL / mg in obese and in non - obese patients, respectively; p = 0.01) and with reduced activity of the antioxidant enzyme SOD (0.4 ± 0.19 vs. 0.76 ± 0.59 Usod / mg in obese and in non - obese patients, respectively; p = 0.05).

Patients with severe LUTS (IPSS score ≥ 20 points) had higher MDA concentration in the bladder wall when compared to the patients with mild LUTS (IPSS < 8 points): 290.93 ± 237.87 vs. 111.93 ± 82.37 pmoL / mg, respectively (p = 0.031). Patients with moderate LUTS (IPSS ≥ 8 and < 20 points) had a mean of MDA concentration of 144.85 ± 112.88 pmoL / mg (intermediate values in - between mild and severe LUTS outcomes, but not statistically significant in the multivariate analysis). Likewise, the diagnosis of OAB (OAB - V8 score ≥ 8 points) had no association with increased OS in the bladder wall (p > 0.05).

MDA concentration was higher in patients with BWT ≥ 3 mm compared to those with BWT < 3 mm (342.03 ± 317.03 vs. 157.97 ± 107.47 pmoL / mg, respectively; p = 0.015). Increased prostate volume (PV) was associated with higher concentrations of MDA: 236.43 ± 217.75 vs. 130.33 ± 66.44 pmoL / mg in patients with PV ≥ 80 cm3 and PV < 80cm3, respectively (p = 0.048). However, IPP was not associated with increased OS in the bladder wall (p > 0.05).

Figure 1 - Severity of bladder outlet obstruction according to the Schaefer's nomogram (Zones V-VI versus III-IV) and MDA concentration in the bladder wall of men aged ≥ 50 years, presenting with LUTS and undergoing open prostatectomy (n = 29)*.



MDA = malondialdehyde; **LUTS =** lower urinary tract symptoms
*** p =** 0.022

Table 2 - Clinical, ultrasound and urodynamic parameters versus oxidative stress markers (MDA, SOD, catalase) in the bladder wall of men aged ≥ 50 years with LUTS and undergoing open prostate surgery*.

Preoperative parameters	Oxidative stress markers (Mean ± SD; p value¥)		
	MDA concentration (pro-oxidant) pmoL/mg	SOD activity (antioxidant enzyme) Usod / mg	Catalase activity (antioxidant enzyme) pmoL/mg
Clinical			
Obesity (BMI ≥ 30kg/m²)			
Yes (n = 4)	216.41 ± 199.62	0.4 ± 0.19	170.88 ± 27.46
No (n = 34)	120.97 ± 101.70	0.76 ± 0.59	317.11 ± 268.98
	p = 0.38	p = 0.05	p = 0.01
LUTS severity			
IPSS≥20 points (n=6)	290.93 ± 237.87	0.69 ± 0.56	181.81 ± 48.59
IPSS<8 points (n=14)	111.93 ± 82.37	0.71 ± 0.95	327.06 ± 215.17
	p = 0.031	p = 0.48	p = 0.68
Overactive bladder symptoms			
OAB-V8 ≥ 8 points (n = 14)	158.16 ± 133.98	0.62 ± 0.39	314.37 ± 354.72
OAB-V8 < 8 points (n = 24)	217.88 ± 211.43	0.74 ± 0.63	281.83 ± 138.66
	p = 0.33	p = 0.46	p = 0.74
Ultrasound			
Bladder Wall Thickness			
≥ 3 mm (n = 30)	342.03 ± 317.03	0.59 ± 0.35	262.15 ± 143.57
< 3 mm (n = 8)	157.97 ± 107.47	0.69 ± 057	283.47 ± 125.18
	p = 0.015	p = 0.53	p = 0.7
Prostate volume			
≥ 80 cm³ (n = 13)	236.43 ± 217.75	0.54 ± 0,31	259.69 ± 102.19
< 80 cm³ (n = 25)	130.33 ± 66.44	0.75 ± 0.61	269.70 ± 157.18
	p = 0.048	p = 0.18	p = 0.81
IPP			
Yes (n = 17)	248.74	0.51 ± 0.29	261.38 ± 125.29
No (n = 21)	136.37	0.81 ± 0.65	270.27 ± 152.60
	p = 0.064	p = 0.095	p = 0.84
Urodynamics			
Reduced bladder sensation			
Yes (n = 7)	202.90 ± 192.37	0.7 ± 0.4	205.66 ± 60.21
No (n = 31)	155.06 ± 106.91	0.67 ± 0.56	280.30 ± 148.67
	p = 0.44	p = 0.85	p = 0.045
Urgency§			
Yes (n = 13)	250.35 ± 245.70	0.62 ± 0.44	245.68 ± 130.89
No (n = 25)	166.14 ± 133.19	0.76 ± 0.68	304.03 ± 150.45
	p = 0.28	p = 0.51	p = 0.25
Detrusor overactivity			
Yes (n = 11)	198.57 ± 206.02	0.65 ± 0.43	262.84 ± 125.15
No (n = 27)	194.73 ± 175.64	0.73 ± 0.74	267.60 ± 146.64
	p = 0.96	p = 0.73	p = 0.92
Severity of bladder outlet obstruction according to the Schäfer nomogram¶			
Zones V and VI (n = 24)	242.74 ± 220.20	0.53 ± 0.29	227.75 ± 65.1
Zones III-IV (n = 5)	114.90 ± 54.08	0.78 ± 0.64	288.89 ± 159.75
	p = 0.022	p = 0.12	p = 0.13
Detrusor underactivity			
Yes (BCI < 100) (n = 17)	222.90 ± 241.21	0.55 ± 0.36	237.39 ± 120.64
No (BCI ≥ 100) (n = 21)	171.83 ± 107.60	0.77 ± 0.63	290.66 ± 151.38
	p = 0.44	p = 0.19	p = 0.24
Post-void residual urine			
≥ 50 mL (n = 22)	270 ± 197.06	0.48 ± 0.29	212.71 ± 99.32
< 50 mL (n = 16)	143.96 ± 154.91	0.97 ± 0.67	344.61 ± 154.01
	p = 0.045	p = 0.005	p = 0.003

MDA = malondialdehyde; **SOD =** superoxide dismutase; **LUTS =** lower urinary tract symptoms; **SD =** standard deviation; **BMI =** body mass index; **IPSS =** International Prostate Symptom Score; **OAB-V8 =** Overactive Bladder-Validated 8-question Screener Questionnaire; **IPP =** intravesical protrusion of the median prostatic lobe; **BCI =** bladder contractility index; *** n =** 38; **¥ =** T test; **§** "urgency" was defined as "the complaint of a sudden compelling desire to pass urine which is difficult to defer" (18); **¶** primary endpoint

Regarding urodynamic parameters, it has been shown that patients with reduced bladder sensation had a statistically significant reduction of the activity of the catalase enzyme in the bladder wall when compared to those patients with normal bladder sensation (205.66 ± 60.21 vs. 280.30 ± 148.67 pmoL / mg, respectively; p = 0.045). On the other hand, parameters such as urinary urgency, detrusor overactivity, reduced bladder compliance or cystometric capacity, and weak stream were not associated with increased OS in the bladder wall (p > 0.05).

Increased MDA concentration and reduced activity of antioxidant enzymes (both catalase and SOD) were observed in the bladder wall of patients with post - void residual urine (PVR) ≥ 50 mL:

MDA: 270 ± 197.06 vs. 143.96 ± 154.91 pmoL / mg; p = 0.045 (Figure-2a),

SOD: 0.48 ± 0.29 vs. 0.97 ± 0.67 Usod / mg; p = 0.005 (Figure-2b),

Catalase: 212.71 ± 99.32 vs. 344.61 ± 154.01 pmoL / mg; p = 0.003 (Figure-2c).

DISCUSSION

To our knowledge, this is the first study investigating OS markers (MDA, SOD and catalase) in the detrusor muscle of humans undergoing open prostate surgery. Clinical factors (LUTS severity and obesity, ultrasound findings (bladder wall thickness ≥ 3 mm, prostate volume ≥ 80 cm3), and urodynamic parameters (BOO severity, post - void residual urine ≥ 50 mL) were associated with either increased MDA concentration or reduced activity of antioxidant enzymes (SOD / catalase) in the bladder wall. Identification of such factors may have clinical relevance, as evidence from animal models suggested a relationship between increased OS and bladder dysfunction (24-26).

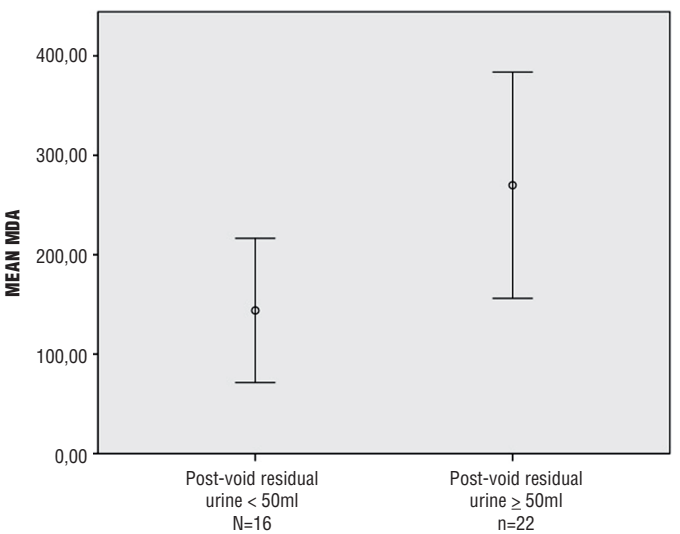
Generation of reactive oxygen species (ROS) and ischemia - reperfusion injury have been proposed as the primary etiological factors in obstruction - induced bladder dysfunction (9). At a molecular level, reactive oxygen species exhibit signaling and cell - function - modifying roles (27). OS occurs when the net flux of reactive oxygen species (ROS) and reactive nitrogen species (RNS) production exceeds the capacity of the cell to detoxify these potentially injurious oxidants. Functional in vitro studies showed that elevated ROS levels impair bladder contractile responses (28, 29).

Several biomarkers have been used in experimental models to assess both urinary and plasma OS, including 8 - hydroxy - 2' - deoxyguanosine (8 - OHdG), MDA, total anti - oxidant capacity (TAC) and glutathione (GSH) (30). SOD and catalase assays have also been carried out on animal tissues to study the effects of partial BOO on the cell's anti - oxidant defense mechanisms (25). In our pilot study, OS biomarkers included MDA, catalase and SOD. Experimental studies showed that both the SOD and catalase activities are calcium - sensitive and changes in their activity would be expected to occur during ischemia, which can result in decreased antioxidant capacity of the bladder smooth muscle and mucosa (31). Hence, there sensitivity and specificity issues related to distinct OS biomarkers and caution should be exercised in interpreting the results of such studies.

There is growing interest on the association between LUTS and systemic conditions such as obesity, diabetes mellitus and metabolic syndrome in men (32, 33). Dibello et al. (33) compared the prevalence of metabolic syndrome in men aged ≥ 50 years with and without a diagnosis of BPH from a large database (UK Clinical Practice Research Datalink - CPRD). Among men with BPH, 26.5% (n = 85.103) had diagnosis of metabolic syndrome, compared with 20.9% without BPH (control group, n = 85.103) (absolute difference of 5.6%, p < 0.001). In our pilot study, data on height and weight of the patients were recorded and allowed the calculation of BMI. Patients with BMI ≥ 30 (n = 4) presented with higher levels of both antioxidant enzymes catalase and SOD in the bladder wall in comparison with those with BMI < 30 (n = 34).

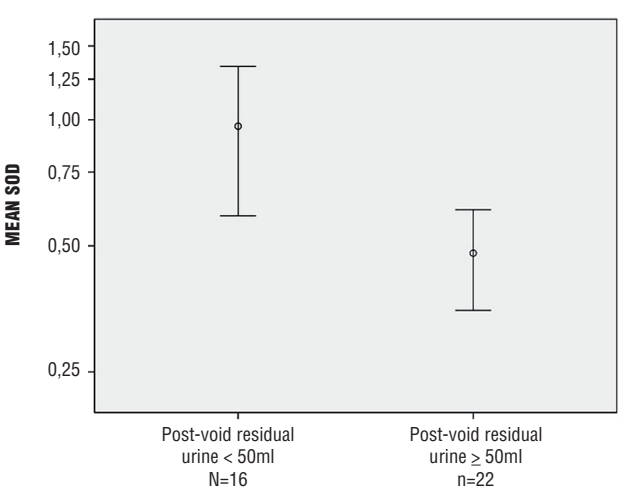
Non - invasive assessment of PVR is usually recommended as a first - line diagnostic tool in men with LUTS (34). Despite the lack of a consensual cutoff, it is known that increased PVR may represent a risk factor for acute urinary retention and / or bladder dysfunction among these patients (35). According to Crawford et al., who studied a total of 3.047 men with BPH over 4.5 years, a baseline PVR of 39 mL or greater was an independent predictor of BPH clinical progression in patients not receiving active treatment for LUTS (36). Our study showed that a PVR ≥ 50 mL was associated with increased OS in

Figure 2a - Increased MDA concentration in the bladder wall of men aged ≥ 50 years, undergoing open prostatectomy and presenting with LUTS and PVR ≥ 50 mL (n = 38)*



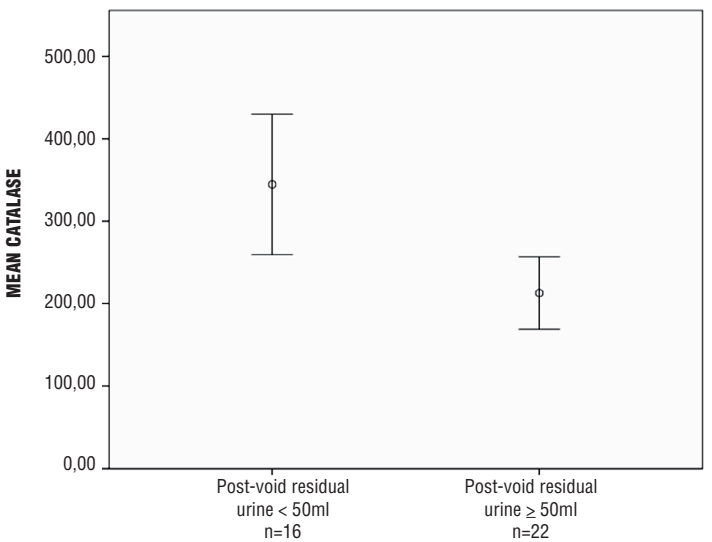
MDA = malondialdehyde; **LUTS =** lower urinary tract symptoms; **PVR =** post-void residual urine
*** p =** 0.045

Figure 2b - Decreased activity of the antioxidant enzyme SOD in the bladder wall of men aged ≥ 50 years, undergoing open prostatectomy and presenting with LUTS and PVR ≥ 50 mL (n = 38)*



SOD = superoxide dismutase; **LUTS =** lower urinary tract symptoms; **PVR =** post-void residual urine
*** p =** 0.005

Figure 2c. Decreased activity of the antioxidant enzyme catalase in the bladder wall of men aged ≥ 50 years, undergoing open prostatectomy and presenting with LUTS and PVR ≥ 50 mL (n = 38)*



LUTS = lower urinary tract symptoms; **PVR =** post-void residual urine
*** p =** 0.003

the bladder wall (higher MDA concentration; reduced activity of both catalase and SOD).

Although not recommended in the routine assessment of LUTS in men without neurological diseases, urodynamics may be important for selected patients in order to define the pattern of voiding dysfunction (34). Incomplete bladder emptying may be caused by detrusor underactivity (DU), bladder outlet obstruction (BOO), or dysfunctional voiding (17, 37). Previous studies showed DU in up to 40% of men aged > 65 years and even up to 48% of men aged ≥ 70 years (38, 39). Despite being a prevalent condition in the elderly population, the origin of DU in men without neurological diseases remains controversial (40). BOO and increased OS have been related to a higher risk of detrusor underactivity in animal models (25, 41, 42). Callaghan et al. demonstrated that partial BOO has significant effects on the activity of both SOD and catalase in the bladder, with variations that are dependent on the severity and duration of the obstruction (25). Our study demonstrated that patients with severe LUTS (IPSS ≥ 20 points; n = 6) and severe BOO (grade V - VI in Schaefer's nomogram; n = 24) had increased MDA concentration in the bladder wall in comparison with patients with mild LUTS (IPSS < 8 points; n = 14) and BOO grade III - IV (n = 5), respectively. These findings may be clinically relevant, as bladder dysfunction has been implicated as a risk factor for persistent LUTS after prostate surgery (2).

Other non - invasive tests, such as ultrasound measurement of IPP, detrusor wall thickness (DWT) or bladder wall thickness (BWT) have been advocated to predict the chances of BOO in the male population (12, 37). Increased DWT is observed in adult men with non - neurogenic LUTS and BOO (12). A prospective study demonstrated that DWT ≥ 2 mm in bladders filled ≥ 250 mL was a predictor of BOO (43). Additionally, Güzel et al. have studied the utility of BWT in men with HPB / LUTS and demonstrated that this parameter is an easy, quick, and repeatable test to predict BOO severity (44). Most probably, due to the high proportion of patients with BOO in our study, we could not demonstrate an association between BWT and obstruction. On the other hand, BWT ≥ 3 mm was associated with increased OS in the bladder wall (p = 0.015).

The main limitation of our pilot study is the small number of patients included. Since the introduction of 5 - alpha - reductase inhibitors in the cli-

nical practice, the number of patients who require open prostate surgery for BPH has dropped significantly (45). On the other hand, BPH and prostate cancer (PCa) are often coexisting in older men (46). Böcking et al. (47) demonstrated that latent prostate cancer would be present in more than 50% of men over the age of 80. In our study, most of the patients underwent open radical prostatectomy (n = 34); notwithstanding, all of them had been followed up at the urology outpatient clinic of the same institution due to BPH symptoms prior to the diagnosis of prostate cancer. In addition, all patients who underwent open radical prostatectomy had low - grade (Gleason 3 + 3 or 3 + 4) organ - confined prostate cancer and concomitant BPH in the pathological examination. Another limitation of our study was the lack of OS analysis in the urothelial layer of the bladder samples. Evidence from animal models suggests that the urothelium is also sensitive to oxidative stress generated by partial obstruction (48). However, as per protocol, our study was aimed at assessing the oxidative stress in the smooth muscle layer (detrusor). In addition, despite the use of previously validated assessment procedures, there is no perfect method for assessing tissue OS. To counteract oxidative and nitrosative stress, human cells employ complex defense mechanisms (27). The interpretation of all OS studies should be done in the light of such imperfection, since there are distinct cellular enzymatic and non - enzymatic antioxidative pathways. At last, the invasiveness of bladder biopsies may also be seen as a limitation. Future research should focus on non - invasive techniques and biomarkers to assess the oxidative stress in the human bladder.

Despite inherent limitations, our pilot study has several strengths. Firstly, our restricted inclusion criteria aimed at patients with higher risk of BOO (76.3% of our study population). Secondly, the technique for retrieving bladder biopsies in our protocol did not require electrocautery and favored the OS analysis. Thirdly, ultrasound and urodynamic assessments were standardized and performed by a single trained researcher, who was blind to the OS analysis and results.

CONCLUSIONS

This pilot study revealed increased MDA concentration in the bladder wall of men with severe LUTS (IPSS score ≥ 20 points), severe BOO (Schaefer's nomogram grade V - VI), BWT ≥ 3 mm and PV ≥ 80 cm3. Reduced activity of the antioxidant enzymes (catalase and / or SOD) was demonstrated in patients with BMI ≥ 30 and in those with reduced bladder sensation on filling cystometry. Increased PVR (≥ 50 mL) was associated with both increased MDA concentration and reduced activity of antioxidant enzymes (catalase and SOD) in the bladder wall.

Novel diagnostic methods targeting oxidative and / or inflammatory pathways may be a reasonable strategy for a more comprehensive evaluation of patients presenting with severe LUTS and BOO. Further studies are still needed to assess the role of non - invasive biomarkers of OS in predicting bladder dysfunction in men with LUTS.

CONFLICTS OF INTEREST

Doctor Márcio Augusto Averbeck reports grants and personal fees from Medtronic, Coloplast, and GSK outside the submitted work.

All the other authors do not have any conflict of interest, including specific financial interests or relationships and affiliations relevant to the subject matter or materials discussed in the manuscript.

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Correspondence address:
Marcio Augusto Averbeck, MD
Rua: Tiradentes, 333 / 6º andar
Porto Alegre, RS, 90560-030, Brasil
Fax: + 55 51 3314 2982
E-mail: marcioaverbeck@gmail.com

ORIGINAL ARTICLE

Vol. 44 (6): 1194-1199, November - December, 2018
doi: 10.1590/S1677-5538.IBJU.2018.0033

Changing bulking agent may require change in injection volume for endoscopic treatment of vesicoureteral reflux

Ali Tekin [1,2], Ismail Yagmur [1,2], Sibel Tiryaki [1,2], Zafer Dokumcu [1], Ibrahim Ulman [1,2], Ali Avanoglu [1,2]

[1] Department of Pediatric Surgery, Ege University Faculty of Medicine, Izmir, Turkey; [2] Division of Pediatric Urology, Ege University Faculty of Medicine, Izmir, Turkey

ABSTRACT

Introduction: Various bulking agents were utilized for endoscopic correction of VUR. A study reviewing multi-institutional data showed that the amount of injection material has increased over time with the purpose of improving success rates, which also resulted in costs. We noticed an opposite trend in our center since we started using a new bulking agent. The aim of this study was to evaluate evolution of our practice with different bulking agents.

Patients and Methods: Records of VUR patients who underwent subureteric injection with polyacrylate polyalcohol copolymer (PPC) and dextronomere hyaluronic acide (DxHA) between 2005 and 2014 were reviewed. Variation of different parameters throughout the study period was evaluated along with the success rate. Success was defined as complete resolution of reflux.

Results: A total of 260 patients with 384 refluxing units were included. The success rate was higher in PPC group compared to DxHA group. There was no statistically significant difference between years regarding distribution of VUR grade, body weight, patient height, and age in PPC group. Despite significant reduction in injection volume, success rate did not decrease through the years with PPC.

Conclusion: Different bulking agents may require different injection volumes to achieve the same success rate in endoscopic treatment of vesicoureteral reflux. Habits gained with previous experience using other materials should be revised while using a new agent.

ARTICLE INFO

Keywords:
Vesico-Ureteral Reflux; Endoscopy; Cakut [Supplementary Concept]

Int Braz J Urol. 2018; 44: 1194-9

Submitted for publication:
January 18, 2018

Accepted after revision:
August 12, 2018

Published as Ahead of Print:
September 15, 2018

INTRODUCTION

Endoscopic subureteric injection has become the most popular surgical method in the management of vesicoureteral reflux (VUR) in children, largely due to lower complication rates and the ease of application (1-4). Essential features of an ideal bulking agent are: easy applicability, inducing less tissue reaction, volume - stability, non - antigenicity, and being non - migratory (4). In recent years, different bulking agents were used for endoscopic correction of VUR and some of them became very popular (3-6). A study reviewing multi - institutional data showed that the amount of injection material has increased over time to improve success rates, although they resulted in higher treatment costs (7). We noticed an opposite trend in our center, since we started using a new bulking agent. The aim of this study was to evaluate progression of our practice with different bulking agents.

PATIENTS AND METHODS

We reviewed the hospital records of VUR patients who had undergone subureteric injection with dextranomer hyaluronic acid (DxHA) and polyacrilate polyalcohol copolymer (PPC) in our institution between 2005 and 2014. Data including patient demographics, injected material volumes, VUR types (primary or secondary), and VUR grades according to the pre - and postoperative voiding cystourethrograms (VCUG) and success rates were similarly recorded. Variation of different parameters throughout the study period was evaluated along with the success rate. Reflux was classified according to the International Reflux Study Committee's Classification System. The procedure was performed under general anesthesia using 8 Fr 6° cystoscope (Storz®, Tutlingen, Germany). Subureteric injection either with Polyacrilate polyalcohol copolymer (PPC) (Vantris®, Promedon, Argentina) or DxHA (Dexell İstem Medikal, Turkey) was administered slowly using a Williams cystoscopic injection needle (Cook Medical, Bloomington, USA) submucosally at the 6 o'clock position of the ureteral orifice until creating a prominent bulge. Evaluation and management of bladder dysfunction was completed before the injection procedure in secondary reflux cases. Success was defined as complete resolution of reflux in VCUG obtained at least three months after the injection. Injection was repeated if persistent reflux above grade 1 was documented. Ultrasonography was performed at the postoperative first, third, and sixth months, and then annually for follow-up of obstructive findings like new onset or increasing hydronephrosis. Statistical analysis was carried out with the SPSS statistical package (SPSS for windows V.16, SPSS, Chicago, IL, USA) and Pearson Chi - square, Mann - Whitney U, Kruskal Wallis tests as required.

RESULTS

A total of 260 patients including 71 patients with 101 refluxing units in DxHA group and 189 patients with 283 refluxing units in PPC group were included in the study. VUR was primary in 73.3% and secondary (bladder - sphincter dysfunction) in 26.7% in DxHA group and primary in 79.9% and secondary in 20.1% in PPC group. Number of patients, mean ages, number of refluxing units, mean injected volumes and success rates are summarized in Table-1. There was no statistically significant difference between groups regarding reflux type, gender, and reflux grade. However, mean injected volume was significantly lower in PPC group ($p < 0.05$). The success rate was higher in PPC group compared to DxHA group ($p < 0.05$, Mann Whitney U test).

In PPC group, which documented a significantly higher success rate, we analyzed the relation between the success rate and the amount of injection material throughout the years of our practice. There was no statistically significant difference between the years regarding distribution of VUR grades, body weight, heights, and age at operation ($p > 0.05$, Kruskal Wallis test) (Table-2). The mean duration of follow-up was 37.9 ± 18.7 months. Overall reflux resolution rate with initial injection using PPC was 91.2% and increased to

Table 1 - Comparison of PPC and DxHA groups.

	PPC	DxHA	p
Number of Patients (G/B) *	189 (111/78)	71 (44/27)	
Mean Age (Years)	4.8 ± 3.8	6.6 ± 3.7	p<0.05***
Number of Refluxing Units (P/S) **	283 (226/57)	101 (74/27)	
Mean Injected Volume (mL)	0.63 ± 0.46	0.97 ± 0.47	p<0.05***
Success (at first injection)	90.5%	62.4%	p<0.05***

***G/B =** Girls/Boys; ****P/S =** Primary/Secondary; ***Mann Whitney U

Table 2 - VUR grade distribution through the years in PPC group.

Years	VUR Grades (PPC Group)					
	1	2	3	4	5	Total
2009	1	5	3	11	1	21
2010	1	8	21	22	14	66
2011	6	8	24	14	16	68
2012	0	7	13	12	9	41
2013	2	9	16	9	9	45
2014	7	5	16	9	5	42
Total	**17**	**42**	**93**	**77**	**54**	**283**

92.4% after repeat injections. Reflux resolution rate and mean injected volumes through the years are summarized in Table-3.

Besides the significant difference between PPC and DxHA groups, mean injected material volume was 0.64 mL per ureter in PPC group; however, it gradually decreased in the study period. Mean injected volume per year decreased from 0.81 mL in the first year to 0.26 mL in the last year of the study period (67.8% reduction). In the meantime, the success rate did not change (Figure-1) ($p > 0.05$, chi - square).

Ureteral obstruction was noted in 8 of 283 injected ureters (2.8%) in 7 patients in PPC group. Obstructions were observed at 1 day to 11 months of time intervals after injection. Four of these patients were managed with temporary double - J stenting. Open ureteroneocystostomy was performed on the other three patients who did not benefit from temporary stenting. During the open surgery a fibrous capsule surrounding

Figure 1 - The success rate did not change significantly through the years.

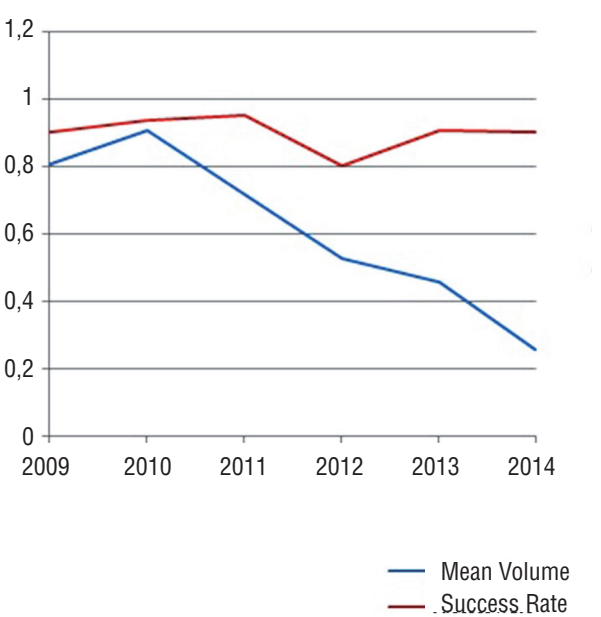

Table 3 - Reflux resolution rate and mean injected volumes through the years in PPC group.

Years	Reflux Resolution Rate	Mean Injected Volume
2009	90.5%	0.81
2010	93.9%	0.91
2011	95.6%	0.71
2012	80.5%	0.53
2013	91.1%	0.46
2014	90.5%	0.26

the substance and mild fibrosis was noted around the ureter, which did not complicate the ureteric dissection. When we retrospectively analyzed these cases, in two of them we found that there were beak sign in VCUG before the treatment that we did not appreciate it. We didn't encounter any obstruction cases in DxHA group so far.

DISCUSSION

Starting with polytetrafluoroethylene (PTFE), many bulking agents with their own advantages and disadvantages have been used for endoscopic correction of VUR (8). Among them, dextranomer hyaluronic acid (Dx / HA) is the most widely used material (7, 9). Its biodegradable nature was suggested to induce minimal inflammation. Endoscopic treatment became the most popular model for VUR especially after its approval by the FDA in 2001. Overall success rate of the Dx / HA is reported between 68 - 92% (8). Polyacrilate Polyalcohol Copolymer is a relatively new material. Short and midterm results are encouraging to use it for the treatment of VUR (10, 11). One multicenter study reported its success rate as 93.8% after the first injection (10). Our success rate of 91.2% at first injection with PPC was also satisfactory, taking into account the high number of units with grade 5 VUR (19.1%) in our series.

After 20 years of experience with other materials, we switched to PPC as a bulking agent in 2009. A retrospective review of our experience with Dx / HA and PPC revealed increased success rate with less material using PPC. Some other studies reported similar results recently (12, 13). We documented a significant reduction (to almost one fourth of the initial volume used early in our experience) in injection volume within the last 5 years of the study period. Initial mean injection volume with PPC was similar to that of the previous agent Dx / HA. We realized in time that we were obtaining a so - called "volcano - type" mound with less material, but it took us some time to stop trying to reach the habitual injection volumes acquired through earlier experience with DxHA. As we got used to this new material's different characteristics, especially its extraordinary compressibility, we gradually decreased injection volume. We admit that we injected more than necessary at the beginning of our experience with this new material. The impression that the amount of material needed was decreasing over the years led us to evaluate our outcomes. Despite this significant change in injection volume, success rate did not decrease through the years with PPC. Two previously reported studies emphasize an opposite trend with Dx / HA (7, 14). Sorensen et al. pointed out a tendency of North American surgeons who used more vials of Dx / HA to achieve success. In their study, most patients were treated with a single vial and only 11% received 3 or more vials initially; however, over time, the number of patients receiving 2 vials significantly increased and the number of cases receiving 3 vials and more tripled (36%) (7). Lee et al. reported an increase in the injected volume in the second half of their experience with Dx / HA (15). Our contrast results with PPC are probably related to the molecular features of the material, such as particle size and compressibility. Particle diameter of PPC is more than 300 µm and with this size, it is larger than most other bulking agents (16).

Vesicoureteral obstruction is a rare but serious complication of endoscopic VUR treatment. Several studies examined the possible reasons of obstruction, mainly focusing on unnoticed refluxing obstructing megaureter, technical aspects, and type of injection material. Ureteral obstruction has been encountered in 7 patients so far after endoscopic treatment in our series. Intraoperative findings of ureteroneocystostomy were consistent with congenital refluxing obstructing megaureter in 2 of them. Aaronson et al. reported 2 cases with obstruction following subureteric injection with Dx / HA, and attributed obstruction to megaureter with the distal aperistaltic segment and cautioned against endoscopic treatment for these cases (17). Obstruction has also been related to the double hit technique with PPC (18). Three of our cases with obstruction were treated with this method. Different studies reported post - injec-

tion obstructions with almost all type of bulking agents mostly of unknown etiology (19-22). Our limited experience with obstructed ureters could not reveal a relation between obstruction and injected material volume. Some recent studies documented late obstructions with PPC and Dx / HA even after 5 years (19). Long term follow-up and randomized prospective studies are necessary in order to clarify this issue.

One of the weaknesses of our study is its retrospective nature and the absence of a control group. There is also no guideline or a study describing where one should stop injecting during the procedure. Instructions defining subureteric injection usually suggest the volcano - like appearance as a goal one must achieve during injection showing the pictures of it, which is not objective at all.

CONCLUSIONS

Different bulking agents may require different injection volumes to achieve the same success rate in endoscopic treatment of vesicoureteral reflux. Polyacrilate polyalcohol copolymer is a new and effective bulking agent with different features, which ensures high success with less material. Habits gained with previous experience in terms of other materials should be revised while using a new agent.

CONFLICT OF INTEREST

None declared.

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Correspondence address:
Ali Tekin, MD.
Department of Pediatric Surgery,
Division of Pediatric Urology
Ege University Faculty of Medicine,
Ege Universitesi Tip Fakultesi Hastanesi Çocuk Cerrahisi A.D
Bornova, Izmir, Turkey
Telephone: + 90 232 390-2800
E-mail: dralitekin@gmail.com

ORIGINAL ARTICLE

Vol. 44 (6): 1200-1206, November - December, 2018
doi: 10.1590/S1677-5538.IBJU.2017.0465

The learning curve of sting method for endoscopic injection treatment of vesicoureteral reflux

Ayhan Dalkiliç [1], Göksel Bayar [2], Hasan Demirkan [3], Kaya Horasanli [3]

[1] Department of Urology, Sisli Hamidiye Etfal Training and Research Hospital, Istanbul, Turkey; [2] Department of Urology, Martyr Prof. Dr. Ilhan Varank Sancaktepe Training and Research Hospital, Turkey; [3] Department of Pediatric Urology, Sisli Hamidiye Etfal Training and Research Hospital, Istanbul, Turkey

ABSTRACT

Objective: To identify how many endoscopic injection (EI) procedures, STING method, must be performed before reaching an ideal success rate when simulation training has not been received.

Materials and Methods: The EI procedures performed by two pediatric urology fellows were investigated. The study excluded patients without primary VUR and those with previous EI or ureteroneocystostomy, lower urinary tract dysfunction, and/or duplicate ureters. The EIs used dextranomer hyaluronate and the STING method, as described by O'Donnell and Puri. Groups number was determined by multiple statistical trials. Statistically significance differences were achieved with one combination that had 35 EI procedures each and with 3 different combination of patients, having 12, 24, and 36 patients, respectively. Therefore, groups were established 12 patients. The first fellow performed 54 EIs, and the second performed 51. Therefore, each of the first fellow's three groups contained 18 EI procedures, and each of the second fellow's 17.

Results: The study included 72 patients and 105 ureter units. When the data from both fellows were combined, each of the three groups contained 35 procedures. For the first fellow, the success rates in the first, second, and third groups were 38.3%, 66.6%, and 83.3% (p = 0.02), respectively, and for the second fellow, the success rates were 41.2%, 64.7%, and 82.3% (p = 0.045), respectively. The increased success rates for both fellows were very similar.

Conclusions: An acceptable rate of success for EI may be reached after about 20 procedures and a high success rate after about 35-40 procedures.

ARTICLE INFO

Keywords:
Learning Curve; Endoscopy; Vesico-Ureteral Reflux

Int Braz J Urol. 2018; 44: 1200-6

Submitted for publication:
April 06, 2018

Accepted after revision:
August 05, 2018

Published as Ahead of Print:
September 05, 2018

INTRODUCTION

Vesicoureteral reflux (VUR) is very common, having an incidence of about 1% in all children (1). VUR is one of the causes of childhood hypertension and chronic renal failure (CRF) (2). In the approach to patients with VUR, the primary aim is not to correct the VUR but to prevent febrile urinary tract infections (UTIs) and CRF due to the formation of scar tissue caused by UTIs (3). For the majority of patients, VUR resolves without requiring any intervention; however, some patients may require surgical treatment, endoscopic injection (EI), or ureteroneocystostomy (4).

The success of an EI procedure having one or more injections is about 85%, and the factors

that reduce the success rate include the presence of a high degree of reflux, duplicate systems, and neuropathic bladder (5). In addition to these patient-linked factors, the success of the EI procedure is affected by the operator's experience (6, 7). As EI is an endoscopic procedure performed by a single operator using a single device and needle, training can be difficult (8). Therefore, ex-vivo and computer-based simulation programs have been used to increase the success rates for EI procedures (8, 9). Those without this training should conduct EI procedures only under expert observation until they have fully learned the procedure.

We hypothesized that the learning curve for EI is longer than has been expected, especially in the absence of ex-vivo or simulation training. The present study aimed to identify how many procedures were required for an operator who had had no ex-vivo or simulation training to reach an acceptable rate of success and an ideal rate of success.

MATERIALS AND METHODS

This study retrospectively evaluated 91 patients who had undergone an EI due to VUR between 2013 and 2016 at our clinic. The study excluded those patients who had not had primary VUR; those who had had previous surgical intervention or EIs for VUR; those who had duplex ureters, CRF, or bladder bowel dysfunction (BBD); and those who had not had at least a 1-year follow up. All EI procedures were completed by the same 2 pediatric urology fellows, each of whom had a 6-month rotation. During their main training periods in urology and pediatric surgery, each fellow had completed three EI procedures under supervision.

Before surgery, a medical history was taken from each patient, and all symptoms were described. In addition, a renal bladder ultrasound (RBUS), a voiding cystourethrography (VCUG), static renal scintigraphy imaging, and creatinine measurements were taken. For those without toilet training, EI was conducted in the presence of the following indications: breakthrough infections (febrile UTI in spite of continue antibiotic prophylaxis), and/or formation of new kidney scar tissue. For those who were toilet trained, EI was conducted in the case of febrile UTI or formation of new scar tissue. All EIs were performed using the STING method described by O'Donnell and Puri (10) and using dextranomer hyaluronic acid (Dx/HA) (Dexell-Vur®; Turkey). The needle was placed under the bladder mucosa about 3 mm below the affected ureteral orifice, at the 6 o'clock position, and the Dexell was injected inside the lumen until adequate mound morphology was attained. Before performing the first EI, each fellow watched at least 15 procedures being performed by a supervisor. Supervisor has not intervened to any case directly while the fellows were performing EI, because both fellows are specialist and have authority for EI. The fellows always decided the amount of material to inject and manipulated the needle in the submucosa. Three months after the EI procedure, each patient underwent a control VCUG. Even if no reflux was seen on this VCUG, each patient was followed up for at least 1 year in terms of infection. Success was defined as no reflux being seen on VCUG, no manifest hydronephrosis being seen on urinary ultrasonography both in the third month and in the first-year control and no new scar on renal scintigraphy was observed in the first-year control. Second EIs, due to unsuccessful, were performed by pediatric urology specialist, so these EIs were not included in the analysis, but first EIs were included to analyze as unsuccessful procedure.

To grade reflux, the international reflux degree system was used. Grades 1, 2, and 3 were classified as low, and Grades 4 and 5 were classified as high. Each patient's age, gender, side and degree and grade of reflux, toilet-training status, dilatation on RBUS, and scar presence on renal scintigraphy were recorded.

The study analyzed 72 patients and 105 EI procedures after exclusion. To identify the ideal number of patients that indicated statistical significance, we placed the patients in a different number of groups. For instance, the 105 EI procedures were placed in groups of 5, 7, 10, 15, 21, and 35, and the 72 patients were placed in groups of 6, 9, 12, 18, 24, and 36. Statistically significance differences were achieved with one combination that had 35 EI procedures each and with 3 different

combination of patients, having 12, 24, and 36 patients, respectively. Therefore, for each fellow (Fellow 1 and Fellow 2), 3 groups were established (Groups 1, 2, and 3), each of which contained 12 patients. For each fellow, the number of EIs performed was divided into 3 groups based on chronological order, with each group containing an equal number of cases. Fellow 1 performed 54 procedures, and Fellow 2 performed 51. Therefore, each of Fellow 1's 3 groups contained 18 procedures, and each of Fellow 2's 3 groups contained 17. This means that the sum of the procedures in Fellow 1's Group 1 and Fellow 2's Group 1 equaled 35, the sum of the procedures in their Group 2s equaled 35, and the sum of the procedures in their Group 3s equaled 35. All patients were evaluated together in terms of demographic and basic information, operation success, and the amount of material injected. In addition, success rates and the amount of material injected were calculated separately for each fellow.

Statistical analysis used the Kruskal-Wallis, one-way analysis of variance (ANOVA), and chi-square tests, and p values less than 0.05 were accepted as statistically significant. All procedures were conducted in accordance with the ethical standards of the institutional and/or national research committees and with the 1964 Helsinki Declaration and its later amendments or with comparable ethical standards. The authors conformed ethic rules of Committee on Publication Ethics and the International Committee of Medical Journal Editors. Human Research Ethics Committee and Institutional Review Board approvals were obtained from Ankara Training and Research Hospital committees. Informed consent was not obtained due to the retrospective nature of the study.

RESULTS

The study included 72 patients and 105 ureter units. The EIs were unilateral in 39 patients and bilateral in 33 patients (66 ureter units). Median age was 7 (1–15) years, there were 22 males (30.5%) and 50 females (69.5%), and there were 77 (73.3%) low-grade reflux ureters and 28 (26.7%) high-grade ones. Of the 105 procedures, 66 (62.8%) were successful. Of the 39 unsuccessful ureter units, 34 received a second injection. Of these 34 second injections, 25 were successful. Each of the 9 ureters that experienced failure of the second injection and the 5 ureters that experienced failure of the first injection and did not undergo a second one received a ureteroneocystostomy procedure. The median follow-up time was 2.5 years (1-4). No major complications (ureterovesical junction stenosis, sepsis, etc.) were observed.

Age, gender, presence of renal scarring, grade, degree of reflux (low or high), side (right or left), laterality (uni- or bilateral) and toilet-training status were similar in all three groups (Table-1). Grade of reflux were similar according to groups for each fellow (Table-2).

Success rates for the EI procedures clearly differed among groups. In the first group, the success rate was 40%; in the second group, it was 65.7%; and in the third group, it was 82.8%. The difference among these groups was statistically significant (p = 0.001) (Figure-1). The mean amount of material used for an EI was 0.48 cc in the first group, 0.92 cc in second, and 0.87 cc in the third (p = 0.011) (Figure-2). Post-hoc analysis showed that the mean amount of material used in the first group was significantly lower than the mean used in the second and third groups, which did not differ significantly from each other.

The total success rates for Fellow 1 and Fellow 2 were 62.9% and 62.7%, respectively. The difference between these two rates was not statistically significant. When the data for each fellow were analyzed by group, the success rates for Fellow 1 were 38.3%, 66.6%, and 83.3% (p = 0.02), and those for Fellow 2 were 41.2%, 64.7%, and 82.3% (p = 0.045) (Figure-1). For both fellows, the amount of material injected was lower in the first group than in the second and third groups (the p value for Fellow 1 was 0.119; for Fellow 2, it was 0.134). The differences among groups were not statistically significant, nor was the difference between fellows (Figure-2).

DISCUSSION

The STING method was first described in 1984 by O'Donnell and Puri (10), and for many years, it was the standard technique used for EI.

Table 1 - Descriptive analyze of the studied population (*: p value is significant under 0.05).

	All	1st Group	2nd Group	3rd Group	p value
Patient/ EI number (n)	72/105	23/35	25/35	23/35	
Female/ Male	74/31	23/12	22/13	29/6	0.121
Median age (years) (min-max)	7 (1-15)	7.5(2-15)	6(1-15)	7(2-14)	0.807
Right/ Left	42/63	14/21	15/20	13/22	0.935
Uni/ Bilateral	39/66	13/22	15/20	11/24	0.702
Low/ High Grade	77/28	27/8	24/11	26/9	0.675
Grade 1/2/3/4/5	5/24/48/13/15	1/10/16/3/5	2/7/15/5/6	2/7/17/5/4	0.971
Renal scar presence (no/ minimal/ extensive)	50/28/27	17/8/10	18/6/11	15/14/6	0.087
No toilet trained / trained patients nu	30/42	11/13	9/15	10/14	0.842

Table 2 - Number of ureter units according to grade of reflux for each fellow.

	1st Fellow				2nd Fellow			
Grade	1st Group	2nd Group	3rd Group	P value	1st Group	2nd Group	3rd Group	P value
1	0	1	2	0.706	1	1	0	0.915
2	5	3	3		5	4	4	
3	8	8	8		8	7	9	
4	2	2	4		1	3	1	
5	3	4	1		2	2	3	

In 2004, Kirsch et al. described a modified STING technique called the hydrodistension implantation technique (HIT) (11). Four years later, a double HIT method was described by authors from the same clinic (12). Systematic reviews have demonstrated that the HIT method has better outcomes than the STING method; however, long-term results from randomized prospective studies are needed (13). For the sake of consistency, the present study did not include patients on whom the HIT or double HIT methods had been used but included only those on whom the STING method had been used.

Dx/HA is the only molecule with FDA permission to be used in EI, and it has become the gold standard molecule for demonstrating the success of endoscopic treatment of VUR (14). In a study comparing three different injection materials (collagen, polydimethysiloxane, and Dx/HA), Dx/HA's success rate after one injection was found to be clearly superior to that of the other

Figure 1 - Variation in EI success rates of all patients according to groups.

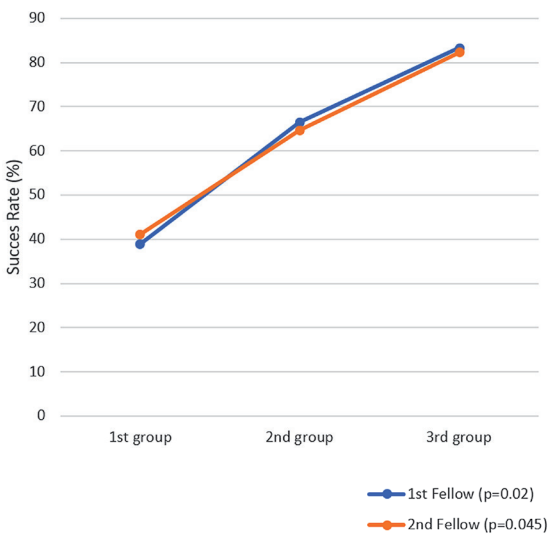
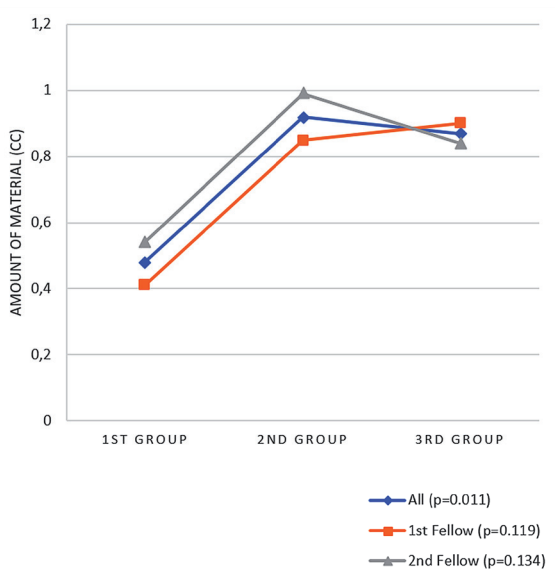


Figure 2 - Variation in mean material injected per ureter unit according to groups.



two molecules (15). For EI procedures, our clinic uses Dexell-Vur® containing Dx/HA of 80-120 µm in size. The success of a second injection after a first unsuccessful injection has been found to be 68%, and the success of a third injection after a second unsuccessful injection has been noted as 34% (5). Factors increasing the failure rate include duplicate ureters and BBD (16, 17). In the present study, the learning curve experienced by operators was the only factor affecting EI success; therefore, the study did not include injections performed after the first one, duplicate systems, and patients with BBD.

Studies related to Dx/HA have shown that the success rate after the first injection varies from 67.5-81.5% (5, 18-20). However, in patients in whom reflux is not observed on a postoperative VCUG, VUR is still observed in the long-term at rates from 13-21% (21). As a result, the success rates reported in the literature may be higher than they should be. We performed a VCUG three months after surgery and again one year after surgery, and we believe that evaluating success at the end of one year accurately reflects the success rate.

Kirsch et al. (22) demonstrated that success rates were 60% in the first 20 and 80% in the last 20 on 292 procedures. They concluded that the success rate was directly related to the learning curve. In the present study, the success rates in the first, second, and third groups were 40%, 65.7%, and 82.8%, respectively. Therefore, the success rate in the second group, 65.7%, was close to what Kirsch et al. noted as an acceptable success rate. The clearly higher success rate for the third group showed that the learning curve for the EI procedure was longer than expected (about 20 procedures) and that the success rate approached the ideal only near the end of this learning curve. Considering that both fellows involved in the study had performed fewer than 5 EIs during training, the number of EIs necessary to reach an acceptable success rate can be calculated as about 20. Each fellow performed another 17 or 18 and 17 EI procedures in their second groups; therefore, the success rate increased again after 35-40 EIs, and the ideal rates were obtained for the procedures performed in the third groups.

One factor affecting the success of an EI procedure is the amount of material injected. According to one study, Dx/HA injections of less than 0.8 mL had success rates of 31.8%, and those of 0.8 mL or more had success rates of 78.9% (7). Some studies of Dx/HA have injected mean amounts of material ranging from 0.9-1 cc in all patients, except for those with ureters suffering

high-grade reflux, to whom 1.3 cc was administered (18, 23). In the present study, the mean amount of material used was 0.48 cc in the first group, 0.92 cc in the second group, and 0.87 cc in the third group. We believe that the second group used the mean amount of material that is required for an ideal success rate. When the data for both fellows were compared, it was observed that the amount of material injected by both fellows increased significantly from the first group to the second group and that the difference between the second group and the third group plateaued. We have talked with the fellows about mean injection material changing, they said that they were afraid of iatrogenic ureterovesical junction obstruction, so they have thought that we can do re-injection in case of failure but iatrogenic ureterovesical junction failure is a more complicated issue. This may be the explanation of low material amount of first 20 cases. In the first 20 cases, they may have placed the needle into the submucosa fairly close to the ureterovesical line or may not have placed the needle deep enough. After about 20 procedures, they learned to make the injections using accurate placement and depth, both of which are needed to provide sufficient space for the material injected. It was noted that although the success rates differed significantly between the second and third groups, the amount of material injected did not. This supports our belief that learning the correct angle and axis is what increased the success rates. We believe that the fellows learned accurate placement after about 20 procedures but did not learn the correct angle and axis until after about 35–40 procedures.

There are several reasons why the learning curve for EI is longer than has been expected. First, EI is an endoscopic procedure, so learning is based solely on sight. Other factors that make learning difficult are the lack of tactile feedback when inserting the needle and the lack of knowledge regarding limits on the amount of material. While working on their first groups, both fellows may have injected less material because they were concerned about the risk of hydronephrosis. Additionally, we must accept that there is a difference in endoscopic training between urologist and pediatric surgeon. In the study, we have shown EI success is similar between urologist and pediatric surgeon regardless endoscopic training background.

The retrospective nature of this study and the fact that it included data from only two operators may be considered limitations. However, it must be remembered that a learning-curve study that has a prospective research design carries the risk of bias. In the future, studies that include more fellows would provide more accurate data.

CONCLUSIONS

Operators who have had no ex-vivo or simulation training may obtain acceptable success rates for EI procedures after about 20 procedures. It may be beneficial for operators learning this procedure by performing it to be observed by an experienced operator for the first 20 procedures. In addition, after 35-40 EI procedures, success rates reach high levels.

CONFLICT OF INTEREST

None declared.

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Correspondence address:
Göksel Bayar, MD
Emek mah, Mehmet akif cad.
Sancaktepe eğitim ve arastirma hastanesi
Sancaktepe, Istanbul
Telephone: + 90 531 584-9749
E-mail: goxelle@yahoo.com

ORIGINAL ARTICLE

Vol. 44 (6): 1207-1214, November - December, 2018
doi: 10.1590/S1677-5538.IBJU.2018.0401

Pelvic floor electromyography and urine flow patterns in children with vesicoureteral reflux and lower urinary tract symptoms

Lida Sharifi-Rad [1, 2], Seyedeh-Sanam Ladi-Seyedian [1], Hossein Amirzargar [1], Abdol-Mohammad Kajbafzadeh [1]

[1] Pediatric Urology and Regenerative Medicine Research Center, Children's Medical Center, Pediatric Center of Excellence, Tehran University of Medical Sciences, Tehran, Iran; [2] Department of Physical Therapy, Children's Medical Center, Pediatric Center of Excellence, Tehran University of Medical Sciences, Tehran, Iran

ABSTRACT

Objective: To determine the different urine flow patterns and active pelvic floor electromyography (EMG) during voiding in children with vesicoureteral reflux (VUR) as well as presenting the prevalence of lower urinary tract symptoms in these patients.
Materials and Methods: We retrospectively reviewed the charts of children diagnosed with VUR after toilet training from Sep 2013 to Jan 2016. 225 anatomically and neurologically normal children were included. The reflux was diagnosed with voiding cystourethrography. The study was comprised an interview by means of a symptom questionnaire, a voiding diary, uroflowmetry with EMG and kidney and bladder ultrasounds. Urine flow patterns were classified as bell shape, staccato, interrupted, tower and plateau based on the current International Children's Continence Society guidelines.
Results: Of 225 children with VUR (175 girls, 50 boys), underwent uroflowmetry + EMG, 151 (67.1%) had an abnormal urine flow pattern. An active pelvic floor EMG during voiding was confirmed in 113 (50.2%) children. The flow patterns were staccato in 76 (33.7 %), interrupted in 41 (18.2%), Plateau in 26 (11.5%), tower in 12 (5.3%) and a bell shape or normal pattern in 70 (31.5%). Urinary tract infection, enuresis and constipation respectively, were more frequent symptoms in these patients.
Conclusions: Bladder/bowel dysfunction is common in patients with VUR that increases the risk of breakthrough urinary tract infections in children receiving antibiotic prophylaxis and reduces the success rate for endoscopic injection therapy. Therefore investigation of voiding dysfunction with primary assessment tools can be used prior to treating VUR.

ARTICLE INFO

Keywords:
Vesico-Ureteral Reflux; Lower Urinary Tract Symptoms; Urinary Bladder

Int Braz J Urol. 2018; 44: 1207-14

Submitted for publication:
June 20, 2018

Accepted after revision:
August 05, 2018

Published as Ahead of Print:
September 25, 2018

INTRODUCTION

The association between vesicoureteral reflux (VUR) and lower urinary tract (LUT) dysfunction especially in older children who present with urinary tract infection (UTI) after toilet training, is well known. The incidence of LUT dysfunction in children with VUR was

reported from 18% to 75% (1). In addition, VUR is diagnosed in one third of UTI patients (2). The overactive bladder (OAB) and dysfunctional voiding (DV) have been described in conjunction with VUR (1). Increasing of intravesical bladder pressure in these conditions can be responsible for the development of VUR in affected patients. It is notable that spontaneous resolution of VUR after treatment of bladder dysfunction has been observed in most of the patients who were diagnosed with VUR after toilet training (2).

It has been found in the recent studies that some children with VUR have been diagnosed with abnormal urodynamic findings including OAB during filling phase and the increased activity of external urethral sphincter during voiding phase (1). Additionally, it has been reported that spontaneous resolution of VUR and cure rate following endoscopic treatment in children with LUT dysfunction and VUR are less than in children with VUR without LUT dysfunction (3-6). Therefore, an undiagnosed and untreated underlying LUT dysfunction can be a considerable cause of surgical failure (7). History taking and physical examination are the hallmark diagnostic tools for evaluation of LUT dysfunction in children. However repeated urine flow studies in conjunction with electromyography (EMG) can also help to confirm diagnosis of different LUT conditions in toilet trained children (8).

The aim of the present study was to evaluate the different urine flow patterns and active pelvic floor EMG during voiding in children who had VUR after toilet training as well as presenting the prevalence of LUT symptoms in these patients.

MATERIALS AND METHODS

After institutional review board approval, we retrospectively reviewed the charts of all patients diagnosed and were treated with VUR after toilet training at our center from Sep 2013 to Jan 2016. A total of 225 anatomically and neurologically normal children were enrolled in the study. Children were selected from patients diagnosed with VUR who had been visited at outpatient pediatric urology clinic at Children's Medical Center, Tehran, Iran. The patients were between 5 to 11 years old. The diagnosis of VUR was confirmed through voiding cystourethrography (VCUG) and was graded according to the International Reflux Study in Children grading system (9). Children with neuropathic bladder, anatomic defects and a history of diagnosis of VUR at birth or early in life before toilet training were excluded from the study. Urine flow patterns, voided volume, maximum urine flow rate (Qmax), average urine flow rate (Qave), presence or absence of EMG activity during voiding phase, and post void residual (PVR) volume were evaluated. Moreover, prevalence of different LUT symptoms was determined.

Evaluations

All participants underwent complete urological work - up as well as physical and neurological evaluations. The study evaluations included urine analysis and urine culture to assess UTI, a voiding diary, a dysfunctional voiding symptom score, uroflowmetry (UF) with EMG, VCUG, kidney and bladder ultrasounds to evaluate upper urinary tract, hydronephrosis, anomalies, bladder capacity and PVR. Patients who had febrile UTI or hydronephrosis on ultrasound underwent VCUG. Expected bladder capacity was measured with regard to International Children's Continence Society (ICCS) recommendation, using the formula mL = [(age in years + 1) × 30] (8).

A filled out LUT symptom and bowel habit questionnaire was obtained from all participated patients / parents to assess any associated LUT symptom, bowel habits, and other associated clinical manifestations. For any included patients, we noted and recorded gender, age, abdominal / flank pain, a history of UTI with or without fever, daytime incontinence, enuresis, urgency, infrequent voiding and / or holding maneuver and bowel dysfunction (constipation alone, fecal soiling alone or constipation plus fecal soiling). Enuresis was defined as wetting the bed at least once a week (10). Constipation was defined with regard to Rome III criteria (11). At least two UF / EMG in separate sessions were performed on all patients in accordance with the ICCS recommendations (8). Any medication

with potential influence on bladder function was discontinued a week prior to performing of UF / EMG. Moreover, bladder ultrasound was performed to assess bladder capacity, PVR volume, the appearance of bladder wall and bladder neck, dilatation of lower ureteral and assessment of rectum for presence of a large stool mass. A written informed consent was obtained from participants.

Urine flow patterns definition

With respect to the ICCS uroflow classification, the uroflow patterns were categorized into bell - shaped, staccato, plateau, interrupted, and tower shaped curves. The ICCS defines an interrupted - shaped curve as discrete segments of urine flow, separated by segments with zero flow. A staccato - shaped curve is defined as irregular and fluctuating during voiding but the flow is continuous and never reaches zero during voiding. A plateau - shaped curve is defined as a flattened and low - amplitude prolonged flow. Tower - shaped curve is defined as a sudden, high - amplitude curve of short duration and normal shape curve is defined as continues bell - shaped curve.

Uroflow / EMG

All UF / EMG studies were performed with the use of a flowmeter with a spinning disc transducer (Tempo, Medtronic Urology, Skovlunde, Denmark) with respect to the ICCS guidelines and on strong need to void. Children voided in their normal position; relaxed posture with upper legs in a horizontal position and use of feet support for smaller children. To record pelvic floor EMG activity during voiding, two integrated biosensor EMG electrodes were placed on the perineum at 3 and 9 o'clock positions. The machine had a high quality audio monitor to recognize motor recruitment activity from activity caused by electrical artifact, such as wire movement or wetting of the electrodes. Children underwent at least two UF / EMG studies in separate sessions. All UF / EMG were done in a calm and equal environment. For each child, data regarding Qmax, Qave, voided volume, flow time and voiding time, as well as EMG activity during voiding were recorded.

Statistical analysis

The Statistical Package of Social Science software (version 18; SPSS, Inc., Chicago, IL) was used for statistical analysis. Categorical data were reported as frequencies and percentages. Continuous data were reported as range and mean ± standard deviation (SD). To analyze data, chi - square or student t - test was executed. A P value of < 0.05 was considered statistically significant.

RESULTS

Study population

Among 463 patients with VUR who were assessed for eligibility, 238 patients with neuropathic bladder, anatomic defects and a history of diagnosis of VUR at birth or early in life before toilet training were excluded; only 225 children met inclusion criteria and were enrolled in the study. The data of 175 girls and 50 boys (mean age: 7.1 ± 2 years, range: 5 to 11) diagnosed with VUR (128 unilateral cases, 102 bilateral cases) were analyzed.

UF / EMG outcomes

Abnormal urine flow patterns were observed in 151 (67.1%) patients. The flow patterns were staccato in 76 (33.7%), interrupted in 41 (18.2%), plateau in 26 (11.5%), tower in 12 (5.3%) and a bell shape or normal pattern in 70 (31.5%) (Table-1).

An active pelvic floor EMG during voiding was confirmed in 113 (50.2%) children. An active pelvic floor EMG during voiding was observed in 69 (61%) patients with a staccato urine flow pattern, 23 (20.3%) patients with an interrupted urine flow pattern, 15 (13.2%) patients with a plateau urine flow pattern and 6 (5.3%) patients with a tower curve. Overall 38 patients (25%) with a staccato, interrupted, tower and plateau urine flow patterns had a quiet pelvic floor EMG during voiding (Table-1).

Mean Qmax and mean Qave were 18.6 ± 8.5 (range: 6.3 to 45) and 9.2 ± 4.3 (range: 2 to 23) mL / sec, respectively. Mean voided volume

Table 1 - Characteristics and urine flow patterns of all patients.

	Patient n. (%)			Urine flow patterns		
	Total	**Normal (%)**	**Staccato (%)**	**Interrupted (%)**	**Plateau (%)**	**Tower (%)**
Age (yr), mean ± SD (range)	7.1± 2 (5-11)	6.8±1.8 (5-11)	7.3 ± 2.1 (5-11)	7.6±2 (5-11)	6.6±2 (5-11)	6.4 ± 1.7 (5-10)
Female (%)	175	54 (30.8%)	62 (35.4%)	34(19.4%)	14 (8%)	11(6.2%)
Male (%)	50	16 (32.6%)	14 (28.5%)	7 (14.2%)	12 (24.4%)	1(2%)
Total	**225**	**70 (31.5%)**	**76 (33.7%)**	**41(18.2%)**	**26 (11.5%)**	**12 (5.3%)**
Bilateral VUR§	102	28 (27.4%)	38 (37.2%)	20 (19.6%)	12 (11.7%)	4 (3.9%)
Unilateral VUR§	128	43 (33.5%)	40 (31.2%)	22 (17.1%)	15 (11.7%)	8(6.2%)
EMG¥ activity during voiding	113	0 (0%)	69 (61%)	23 (20.3%)	15 (13.2%)	6(5.3%)

§ = vesicoureteral reflux; **¥ =** electromyography

was 203 ± 109 mL (range: 70 to 620) and mean PVR volume was 25.5 ± 22.8 mL (range: 0 to 105). Also, mean voiding time was 24.7 ± 14 sec (range: 6 to 76). Mean PVR volume was significantly higher in patients who had abnormal urine flow pattern in comparison with patients who had normal flow curve (32.3 ± 27.6 mL vs. 17.3 ± 5 mL, $P < 0.04$). There was no significant difference in UF measures between children who had abnormal urine flow pattern with and without positive EMG activity during voiding (Table-2). Of UF measures, mean voided volume in children with interrupted flow curve was significantly higher than in children with other curves ($P < 0.05$). Additionally, mean Qmax and mean Qave were significantly lower in children with plateau flow curve in comparison to children with normal curve ($P < 0.05$).

Among different grades of VUR, grades II and III were more frequent in ureters of patients with and without abnormal urine flow pattern (Table-3).

We reanalyzed the data of two different age groups (5 - 7 versus 8 - 11 years of age). There were 144 patients (64%, 26 boys and 118 girls) in the age range of 5 to 7 years old and 81 patients (36%, 23 boys and 58 girls) in the age range of 8 to 11 years old. There is no significant difference between the two age groups according to normal and abnormal urine flow patterns and EMG activity during voiding.

Prevalence of lower urinary tract symptoms

Urinary tract infection, enuresis and constipation, respectively were more frequent features in patients with VUR. Of 225 children, 201 (89.3%) had a UTI history of whom 187 (93%) had at least 1 febrile UTI history. Constipation and enuresis were reported in 56 (24.8%) and 66 (29.3%) patients, respectively. In addition, 34 (15.1%) patients had urgency and 35 (15.5%) patients had daytime incontinence. Details of prevalence of LUT symptoms in VUR patients who had different urine flow patterns are shown in Table-4.

DISCUSSION

There is a known association between VUR and LUT dysfunction in older children after toilet training. However, the exact relationship and natural history between VUR and LUT dysfunction are controversial. Prior studies have reported that children with LUT dysfunction and VUR have a higher frequency of UTIs (1). Moreover, they have a lower cure rate following endoscopic treatment (1, 12). One of the primary causes of surgical failure which prevents resolution of VUR in affected patients is an untreated underlying LUT condition (12). With respect to the association between LUT dysfunction and VUR, some authors have suggested that all children with VUR should be evaluated for abnormal urodynamic findings (7, 13).

UF with / without EMG is frequently used as a simple and non - invasive method to assess LUT function in pediatric urology practice. The appearance of the urine flow pattern accompanied with clinical neuro - urological evaluations, voiding diary and assessment of PVR through bladder

Table 2 - Comparison of uroflowmetry measures and post-void residual volume between patients with normal and abnormal urine flow patterns.

Urine flow pattern	Voided volume (mL)	Maximum urine flow rate (mL/sec)	Average urine flow rate (mL/sec)	Voiding time (s)	Residual volume (mL)
Bell shape	190 ± 113	20.6 ± 7.6	10.9 ± 4.2	23.5 ± 14	17.3 ± 5
Staccato	194 ± 117	20.1 ± 10.3	8.4 ± 4.1	21.7 ± 9.3	29.8 ± 24.8
P*	0.581	0.839	0.054	0.623	0.167
Bell shape	190 ± 113	20.6 ± 7.6	10.9 ± 4.2	23.5 ± 14	17.3 ± 5
Interrupted	265 ± 87	15.1 ± 6.4	8.3 ± 4.4	27.7 ± 18.6	45 ± 44
P*	0.540	0.014	0.082	0.455	0.332
Bell shape	190 ± 113	20.6 ± 7.6	10.9 ± 4.2	23.5 ± 14	17.3 ± 5
Plateau	209 ± 74	12.4 ± 5.3	6.5 ± 3.6	28.6 ± 14.3	30.8 ± 13.6
P*	0.351	0.01	0.02	0.450	0.299
Bell shape	190 ± 113	20.6 ± 7.6	10.9 ± 4.2	23.5 ± 14	17.3 ± 5
Tower	133 ± 51	34.9 ± 6.6	14.6 ± 5.3	16.9 ± 6.4	9.2 ± 4
P*	0.053	0.034	0.014	0.04	0.04

Mean±SD; * Student t test

Table 3 - Lower urinary tract symptoms in different flow groups of patients.

	Patient n. (%)			Urine flow patterns		
	Total	Bell shape	Staccato	Interrupted	Plateau	Tower
Urinary tract infection n.%	201	67(34.8%)	72(35.8%)	32(16.6%)	21(10.9%)	9(4.4%)
Constipation n.%	56	20(35.7%)	18 (32.1%)	9(16%)	5(8.9%)	4(7.1%)
Daytime incontinence n.%	35	2(5.7%)	19(54.2%)	6(17.1%)	3(8.5%)	5(14.2%)
Nocturnal enuresis n.%	66	19(28.7%)	25(37.8%)	9(13.6%)	7(10.6%)	6(9%)
Urgency n.%	34	3(6.8 %)	12(35.2%)	4(11.7%)	3(6.8%)	12(35.2%)
Holding maneuver n.%	56	2(3.5%)	29(51.7%)	19(21.4%)	6(10.7%)	0

ultrasound, can often provide enough information to decide the diagnosis and management (12). Mostly prior studies have used invasive urodynamic evaluation to assess patients with VUR. This study was undertaken to evaluate urine flow curves and measures in children who had VUR after toilet training by non - invasive UF / EMG. Additionally, the prevalence of LUT symptoms in these patients was determined. The results showed an abnormal urine flow pattern in 67.1% of patients. More than 50% of patients had a positive EMG activity during voiding. A staccato urine flow curve was seen in 33.7% of patients and also 18.2% of patients had interrupted urine flow curve. Only, 31.5% of patients had a normal urine flow curve. In addition, UTI, enuresis and constipation respectively, were more frequent manifestations in patients with VUR.

As regard to abnormal urine flow pattern, staccato and interrupted curves were more frequent in our patients. According to the ICCS guidelines, staccato urine flow pattern presents DV and interrupted pattern shows underactive bladder (8). Each condition involves urinary stasis and can

Table 4 - Grade of VUR in different urine flow patterns.

	VUR grade I (unit)	VUR grade II (unit)	VUR grade III (unit)	VUR grade IV (unit)	VUR grade V (unit)
Bell shape	4	35	52	10	1
Staccato	5	49	46	17	3
Interrupted	4	21	27	6	3
Plateau	0	12	21	3	0
Tower	0	5	12	3	2

be associated with high storage volume. Functional bladder outlet obstruction in children with LUT dysfunction can cause voiding difficulty and storage symptoms as a consequence of bladder changes secondary to obstruction (8). Accordingly, intravesical pressure rises resulting in production and persistence of VUR (14). Ural et al. compared the clinical, demographic, urodynamic and prognostic characteristics related to VUR in 348 patients with idiopathic LUT dysfunction or DV. They reported that median maximum filling pressure was higher in the refluxing group compared to the non - refluxing group (40.0 vs. 34.0 cm H2O). Moreover, increased intravesical pressure could be the primary factor for inducing reflux in idiopathic LUT dysfunction (15).

UTI commonly occurs in children with VUR. The close association between VUR and UTI, especially febrile UTI, has been discussed in the literature (16). The results of the present study reveal that nearly 90% of our patients had a history of UTI. This finding is the most frequent feature in patients with normal and abnormal urine flow curve. Although 31.5% of our patients had normal urine flow pattern, most of these patients complained of at least a LUT symptom such as constipation and / or enuresis. On the other hand, several studies have shown correlation between VUR and bladder bowel dysfunction (BBD) (17). VUR and constipation are well - recognized co - morbidities in children with bladder dysfunction. Treating constipation on the other hand has been shown to resolve urinary symptoms such as daytime wetting, enuresis and UTI up to 90% (18). In a survey by Koff et al. the influence of functional bladder and bowel disorder on the natural history of patients with primary reflux was assessed (19). The authors evaluated 143 pediatric patients who had either spontaneous resolution of VUR or had breakthrough infection which led to surgical management. They reported the rate of BBD was higher in children with breakthrough infections (77% vs. 23%). Moreover, they concluded that BBD adversely affected the results of reimplantation and was present as a risk for recurrent UTI after resolving reflux (19).

Additionally, the less cure rate of endoscopic treatment of VUR in children with BBD in comparison with children without BBD has been reported (4, 17). The results of mentioned studies strongly support that urodynamic evaluation can provide valuable information regarding the diagnosis, treatment and prognosis of children with VUR. Concomitant treatment for bladder dysfunction including pharmacotherapy, standard urotherapy (hydration, diet, timed voiding and toilet training) and biofeedback that can effectively help to spontaneously cure VUR or to achieve optimal results of anti - reflux surgery (12, 20, 21). Van Batavia et al. described their experience using dextranomer / hyaluronic acid copolymer in the patients whose VUR persisted despite targeted therapy for their LUT condition. They reported that the highest resolution rates of VUR were seen in patients with either DV or detrusor underutilization or in patients who had least symptoms prior to injection (3).

The main limitations of this study were small sample size and single center study. In addition, this was not a prospective study and we

did not include a control group. A prospective study with a larger sample size is needed to offset these limitations.

CONCLUSIONS

The results of present study have shown that two third of patients who had VUR after toilet training present an abnormal urine flow pattern. Additionally, more than 50% of the patients had an active pelvic floor EMG during voiding. Although one third of patients had normal urine flow pattern, most of mentioned patients complained of at least a LUT symptom. Therefore, evaluation and treatment of LUT dysfunction should be an essential part of the initial assessment and management of a child with VUR. Furthermore, treatment of LUT dysfunction would be supported to postpone definite surgical correction in these patients in order to improve surgical outcome.

ABBREVIATIONS

BBD = bladder bowel dysfunction
DV = dysfunctional voiding
EMG = electromyography
ICCS = International Children's Continence Society
LUT = lower urinary tract
OAB = overactive bladder
Qave = average urine flow rate
Qmax = maximum urine flow rate
VCUG = voiding cystourethrolgraphy
VUR = vesicoureteral reflux
UTI = urinary tract infection

ACKNOWLEDGMENT

We are highly grateful to Mrs. S. Lotfi for her precise final linguistic revision of the manuscript.

CONFLICT OF INTEREST

None declared.

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Correspondence address:
Abdol-Mohammad Kajbafzadeh, MD
Pediatric Urology and Regenerative Medicine Research Center
No. 62, Dr. Qarib's St, Keshavarz Blvd
Tehran 14194 33151, Iran
Telephone: + 98 21 6656-5400
E-mail: kajbafzd@sina.tums.ac.ir

ORIGINAL ARTICLE

Vol. 44 (6): 1215-1223, November - December, 2018
doi: 10.1590/S1677-5538.IBJU.2018.0128

Artificial sphincter "BR – SL – AS 904" in the treatment of urinary incontinence after radical prostatectomy: efficacy, practicality and safety in a prospective and multicenter study

Salvador Vilar Correia Lima [1, 2], Evandilson Guenes Campos de Barros [1], Fabio de Oliveira Vilar [1], Flavia Cristina Morone Pinto [2], Thomé Décio Pinheiro Barros [1, 2], José Carlos Truzzi [3], Luiz Gustavo M. de Toledo [4], Francisco Kanasiro [5], João Luiz Amaro [6]

[1] Serviço de Urologia, Hospital das Clínicas, Universidade Federal de Pernambuco, UFPE, Recife, PE, Brasil; [2] Departamento de Cirurgia, Centro de Ciências da Saúde, Universidade Federal de Pernambuco, UFPE, Recife, PE, Brasil; [3] Departamento de Urologia, Instituto do Câncer Dr. Arnaldo Vieira de Carvalho, São Paulo, SP, Brasil; [4] Santa Casa de Misericórdia de São Paulo, São Paulo, SP, Brasil; [5] Serviço de Urologia, Hospital Santa Marcelina, Porto Velho, RO, Brasil; [6] Disciplina de Urologia, Unesp - Universidade Estadual Paulista, São Paulo, SP, Brasil

ABSTRACT

Purpose: The objective of the present study is to test the efficiency and practicality of a new artificial sphincter "BR - SL - AS - 904" in the control of urinary incontinence in post - PR patients and to evaluate their complications.

Patients and Methods: Fifteen patients with incontinence after one year of radical prostatectomy were included prospectively. All patients underwent artificial urethral sphincter (AUS) implant "BR - SL - AS - 904" according to established technique. Independent variables such as free urinary flow, PAD weight test, ICIQ - SF score and urinary symptoms through the IPSS score were compared in different follow-up moments.

Results: Patients submitted to AUS implantation did not present trans - operative or post - operative complications related to the surgical act such as: infection, hematoma, erosion or urinary retention. Device was inert to the body during the follow-up, showing an excellent adaptation of the patients, besides the easy handling. The mean age was 68.20 years 40% of the patients had systemic arterial hypertension, 6.7% diabetes mellitus, 6.7% were hypertensive and diabetic, 13.4% were hypertensive, had diabetes and hypercholesterolemia and 26.7% patients had no comorbidities. It was evidenced that the urinary flow peak during the follow-up remained stable. Decreased averages and median PAD weight test were 135.19 to 75.72 and 106.00 to 23.50, respectively. The IPSS score decreased and the quality of life increased (12.33 to 3.40 and 2.50 to 3.20 respectively). The ICQF - SF questionnaire score also showed a decrease, ranging from 16, 71 to 7.33.

Conclusion: The artificial sphincter implant "BR - SL - AS 904" was reproducible, safe and effective in the control of urinary incontinence in post - PR patients.

ARTICLE INFO

Keywords:
Urinary Incontinence; Urinary Sphincter, Artificial; Prostatic Neoplasms

Int Braz J Urol. 2018; 44: 1215-23

Submitted for publication:
March 06, 2018

Accepted after revision:
June 26, 2018

Published as Ahead of Print:
September 20, 2018

INTRODUCTION

Prostate cancer is the most frequently diagnosed non - skin cancer in the United States and the third leading cause of cancer deaths. In 2017, 1.688.780 new cancer cases and 600.920 cancer deaths were projected to occur in the United States (1). Radical prostatectomy (PR) is the currently treatment with satisfactory cancer results in patients with localized or locally advanced prostate neoplasia without major comorbidities. However, the main complication PR - associated is the involuntary loss of urine, ie, urinary incontinence (UI). After one year of persistent UI pos - PR, the artificial sphincter implant is the main treatment option.

AMS 800 artificial sphincter is currently considered the gold standard treatment for men with UI. However, this system has high complexity for the implantation of the device. It is necessary to perform a high number of procedures to obtain satisfactory rates of urinary continence associated with acceptable rates of surgical complications (2). In a retrospective study, from January 1972 to September 2015, 27.096 cases were included from the AMS 800 implants, of which 5.723 required either revision or explantation (21.1%). Younger age and penoscrotal approach were associated with higher device explantation and revision rates, while use of a tandem cuff was associated with higher explantation rates (3). In addition, another limiting factor for this type of device is that the operating mechanism is static, ie, the pressure exerted on the urethra through the cuff is constant as the patient is at rest, exercising, coughing or performing maneuvers that increase abdominal pressure (personal findings). Finally, those implants have a high economic cost making its accessibility quite limited, especially for those patients assisted in the public health system.

The BR - SL - AS 904 sphincter was developed in order to reduce the restrictions of the currently available devices, to improve the postoperative results and to make the implantation of an artificial urinary sphincter feasible and accessible.

PATIENTS AND METHODS

This was a prospective, multicenter, non-randomized trial with patients with urinary incontinence after radical retropubic prostatectomy and submitted to artificial urethral sphincter implant "BR - SL - AS - 904". The trial was carried out in accordance to the National Council of Health, the Helsinque Declaration and the Nuremberg Code for human experiment. The study is also listed on www.clinicaltrials.gov, and was approved by the National Ethics Committee in Research (CONEP # 814.933). The non - inclusion of a control group was discussed and accepted by the Ethical Committee of the Institution.

Patient eligibility criteria

Fifteen patients with moderate and severe incontinence after one year of radical prostatectomy were included prospectively. All patients underwent artificial urethral sphincter (UE) implant "BR - SL - AS - 904" according to established technique. Patients submitted to previous radiotherapy; patients with urethral stenosis or previously submitted to internal urethrotomy due to vesico - urethral anastomosis stenosis were excluded.

BR - SL - AS 904 sphincter appearances

The proposed device operating mechanism including two parts: constriction - pumping system and activating valve (Figure-1). The resting device maintains urethral compression preserving urinary continence. During pumping, the fluid present in the device is displaced from the urethral cuff to the reservoir located in the peritoneal cavity and through a flow reducing system it slowly returns to the cuff, causing it to remain deflated for about three minutes allowing urination (Figure-2).

Surgical technique and device's implant

The implant of the device was performed through two incisions: one perineal and one inguinal. In the perineal incision, a five - centimeter incision was made at the bulbar urethra level, allowing the passage of the constrictor balloon. After the cuff is passed, it is locked through the safety catch. After the perineal surgical time, an inguinal incision was made close to the external inguinal ring, dissected by anatomic planes, with communication between the inguinal and perineal incisions, parallel to the inguinal canal, forming a tunnel through which the device is carefully introduced. In the inguinal region, an

Figure 1 - Appearance of the disassembled BR-SL-AS 904 sphincter (A), consisting of the following parts: 1; 2; 4; and 5: constriction-pumping system and 3: activating valve. B) sphincter ready to be implanted after air removal by injecting 15ml of saline through the activator valve (B).

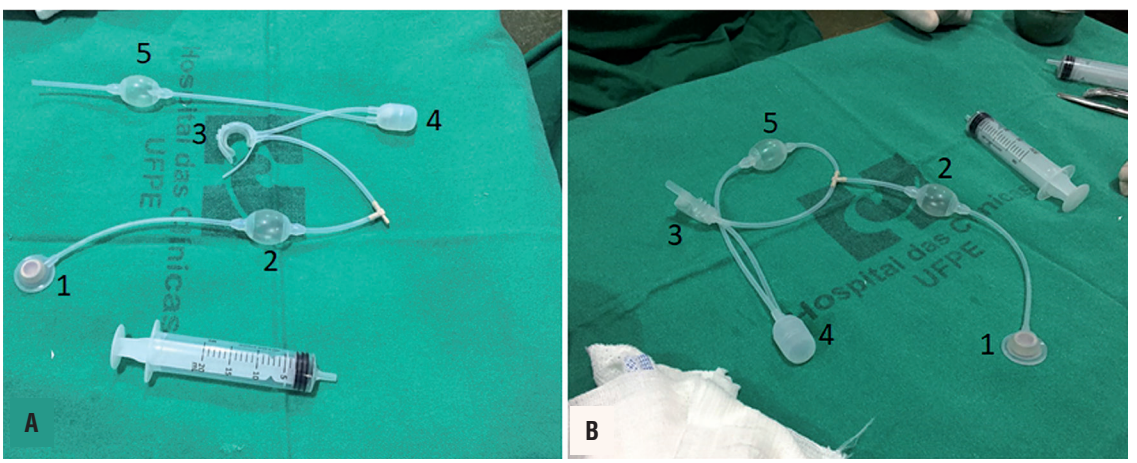
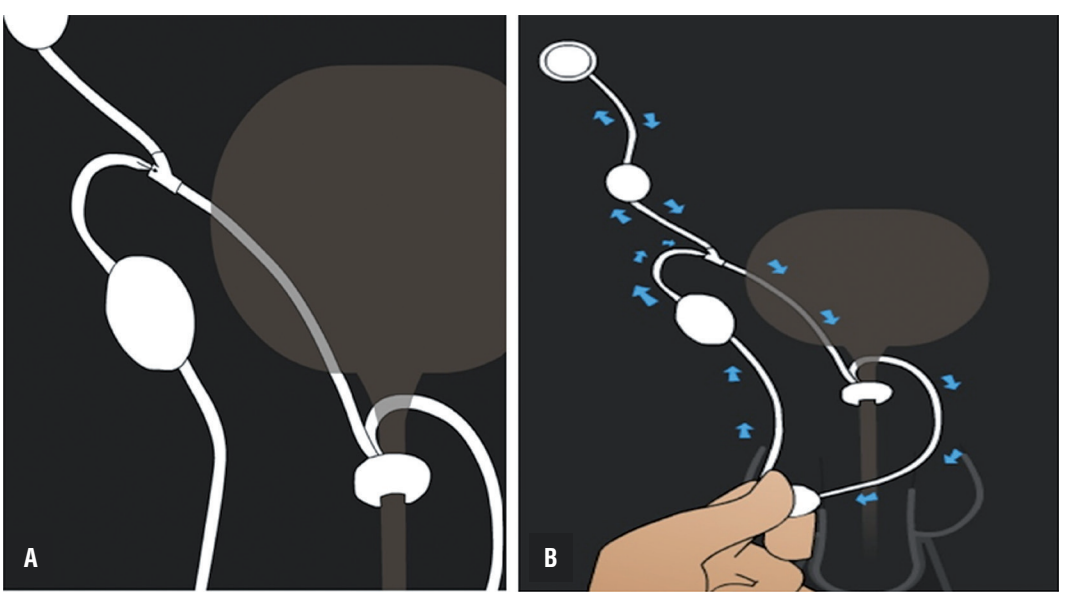


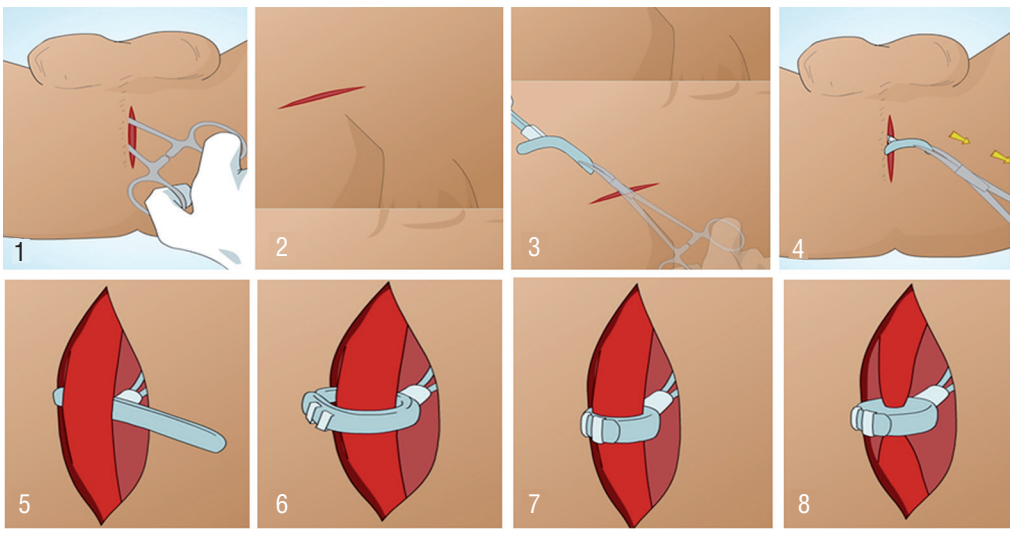
Figure 2 - A) Pressure reducing system and B) Operating system. The resting device maintains urethral compression preserving urinary continence.



(A). During pumping, the fluid present in the apparatus is displaced from the urethral cuff to the reservoir located in the peritoneal cavity and through a flow reducing system it slowly returns to the cuff, causing it to remain deflated for about three minutes allowing urination (B).

incision was made in the aponeurosis of the external oblique muscle to gain access to the Retzius space. This space is dissected carefully forming a retropubic virtual cavity, where the reservoir of the urethral sphincter would be placed. After the reservoir was allocated in the Retzius space, a new subcutaneous tunnel was made towards the patient's flank. After passage of the device through the conduit formed we carefully allocate the periurethral cuff around the bulbar urethra and lock the urethral cuff using the safety clips (Figure-3). The wound suture was performed by planes

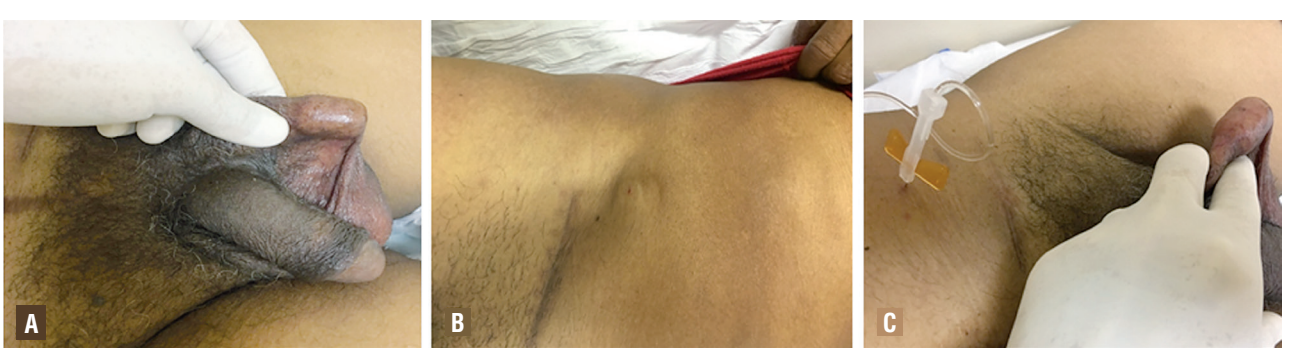
Figure 3 - Surgical technique and device implantation steps: 1-2) Perineal and inguinal incision, respectively; 3) Inguinal tunnel construction and device passage; 4) Device in the perineal region; 5-8) Periurethral cuff being activated and locked.



with vicryl 3 - 0 and skin with 4 - 0 mononylon. The surgical stitches were removed on the tenth postoperative day and the device activated on the thirtieth day by infusing 15 mL of distilled water into the system through the activation valve. After activation of the system, the patient was instructed to manually activate the pump located in the scrotal sac to start voiding (Figure-4).

independent variables: free urinary flow, PAD weight test, quality of life through ICIQ SF score; urinary symptoms through the IPSS score, all of them in the different moments of the urologist evaluations and lastly possible complications inherent to the implantation of the device such as infections, erosions and device failure.

Figure 4 - Aspect of the sphincter BR-SL-AS 904 (Pump), activation valve (B). Surgical stitches were removed on the tenth postoperative day and the device activated on the thirtieth day by infusing 15 ml of distilled water into the system by the activation valve (C).



Clinical and urological outcomes

As an independent variable, the implantation of the device was evaluated, and as

Statistical analyses

Data were analyzed descriptively and inferentially. The descriptive analysis was through

absolute frequencies in the categorical variables and the statistics: mean and standard deviation. The inferential analysis was performed through the F (ANOVA) tests for repeated measures and Friedman's test for the comparison between the evaluations. In the case of significant difference by the F test (ANOVA), multiple Bonferroni comparisons were obtained. The verification of the normality hypothesis was through the Shapiro - Wilk test. The margin of error used in the statistical test decisions was 5%. The data were tabulated in Excel® and analyzed in the SPSS (Statistical Package for the Social Sciences, IBM® statistics, version 23.0).

RESULTS

Fifteen patients underwent implantation of AUS at the three centers participating in the study. The mean age was 68.2 ± 7.5 years. In comorbidities analysis, it was observed that 40% of the patients had hypertension, 6.7% presented diabetes mellitus, 6.7% were hypertensive and diabetic patients, 6.7% had chronic renal insufficiency, 13.4% were hypertensive, diabetic and had hypercholesterolemia, and 26.7% of the patients had no comorbidities. The mean postoperative follow-up was 192.71 months. These data are described in Table-1.

Analyzing the complaints of the patients studied, it was identified that two thirds of them did not have voiding urgency, this index remained stable until the end of the study. The percentage of patients classified with the light score on the IPSS scale was 40.0% at visit 1, 46.7% after device activation and 80.0% at visit 4. The percentage of those classified as moderate had a variation of 33.3% at visit 1 to 20% at visit 4. The severe classification in the IPSS was 26.7% in the pre - op, and zero at visit 4. In the analysis of the quality of life an improvement was identified in "well and happy", as well as a decrease in "unhappy and terrible" scores during follow-up visits. Data described in Table-2.

Table-3 presents the statistics related to the different clinical and urological variables. Patient's weight and BMI remained stable during the course of the study, as well as the number of daily urinary frequency and free peak flow. It was possible to verify a statistically significant difference (p = 0.016) in the IPSS score: highest mean in the preoperative period (12.3) and the lowest in the visit 4 (3.4) (Figure-5). Although there was no statistical relevance for the other variables, it was observed an objective improvement in the scores of involuntary urinary loss and improvement of quality of life with maintenance of urinary flow force without obstructive symptoms.

The device was inert to the body throughout

Table 1 - Mean age and medical comorbidities.

Outcomes	Total
n	**15**
Age (years): mean ± SD	68.20 ± 7.56
Comorbidity	
Chronic Renal Insufficiency	1
Hypertension	6
Hypertension + Diabetes Mellitus	1
Hypertension + Diabetes Mellitus + Dyslipidemia	2
Diabetes Mellitus	1
None	4
Satisfaction rate	
Unsatisfied	15
Post-surgery (weeks)	192.71 ± 100.88

Table 2 - Urinary urgency, IPSS and quality of life per urologist visit.

	Urologist visit				
	Pre-op	Visit 1	Visit 2	Visit 3	Visit 4
Outcome	n	n	n	n	n
TOTAL	15	15	15	15	15
Urinary urgency					
Yes	5	4	6	3	1
No	10	11	9	10	9
Not informed	-	-	-	2	5
IPSS					
Mild (0 - 7)	6	8	7	9	12
Moderate (8 - 19)	5	5	7	5	3
Severe (20 - 35)	4	2	1	1	-
Quality of life					
Unhapy	4	4	1	3	1
Awful	4	3	1	1	2
Discomfort	3	-	4	4	3
Generally well	1	7	3	4	3
Regular	2	1	3	1	-
Happy	-	-	2	-	1
Nor informed	1	-	1	2	5

The Outpatient Follow-up was performed every 30 days (Visit).

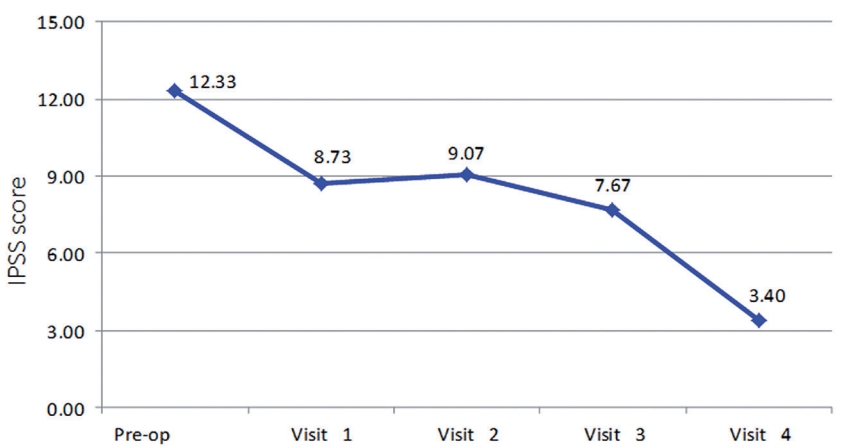
Table 3 - Statistics related to the different clinical and urological variables.

	Urologist evaluation					
Outcome	Pre-op	Visit 1	Visit 2	Visit 3	Visit 4	P-value
Weight	74.56 ± 16.06	69.50 ± 10.80	78.20 ± 17.00	76.20 ± 14.14	74.87 ± 7.33	$p^{(1)} = 0.420$
BMI	26.61 ± 4.09	25.19 ± 3.57	26.91 ± 4.24	26.96 ± 3.74	26.59 ± 3.38	$p^{(1)} = 0.487$
Daily urination	5.47 ± 2.95	4.87 ± 2.50	4.07 ± 2.76	5.85 ± 1.68	4.89 ± 1.27	$p^{(2)} = 0.206$
Urinary free flow	19.30 ± 7.44	21.89 ± 11.04	16.97 ± 7.90	16.27 ± 8.70	21.14 ± 11.35	$p^{(1)} = 0.812$
PAD test	135.19 ± 159.54	94.90 ± 77.15	162.53 ± 217.53	110.37 ± 126.90	75.72 ± 95.29	$p^{(2)} = 0.092$
IPSS score	$12.33 \pm 7.57^{(A)}$	$8.73 \pm 6.08^{(AB)}$	$9.07 \pm 7.20^{(AB)}$	$7.67 \pm 6.62^{(B)}$	$3.40 \pm 3.92^{(C)}$	$*p^{(1)} = 0.016$
Quality of life	2.50 ± 1.40	2.87 ± 1.46	3.86 ± 1.46	2.92 ± 1.32	3.20 ± 1.40	$p^{(2)} = 0.266$
ICIQ-SF	16.71 ± 2.69	14.20 ± 5.78	10.47 ± 7.1	11.47 ± 7.31	7.33 ± 7.17	$p^{(1)} = 0.126$

(*) Mean $P \leq 0.05$. (1) One way ANOVA; (2) Friedman's test. Different letter at the same line means statistically different.

the initial follow-up of the study, showing an excellent adaptation of the patients. After clarification at the device activation visit, the patients were able to handle the device without difficulties, thus demonstrating its practicality. At the second follow-up visit, four patients reported a return of urinary loss and difficulty in activating the device, probably due to a mechanical problem. After information and clarification, two patients decided to exchange for a new device, while two other patients chose to remain with it. In the trans - operatory period, it was observed a fracture of the "T" connection hoses in both exchanged devices. Those complications occurred after the fourth month of surgery. There was no complication related to the surgical procedure or the reaction between the device and the organism, due to the mechanical problem (material fatigue). At the moment, these patients are in clinical follow-up according to established protocol. This event was communicated to the ethics committee of the participating center.

Figure 5 - IPSS score per medical evaluation.



DISCUSSION

Patients undergoing radical prostatectomy (RP) may suffer of urinary incontinence (UI) mainly due to damage sustained to the distal urethral sphincter, essentially producing stress urinary incontinence. In men, the artificial urinary sphincter (AUS) is currently considered the gold standard treatment for UI (4, 5). In our study, we proposed a new device for UI treatment after RP, through the analysis of the learning curve of the device implant, and through the dependent variables such as PAD weight test, ICIQ SF score, IPSS score and main related complications.

After more than 25 years of widespread use, the modern version of the AMS800 has proven to be a reliable surgical option for the management of non - neurogenic UI in men (6). However, large amounts of data regarding efficiency, complications, and patient satisfaction have been published after artificial sphincter implantation, but the quality of these reports does not meet current standards of evidence-based medicine (7-9). The AMS800 remains the gold standard but does not have the ability of rearrange the cuff in case of postoperative urethral atrophy (10, 11) and no option to adjust the issued pressure of the device after activation. The artificial sphincter BR - SL - AS 904, unlike the commercially available sphincters, has a pressure transmission system, where a reservoir in the patient's abdominal cavity has a direct connection with the urethral cuff. Therefore, the pressure exerted on the reservoir is transmitted through the hydraulic system to the urethral cuff, maintaining the continence of the patient only during stress. This device had already been described experimentally and the authors suggests that the direct pressure transmission to the cuff is an interesting concept to improve clinical outcomes of hydraulic sphincters (6). All surgeons reported the implantation of BR - SL - AR 908 being strai-

ghtforward, fast, feasible and reproducible, with also a short learning curve to achieve mastery (personal findings). Unlike the facility offered by our device, the gold standard sphincter (AM800) is still provided in several boxes and separated components to assemble during the procedure with a constraining preparation (12). In addition, none of patients submitted to our implant presented trans - operative or postoperative complications related to the surgical procedure, using Clavien - Dindo score (13).

Mean age of the population at surgery was 68.2 years (SD 7.5) and did not appear to be related to any complications, however it is unclear why younger age would lead to higher complication rates. One potential explanation is that younger patients in this series had longer documented follow-ups, and consequentially a higher chance for complications and need for revision surgery. There are limited reports that have specifically examined the effect of age on AUS outcomes (14, 15). While one study did demonstrate that octogenarians were more likely to experience erosion or infection compared with younger patients (28), the 5 - year device survival rates were comparable to those reported in younger men (63% to 70%). As such, current evidence remains unclear to recommend making decisions on AUS placement and outcomes solely based on age.

We sought to identify risk factors for AUS complications such as prior radiation, or comorbid conditions as diabetes and hypertension. This study could not identify any statistically significant risk factors for AUS complications. To do so, it will be necessary a higher number of patients and studies. However, we could notice in our study that patient comorbidities, in particular diabetes (40%) and hypertension + diabetes (13%), had similar rates of urinary urgency, IPSS and reported quality of life. In particular, pelvic radiation and high blood pressure have been considered potential risk factors for AUS treatment failure and complication but our study did not have any case of it (16, 17).

In general, there was an improvement in urinary urgency (5 patients at pre - op and only 1 at fourth visit); better IPSS score (6 patients with mild score at pre - op and more 6 at visit 4) and finally a better quality of life (just one patient reported being unhappy at visit 4). Assessment of urinary continence was based on daily urination, urinary free flow, PAD weight test, IPSS score, quality of life and ICIQ - SF values. Although not statistically significant when the comparative analysis tests were applied over the following-up, an improvement in the scores of involuntary urinary loss and quality of life was observed with preservation of urinary flow force without the occurrence of obstructive symptoms. Previous studies assessed functional and quality of life outcomes by PAD use or no validated questionnaires measuring urinary incontinence, frequency and nocturia but did not address long - term functional outcomes for health related quality of life (18-20). Our results are similar to other series with patients reporting an improvement in urinary incontinence and quality of life.

In our study, four different devices (26.7%) had mechanical problem and needed to be removed. However, from 15 cases those four were the only where complication were found. Recently a retrospective study identified 27.096 cases of which the main complications were: erosion of the cuff (4%); loss of fluid (3.8%); cuff atrophy (2.4%); infections (8%) and herniation of the pump (0.2%) (3). In contrast, there were no cases of infection related to device implantation during the follow-up period of this study. There was also no evidence of urethral erosion or appliance extrusion. The device was inert, without triggering inflammatory reactions or infectious processes. Another study analyzing 1.082 artificial sphincter implants followed up for 4.2 years, re - operated 125 patients (11.6%) due to malfunction of the device (21). The urethral cuff was the component that failed the most (46.1%), followed by the abdominal reservoir (22.6%) and the pump (9.6%). Rupture of the tubes were not observed. As the rupture of the ducts is an uncommon complication and the number of patients analyzed was low, we believe that after an improvement in the quality of the T - connection, it would be possible to promote satisfactory urinary continence. However, larger cohort studies with longer follow-up are needed to assess the device efficacy and safety.

CONCLUSIONS

According to the present study, the artificial sphincter implant BR - SL - AS 904 was reproducible, safe and efficient in the control of urinary in-

continence in patients after radical prostatectomy. Improvements in both quality and of the implant material and increase the number of patients can make this treatment modality very attractive and widely practiced.

CONFLICT OF INTEREST

None declared.

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Correspondence address:
Salvador Vilar Correia Lima, M.D, PhD
Departamento de Cirurgia
Centro de Ciên. da Saúde, Univ. Fed. de Pernambuco, UFPE.
Av. Prof. Moraes Rego, nº 1235
Cidade Universitária, Recife, PE, 50670-901, Brasil
Fax: +55 81 2126-3649
E-mail: urology@salvador.net

ORIGINAL ARTICLE

Vol. 44 (6): 1224-1233, November - December, 2018
doi: 10.1590/S1677-5538.IBJU.2017.0620

Simplified method using kidney / ureter / bladder x-ray to determine the appropriate length of ureteral stents

Makoto Taguchi [1], Kenji Yoshida [1], Motohiko Sugi [1], Hidefumi Kinoshita [1], and Tadashi Matsuda [1]

[1] *Department of Urology and Andrology, Kansai Medical University, Osaka, Japan*

ABSTRACT

Purpose: To investigate a method to determine the appropriate length of ureteral stents, given that the stent length may lead to exacerbation of urinary symptoms if the stent crosses the bladder midline.

Materials and Methods: We retrospectively reviewed the position of the distal curl of the ureteral stent using kidney/ureter/bladder (KUB) radiographs after ureteroscopic lithotripsy in 165 patients who underwent placement of 24- or 26-cm ureteral stents. According to the KUB findings, we categorized the position of the distal curl of the ureteral stent into two groups. In Group 1, the stents did not cross the midline (appropriate length); in Group 2, the stents crossed the midline (inappropriate length). We assessed several patient parameters (sex, height, body mass index, and stone side) and the index of ureteral length using KUB radiographs ("C-P") and computed tomography (CT, "P-V"). Multivariate analysis was performed to identify the most significant factors affecting the position of ureteral stents. We also calculated the cutoff points of the receiver operating characteristic (ROC) curve of C-P and P-V for the position of ureteral stents.

Results: The multivariate analysis showed that C-P was the most significant factor affecting the position of ureteral stents ($p < 0.001$) in patients with 24- and 26-cm ureteral stents. Comparison of the ROC curves of C-P and P-V showed that C-P was superior to P-V ($p < 0.01$) in patients with 24- and 26-cm stents.

Conclusion: The use of KUB radiographs was effective and simple in determining the appropriate length of ureteral stents.

ARTICLE INFO

Keywords:
Kidney; Ureter; Urinary Bladder

Int Braz J Urol. 2018; 44: 1224-33

Submitted for publication:
November 22, 2017

Accepted after revision:
July 09, 2018

Published as Ahead of Print:
August 03, 2018

INTRODUCTION

Since Zimskind et al. (1) introduced ureteral stents in 1967, such stents have become widely used for the maintenance of renal function, pain relief, and the treatment of urinary tract infections. However, many complications of ureteral stenting have been reported, such as incomplete emptying, bladder pain, frequency, hematuria, and migration. In one study, ureteral stenting reportedly decreased the urination-related quality of life (QOL) in 80% of patients who underwent ureteral stenting (2). Several factors have been investigated for their effects on ureteral stent - related symptoms, including stent length, (3, 4) diameter, (5-7) material, (7) softness, (8) position, (9) and loop completeness (3). Among these factors, determination of the most appropriate ureteral stent length assumes importance in reducing stent-related complications. Some studies have revealed that placement of overly long ureteral stents that cross the bladder midline can lead to worsening of

urinary symptoms (3, 10, 11). Therefore, we consider the position of the ureteral stent to be an important factor in stent-related surgery. In the present study, we evaluated a method to determine the appropriate ureteral stent length and ensure that the stent does not cross the bladder midline.

The optimal method for determining the appropriate ureteral stent length remains unclear. In previous reports, the appropriate ureteral stent length for each patient was calculated by three different methods. The first is direct measurement of the ureter itself using a guide wire or ureteral catheter (12-16). The second involves measurement of the distance from the pelviureteric junction (PUJ) to the vesicoureteric junction (VUJ) by either retrograde or intravenous pyelography (16-19). The third method provides an estimation of the appropriate stent length using a formula based on the patient's height. The patient's height is reportedly a more reliable guide for obtaining an appropriate ureteral stent length than direct ureteral measurement using a guide wire and ureteral catheter (12, 13, 15, 16, 18, 19). However, there is no standard and simplified method for determining the appropriate ureteral stent length that prevents a decline in urination-related QOL. Moreover, in some hospitals, assorted lengths of ureteral stents are not stocked, and preoperative prediction of ureteral stent lengths is often needed. In this study, therefore, we measured the distance between two points on a kidney / ureter / bladder (KUB) radiograph using retrospective data and evaluated predictors to place ureteral stents (of lengths 24 and 26 cm) so as not to cross the bladder midline. We have developed a predictive and simplified method for determination of the appropriate length of ureteral stents using KUB radiographs with the aim of reducing urination-related symptoms and concomitant QOL.

MATERIALS AND METHODS

Study population

This study was approved by our institutional review board (authorization number: H160741). From January 2013 to December 2015, 168 of 204 patients who underwent ureteroscopic lithotripsy and ureteral stent insertion were enrolled. At the end of the procedure, each patient underwent placement of a ureteral stent (Inlay Optima; C.R. Bard Inc., NJ, USA or Polaris Ultra; Boston Scientific, MA, USA). The diameter of all ureteral stents was 6F and the length was 24 or 26 cm according to the surgeon's discretion. All the stents were placed with full curls in the bladder and kidney.

The exclusion criteria were severe body deformity or disability, a duplicate collecting system, renal ectopia, reimplantation using a psoas hitch, vaginal vault eversion beyond the introitus, and a proximal loop in the upper calyx (Figure-1).

Patient parameters

We assessed several parameters to evaluate the correlation between these characteristics and the appropriate ureteral stent length. Patient demographics including age, gender, height, body weight, body mass index (BMI), and stone side were reviewed. We calculated the length as the index to choose appropriate ureteral stent length for not crossing the bladder midline using KUB radiographs and computed tomography (CT).

Measurement of index using KUB radiographs

We measured the index using preoperative KUB films. KUB filming conditions were standardized at maximum inspiration in the supine position, and imaging was performed at 70 kV and 132 mA. The index used in this study was the length from the central renal point to the midpoint of the superior margin of the pubis (C-P), measured on KUB films (Figures 2A and B). The central renal point was defined as the midpoint of the distance from the extremitas superior renis to the extremitas inferior renis.

Measurement of index using CT

All patients were scanned with a 64-slice CT scanner (120 kV, 200 mA, and 5-mm slice thickness). We also calculated the length from the PUJ to the VUJ (P-V) using CT and the Pythagorean theorem and compared this method with the above-described method to determine which more effectively predicts the appropriate ureteral stent length. The CT index was calculated using Cares-

Figure 1 - Flowchart of inclusion process.

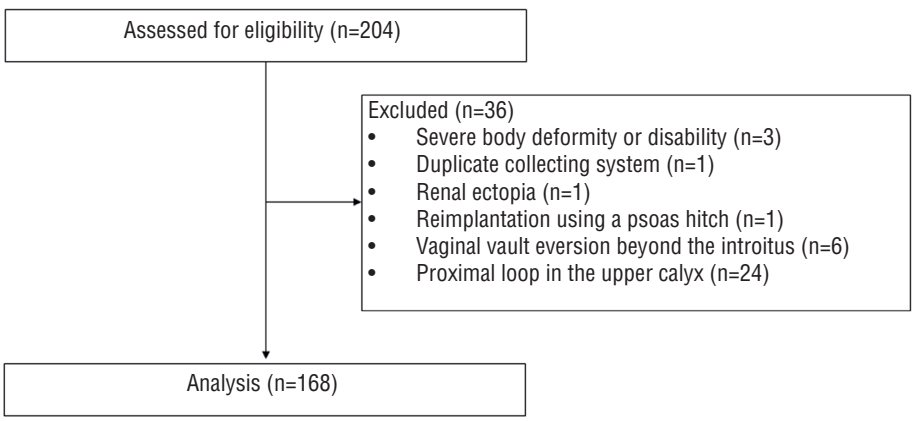


Figure 1 Flowchart of inclusion process.

tream Vue PACS (Carestream Health, Rochester, NY, USA), and all CT images were reviewed by a single urologist (M.T.) with 5 years of experience as an urologist. First, in the CT slice showing the PUJ, we marked the point of the PUJ (Figures 2C and D, star). Next, in the CT slice showing the VUJ, we marked the corresponding point for the PUJ slice (Figures 2E and F, star) and measured the distance from the VUJ (Figures 2E and F, square) to the marked point (Figures 2E and F, star) in the CT slice showing the VUJ. We defined this length as the short side of a right-angled triangle (Figures 2E and F; from star to square). We then defined the length of the long side of a right-angled triangle, calculated by the total number of slices between the slice showing the PUJ (Figure-2G, star) and the VUJ (Figure-2G, square). All slices were 5 mm thick (Figure-2G). Finally, we calculated the length of P-V using the Pythagorean theorem ([P - V]2 = [short side] 2 + [long side] 2).

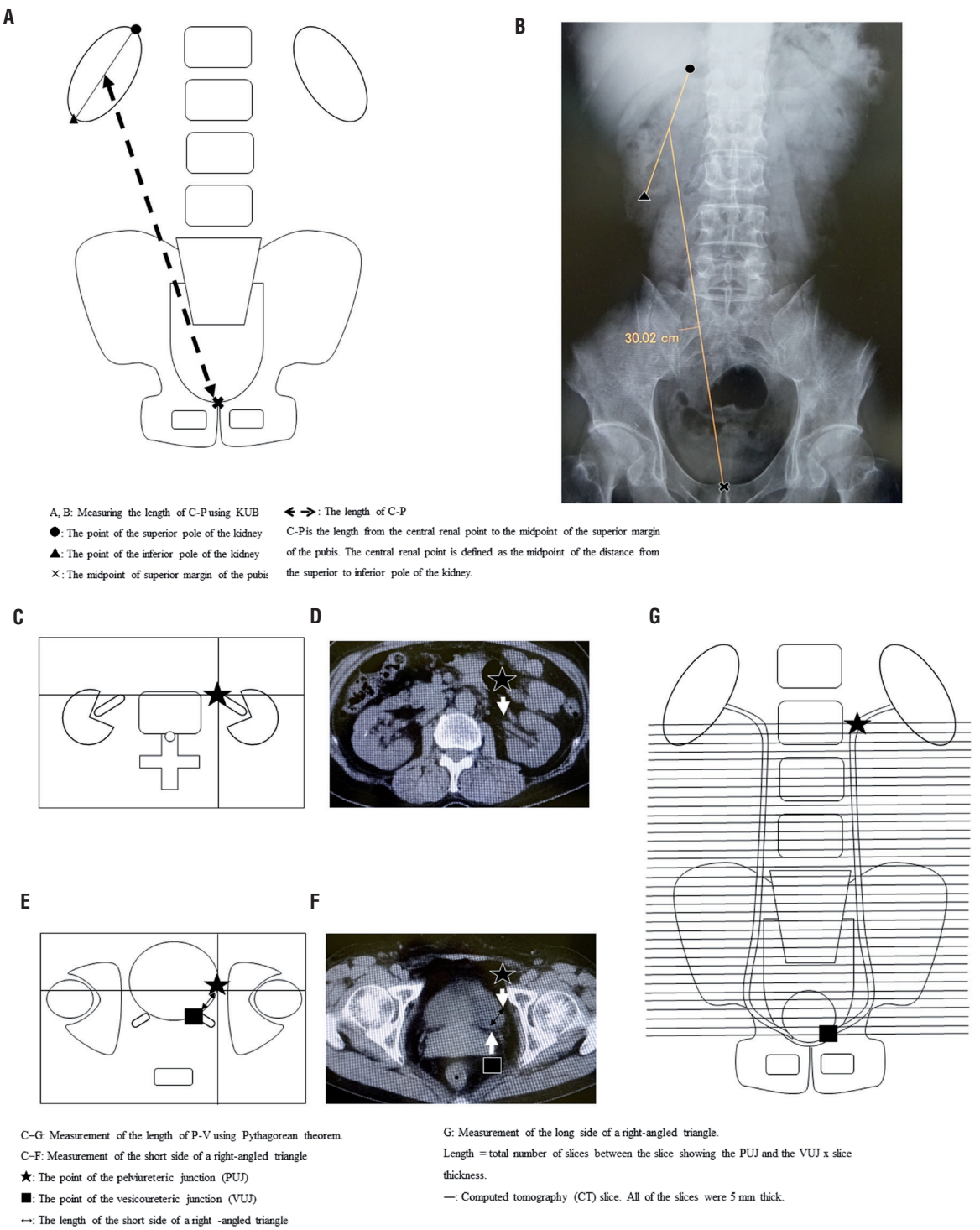
Definition of appropriate ureteral stent length

We routinely obtained KUB films to confirm the presence of residual stones on postoperative day 1. We retrospectively reviewed the position of the ureteral stents using these KUB films. All KUB films were reviewed by a single urologist (M.T.). We categorized the patients into two groups according to the position of the distal curl of the ureteral stent on the KUB films using the technique described by Giannarini et al. (11) In Group 1, the stent did not cross the midline (appropriate length of ureteral stent, Figure-3A); in Group 2, the stent crossed the midline (inappropriate length of ureteral stent, Figure-3B).

Statistical analysis

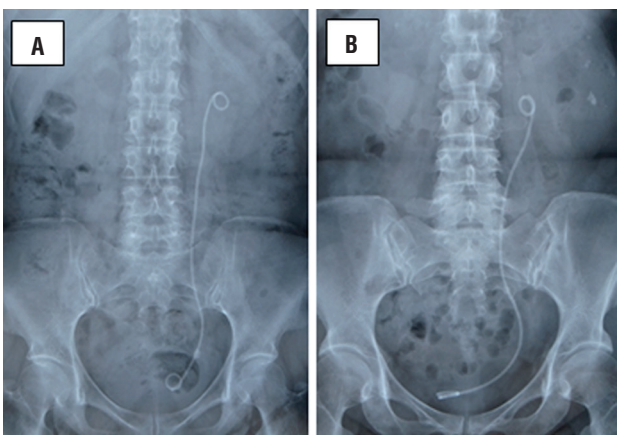
In each group, both of which included patients with 24- and 26-cm ureteral stents, we evaluated the correlation between the position of the ureteral stents and various patient parameters: age, gender, height, body weight, BMI, stone side, C-P, and P-V. Univariate analysis was performed using either the Mann-Whitney U-test or the $\chi 2$ test to evaluate the correlation between the position of the ureteral stents and patient parameters. Multivariate analysis was performed

Figure 2 (A-G) - Measuring the length of C-P and P-V.



A, B - C-P is the length from the central renal point to the midpoint of superior margin of the pubis. Central renal point is defined as the midpoint of distance from extremitas superior renis to extremitas inferior renis. / **C, D, E, F, G -** Measuring the length of P-V using Pythagorean theorem. / **C, D, E, F -** Measuring the short side of a right-angled triangle / **G -** Measuring the long side of a right-angled triangle. / **It length =** the total number of slices between the slice showing the PUJ and the VUJ × slice thickness.

Figure 3 (A, B) - Classification of the intravesical ureteral stent position. (A) Not crossing midline (Group 1). (B) Crossing midline (Group 2).



using a logistic regression model to identify the most significant factors affecting the position of the ureteral stents. Furthermore, we calculated the cutoff points of the receiver operating characteristic (ROC) curve, area under the ROC curve (AUROC), and 95% confidence interval (CI) of the C-P and P-V for the position of the ureteral stents. Data were analyzed using the IBM SPSS Statistics V21.0 software package.

RESULTS

Table-1 shows the patients' demographic data. In Group 1, 46 (59.0%) and 46 (51.1%) patients had 24- and 26-cm indwelling stents, respectively. In Group 2, 32 (41.0%) and 44 (48.9%) patients had 24- and 26-cm indwelling stents, respectively.

Table-2 shows the results of the univariate and multivariate analyses performed to evaluate the correlation between the position of the ureteral stents and patient parameters. Comparison of Groups 1 and 2 using univariate analysis revealed no significant differences in age, gender,

Table 1 - The demographic data of patients with indwelling 24-cm and 26-cm ureteral stents.

	24 cm ureteral stents	26 cm ureteral stents
	n (%) or median (range)	
Patients	78	90
Age (years)	62.5 (92-33)	55 (26-84)
Gender		
male	29 (37.2)	85 (94.4)
female	49 (62.8)	5 (5.6)
Height (m)	1.57 (1.39-1.81)	1.65 (1.45-1.85)
Body weight (kg)	59.2 (30.2-118.2)	63.3 (39-108.6)
BMI (kg/m^2)	24.2 (15.4-35.2)	23.7 (17.8-36.1)
Stone side		
left	52 (66.7)	58 (64.4)
right	26 (33.3)	32 (35.6)
Ureteral stent position		
crossing midline	32 (41.0)	44 (48.9)
not crossing midline	46 (59.0)	46 (51.1)

BMI = body mass index.

height, body weight, BMI, or stone side in either the 24- or 26-cm group. However, C-P and P-V were significantly longer in Group 1 (not crossing midline) than Group 2 (crossing midline) in both the 24- and 26-cm groups ($p < 0.001$).

According to our multivariate analysis, C-P was the most significant factor affecting the position of the ureteral stents in both the 24- and 26-cm groups ($p < 0.001$ for both) (Tables 2A and B, respectively).

Table 2A - Multivariate analysis of patients with 24 cm ureteral stents.

	Group 1 (Not crossing midline)	Group 2 (Crossing midline)	Univariate analysis[a]	Multivariate analysis[b]		
			p-value	p-value	OR	95% CI
Gender						
male	17 (37.0)	12 (37.5)	0.98			
female	29 (63.0)	20 (62.5)				
Height (m)	1.59 (1.41-1.81)	1.57 (1.39-1.78)	0.69			
BMI (kg/m^2)	24.3 (17.1-32.0)	23.8 (15.4-35.2)	0.99			
Stone side						
left	31 (67.4)	21 (65.6)	0.92			
right	15 (32.6)	11 (34.4)				
C-P	28.5 (25.5–33.9)	26.1 (19.9–28.8)	<0.001	<0.001	7.445	2.689-20.612
P-V	20.2 (16.7–25.3)	19.1 (14.7–21.2)	<0.001	0.331	0.966	0.901-1.036

[a] Mann-Whitney U-test; [b] Logistic regression analysis; **OR =** odds ratio; **CI =** confidence interval

Table 2B - Multivariate analysis of patients with 26 cm ureteral stents.

	Group 1 (Not crossing midline)	Group 2 (Crossing midline)	Univariate analysisa	Multivariate analysisb		
			p-value	p-value	OR	95% CI
Gender						
male	42 (91.3)	43 (97.7)	0.18			
female	4 (8.7)	1 (2.3)				
Height (m)	1.66 (1.47-1.80)	1.64 (1.45-1.85)	0.24			
BMI (kg/m^2)	23.5 (17.8-36.1)	24.1 (19.7-35.2)	0.40			
Stone side						
left	29 (63.0)	29 (65.9)	0.78			
right	17 (37.0)	15 (34.1)				
C-P	30 (26.0–34.8)	28.2 (24.6–29.9)	<0.001	<0.001	3.003	1.701-5.301
P-V	21.0 (17.7–27.1)	19.5 (17.4–22.3)	<0.001	0.273	1.018	0.986-1.051

[a]Mann-Whitney U-test; bLogistic regression analysis; **OR =** odds ratio; **CI =** confidence interval

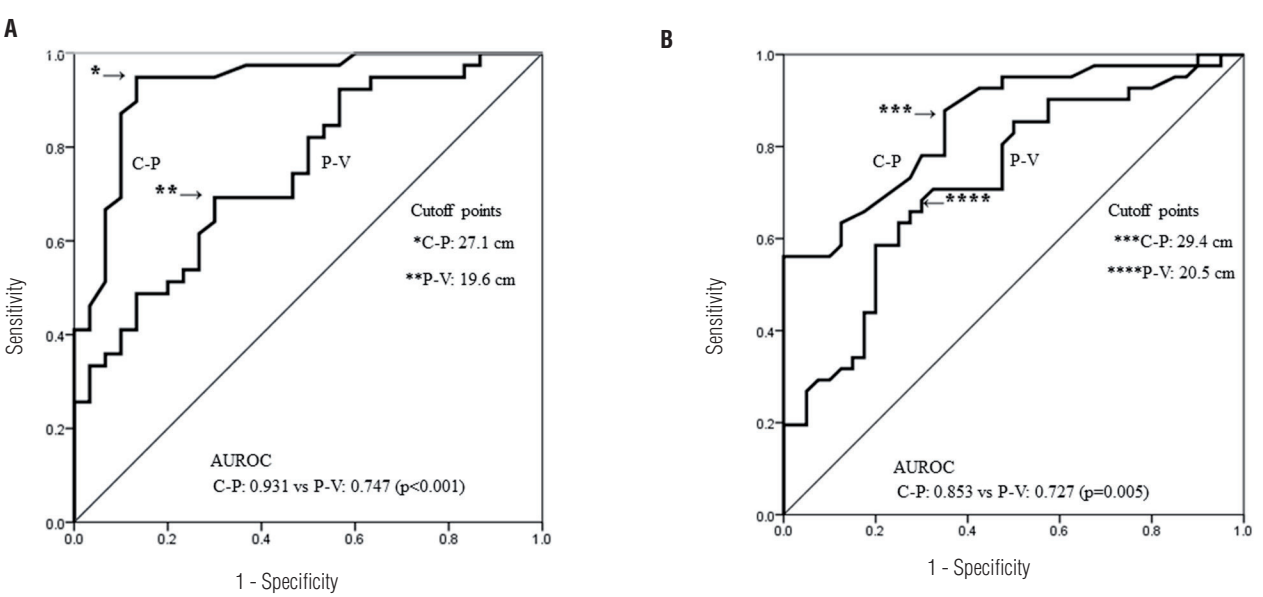
Figure-4 shows the comparison of the ROC curves of C-P and P-V and the AUROC in the patients with 24- and 26-cm ureteral stents. The cutoff points of the ROC curve of C-P and P-V in the patients with 24-cm ureteral stents were 27.1 and 19.6 cm, respectively, and those in the patients with 26-cm stents were 29.4 and 20.5 cm, respectively. Comparison of the ROC curves of C-P and P-V showed that C-P was superior to P-V in both the 24- and 26-cm groups ($p < 0.01$).

DISCUSSION

Determination of the appropriate ureteral stent length is very important for reducing stent--related complications. However, few reports have addressed this topic, and no guidelines regarding ureteral stents exist. In this study, we evaluated several patient parameters that we considered relevant when choosing the appropriate stent length. We found that the method using KUB films was more useful and simpler than the methods using patient height or CT for determining the appropriate stent length. We measured the C-P length using KUB films. In this study, we found that a 26-cm ureteral stent is appropriate for patients with a C-P of ≥ 29.4 cm, that a 24-cm ureteral stent is appropriate for patients with a C-P of 27.1 to < 29.4 cm, and that a 22-cm ureteral stent may be appropriate for patients with a C-P of < 27.1 cm. The appropriate ureteral stent length was short if the C-P length was shorter. Because we considered that a short C-P or P-V means that the ureter length is also expected to be short, the appropriate ureteral stent length was also short.

Some studies have reported that crossing of a ureteral stent over the bladder midline may lead to worsening of urinary symptoms (3, 10, 11). Rane et al. (3) investigated the correlation between the position of the ureteral stent and stent-related symptoms in 60 patients and reported that a ureteral stent that crosses the bladder midline causes significantly more frequency and urgency. Ho et al. (10) evaluated whether the ureteral stent length affects stent-related symptoms after placement of stents in 87 patients. They discovered that the

Figure 4 (A, B) - Receiver operating characteristic curves for success of ureteral stenting of KUB and CT, and area under the receiver operating characteristic curve (AUROC).



A - 24-cm ureteral stents.

B - 26-cm ureteral stents.

ureteral stent length was associated with the position of the distal loop of the stent and reported that a longer stent crossing the bladder midline causes more irritative symptoms. Giannarini et al. (11) assessed the predictors of morbidity in 84 patients with indwelling ureteral stents. Using multivariate analyses, they reported that the location of the distal loop of the ureteral stent (not crossing the bladder midline) had the strongest association with ureteral stent-related symptoms. Therefore, we consider that crossing of a ureteral stent over the bladder midline may lead to worsening of urinary symptoms and that choosing the most appropriate ureteral stent length for each patient is important to improve stent-related symptoms.

Table-3 shows reported clinical studies to choose the appropriate ureteral stent length not crossing the bladder midline. Pilcher and Patel (13) reported that the patient's height is a more reliable guide to choosing the most appropriate ureteral stent length than is direct ureteral measurement using a guide wire and ureteral catheter. They compared the accuracy of a patient height--based formula for choosing the correct ureteral stent length with that of direct ureteral length measurement. In their study, the patient's height correctly predicted the appropriate stent length in the majority of ureters, and direct ureteral measurement oversized the ureteral stent length in 83% of cases (13). Additionally, Ho et al. (20) found a 22-cm ureteral stent to be more appropriate for patients of < 175 cm in height, who comprised nearly 90% of their study population. Lee et al. (21) also reported that a 22-cm stent was appropriate for patients of < 175 cm in height. Conversely, Jeon et al. (16) found direct measurement of the ureteral length to be a more reliable method than determination of the stent length according to patient height. Wills et al. (17) reported that measurement of the ureteral length by intravenous

Table 3 - Clinical studies performed to choose the appropriate ureteral stent length that does not cross the bladder midline.

Study	n	Methods to choose stents	Outcome
Pilcher and Patel (13)	41	Ureteral catheter vs patient's height	Patient's height was a more reliable guide.
Ho et al. (20)	408	Comparing patient's height and stent position	Patient's height could predict the ideal stent length.
Lee et al. (21)	70	Comparing patient's height and stent position	A 22 cm ureteral stent was appropriate for Korean patients smaller than 175 cm in height.
Jeon et al. (16)	70	Direct measurement using guidewire vs patient's height	Direct measurement of ureteral length using guidewire was easy and reliable. Patient's height did not correlate well with appropriate ureteral length.
Wills et al. (17)	40	Comparing with the ideal stent length and the length of the ureter measured on intravenous urography	Measuring on intravenous urography had the correlation with the ideal stent length.
Barrett et al. (22)	59	Patient's height vs L1-L5 height vs length measured on CT	CT measurements could be used to choose the appropriate stent length.
Our study	168	Comparing predictors (sex, patient's height, BMI, side, KUB radiograph, CT) to determinate the appropriate length of ureteral stent.	KUB radiograph and CT were significant factor affecting the position of the ureteral stents according to our multivariate analysis.

CT = Computed tomography, **KUB =** kidney/ureter/bladder

urography is useful. However, this method requires a full-length intravenous urography film, and tracing the curved ureter viewed on a retrograde or intravenous pyelography film is difficult (21). Therefore, we considered that establishment of a simple method with which to determine the appropriate ureteral stent length was necessary and recommend the herein-described method using KUB films, which we consider more useful and simpler than other methods.

Barrett et al. (22) reported using CT to choose the most appropriate stent length; in this technique, the ureteral length can be measured by identifying the location of the ureter in each CT slice. We referred to this method to measure the index using CT in this study. However, this method requires considerable time and effort. Moreover, CT has some limitations such as radiation exposure, measurement error associated with slice thickness, and the need for precise measurement using rendering software. Furthermore, the location of the ureteral orifices differs according to whether bladder filling is performed, (23) although bladder filling was not a standard of care in the present study. Therefore, we consider that we should investigate a more useful method than CT to choose the appropriate ureteral stent length.

This report is the first to calculate cutoff points for determination of the appropriate length of ureteral stents. We have herein introduced our method using KUB films, which is inexpensive and less invasive.

This study has some limitations. First, it was a retrospective and non-randomized trial, and the choice of the ureteral stent was entirely dependent upon the operator. Second, we did not standardize the type of ureteral stents, and the coiling patterns varied among the stents. Third, we did not use 22- and 28-cm ureteral stents and thus did not evaluate the appropriate C-P length for stents of these lengths. Fourth, we did not evaluate the patient's ureteral stent-related symptoms. Future studies should involve reassessment using a 22-cm ureteral stent and evaluation of ureteral stent-related symptoms. Fifth, the method of measurement of the index using CT did not use the coronal plane, and the method using the Pythagorean theorem might be complicated. If we use other methods when measuring the index using CT, there would be a possibility that CT is superior to KUB. Therefore, it is controversial whether these parameters could be transposed to tomography. Finally, the renal shadow was occasionally unclear because of bowel gas. Therefore, some preoperative KUB films were seldom needed. In this study, we could measure the index of all patients using KUB films because we obtained some KUB films as a preoperative assessment, and only one or two films were needed to measure the index in most cases. Furthermore, all KUB films were reviewed by a single urologist and we have not confirmed whether other urologists can measure the index using KUB. We do not consider these methods to be complicated. However, future studies should involve reassessment in multiple centers.

CONCLUSIONS

We consider that our method using KUB radiographs is useful and simple to determine the appropriate ureteral stent length. Furthermore, we can preoperatively choose an appropriate ureteral stent length compared with direct ureteral measurement using a guide wire and ureteral catheter. However, this study has some limitations and we could not conclude that the method of measurement of the index using KUB is superior to CT.

ABBREVIATIONS

KUB = kidney, ureter, bladder X-ray
ROC = receiver operating characteristic
QOL = quality of life
PUJ = pelviureteric junction
VUJ = vesicoureteric junction
BMI = body mass index
CT = computed tomography
AUROC = area under the receiver operating characteristic curve
CI = confidence interval

CONFLICT OF INTEREST

None declared.

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Correspondence address:
Makoto Taguchi, MD
Department of Urology and Andrology, Kansai Medical University, 2-5-1 Shinmachi
Hirakata, Osaka 573-1010, Japan
Fax: + 81 72 804-2068
E-mail: taguchim@takii.kmu.ac.jp

ORIGINAL ARTICLE

Vol. 44 (6): 1234-1242, November - December, 2018
doi: 10.1590/S1677-5538.IBJU.2017.0656

Vitamin C inhibits crystallization of struvite from artificial urine in the presence of *Pseudomonas aeruginosa*

Muhammed A. P. Manzoor [1], Surya Ram Duwal [2], M. Mujeeburahiman [3], Punchappady-Devasya Rekha [1]

[1] Yenepoya Research Centre, [2] Department of Biochemistry, [3] Department of Urology, Yenepoya Medical College, Yenepoya University, Mangalore, Karnataka, India

ABSTRACT

Background: Formation of struvite stones is associated with urinary tract infection by urease-producing bacteria. Biogenic crystal growth in natural and synthetic materials is regulated by the action of inhibitors, ranging from small ions, molecules to large macromolecules.

Materials and Methods: We report the dynamics of *in vitro* crystallization of struvite in presence of vitamin C in synthetic urine using single diffusion gel growth technique. Sodium metasilicate gel of specific gravity 1.05 and the aqueous solution of ammonium dihydrogen phosphate were used as the medium for growing the struvite crystals. The crystallization process was induced by a urease positive struvite stone associated *Pseudomonas aeruginosa* to mimic the infection leading to stone formation. The grown crystals were characterized by ATR-FTIR and powder XRD. The surface morphology was analysed through FE-SEM for comparison between treatments.

Results: We observed decrease in number, dimension, and growth rate of struvite crystals with the increasing concentrations of vitamin C. Crystals displayed well-defined faces and dendritic morphology of struvite in both control and biogenic systems.

Conclusion: The results strongly suggest that, vitamin C can modulate the formation of struvite crystals in the presence of uropathogenic bacteria.

ARTICLE INFO

Keywords:
Struvite; Pseudomonas aeruginosa; Ascorbic Acid

Int Braz J Urol. 2018; 44: 1234-42

Submitted for publication:
December 06, 2017

Accepted after revision:
February 13, 2018

Published as Ahead of Print:
March 10, 2018

INTRODUCTION

Struvite or magnesium ammonium phosphate stone is associated with infections by urease-producing bacteria and accounts for about 10-15% of all kidney stones (1). The enzyme urease produced by the bacteria can cause super-saturation and crystallization of Mg^{2+} and PO_4^{3-} as carbonate apatite ($Ca_{10}(PO_4)_6.CO_3$) and struvite ($MgNH_4PO_4.6H_2O$), respectively (2, 3). Struvite crystals can aggregate and form large crystals in the branches of the collecting system, to form large aggregates called staghorns. If untreated, they can cause significant kidney damage, and can sometimes also be life-threatening due to loss of kidney function (4).

Previous study from our group reported the high diversity of mixed stones having two or more than two types of mineral compositions among kidney stone patients (5). Struvite stones can be present as pure types or along with other compositions such as calcium oxalate and hydroxyapatite crystals. Struvite crystallization is mediated by the urease producing bacteria such as: S*taphylococcus* (Gram-positive), *Proteus, Pseudomonas, Providencia* and *Klebsiella* (Gram-negative). In addition, certain species of *Serratia*, *Corynebacterium* and *Morganella* also produce the enzyme urease

which can lead to stone formation. Treatment of struvite stones involves stone removal followed by antibiotic therapy to eliminate bacteria from the urinary tract (6). Crystal growth in biogenic, natural and synthetic medium is regulated by the action of various inhibitors. *In vitro* crystallization of struvite has been increasingly investigated over the last years (7-9). However, bacterially induced struvite crystallization and its inhibition studies have higher implications in healthcare (6, 8, 10).

Compounds such as curcumin and vanillic acid can inhibit the growth of struvite in bacteria--induced crystallization *in vitro* (6, 11). Ascorbic acid, or vitamin C, is an essential micronutrient required for the normal metabolic functions and acts as an electron donor or reducing agent in biochemical reactions (12). Studies on vitamin C in kidney stone disease have shown mixed results with respect to oxalate metabolism and excretion (13, 14). It was initially reported that vitamin C can reduce the urinary pH (15, 16), however, others have found it as an ineffective urinary acidifier (17, 18).

Here we report the role of vitamin C on crystallization and pathogenesis of struvite crystal caused by *P. aeruginosa* isolated from the infectious kidney stone. To best of our knowledge there are no reports on uropathogenic *P. aeruginosa* induced struvite crystallization and inhibition of the same by vitamin C.

MATERIALS AND METHODS

Chemicals and Reagents

Ammonium di-hydrogenphosphate ($NH_4H_2PO_4$), sodium metasilicate (Na_2SiO_3), magnesium acetate solution ($C_4H_6MgO_4$) and vitamin C were of analytical grade and purchased from commercial sources.

Bacterial Strain and Culture Conditions

All the procedures performed in studies involving human participants were approved by the Institutional Scientific Review Board (YRCSRB/034/17) and Institutional Ethics Committee (YUEC.No.2016/022) of the Yenepoya University. *Pseudomonas aeruginosa* strain YU22S, previously isolated from the stone culture of a patient with struvite stone was used. *P. aeruginosa* was tested for urease activity using urea agar and phenol hypochlorite assay (secondary screening). The bacteria were cultured on tryptic soy broth (TSB) for 18h at 37°C and cells were harvested by centrifugation (6000rpm, 8 min). Density of the suspension was determined using McFarland standards and spectrophotometrically (OD 550nm). The cells were suspended in synthetic urine (artificially prepared aqueous solution with mineral compositions for simulating the urine) to an appropriate concentration (10^5cells/mL).

Preparation of Synthetic Urine

The synthetic urine used for the crystallization was prepared according to Griffith et al., (19) (g/L): $CaCl_2$ $2H_2O$, (0.651); $MgCl_2$ $6H_2O$, (0.651); NaCl, (4.6); Na_2SO_4, (2.3); KH_2PO_4, (2.8); KCl, (1.6); NH_4Cl, (1.0); sodium citrate, (0.65); sodium oxalate, (0.02); urea, (25.0); creatine, (1.1); and TSB, (10). The content of the mineral components in the synthetic urine corresponds to mean concentration found in 24h period in normal human urine.

Experimental Setup

The single diffusion gel growth technique was used to study the growth and inhibition of struvite crystals as described elsewhere (8, 20). Briefly, sodium metasilicate (SMS) solution of specific gravity 1.05 was used to prepare the gel. An aqueous solution of ammonium di-hydrogenphosphate (0.5M) was mixed with the SMS solution in appropriate amount, so that the gel pH was set at 7.0 in test tubes (140mm length and 25mm diameter). To this, 20mL supernatant solutions of 0.5M magnesium acetate in synthetic urine along with different concentrations of vitamin C were gently poured on the gels without disturbing it. Bacteria suspended in synthetic urine at a density of $5x10^5$ CFU/mL was used for the groups having bacterial addition. All the procedures were done aseptically. Bacterial growth in the synthetic urine was assessed every 24h by plating on trypticase soy agar (TSA) to monitor the viability. All the experiments were performed in triplicates at 37±0.5°C. During the experiment, pH of the samples was measured using a digital pH meter. Additionally, samples from different stages of the crystallization

process were observed under a light microscope. Macro-morphology of grown crystals was recorded using stereomicroscope (Carl Zeiss, Göttingen, Germany).

Characterization of Struvite Crystals

Samples from different stages of the crystallization process were collected and used for investigations. The grown crystals were harvested from the test tube set up and characterized by Field Emission Scanning Electron Microscope (FE-SEM), Attenuated Total Reflectance- Fourier transform infrared spectroscopy (ATR-FTIR), and X-ray diffraction (XRD). ATR-FTIR of the amorphous crystals was directly recorded using the instrument (Shimadzu IR Prestige-21) and compositions were determined by the FTIR spectra at mid frequency range (4000-400cm^{-1}) at 4cm^{-1} resolution. The XRD patterns were recorded with a Rigaku MiniFlex 600 laboratory diffractometer using a Cu-Kα radiation (λ=1.5406Å). Diffraction patterns were registered within the 2^{o} angle range from 10 to 80° and the phase identification was calculated from the diffractograms. The microstructure and morphology were observed using FE-SEM (Carl Zeiss, Germany).

Statistical analysis

All the experiments were performed in triplicates. Continuous variables are reported as means ± standard deviation. Pearson's correlation was used to correlate the amount of struvite crystals formed in control and test groups. The value of $p<0.05$ was considered statistically significant. Statistical analysis was performed using SPSS, Version 22.0. (IBM Corp).

RESULTS

Inhibitory Activity of Vitamin C during Bacteria-induced Struvite Crystallization.

The early stages (2-24h) of struvite crystallization are given in Figure-1. During this interval, struvite exhibited typical hemimorphic morphology of coffin-lid shape along with bacterial cell. In the presence of different concentrations of vitamin C, crystal formation was delayed compared to control. In both control and vitamin C treated groups, dendritic and X-shaped crystals were formed. However, in the presence of vitamin C, the crystals formed were comparatively smaller and lesser in numbers.

Due to the urease activity, at higher pH, the crystals frequently formed twins, and large dendritic branches (Figure-1A, image a6). The time--resolved experiments in the gel media showed the growth of small dendritic type crystals at the end of the first day in the gel at the gel-liquid interface. The initial pinpoint crystals tend to nucleate randomly throughout the test tube and in its surfaces (Figure-2). With the increase in time, the amount of crystals and size gradually increased. However, vitamin C concentrations and the weight of the struvite crystals showed significant inverse correlation ($r= -0.962$, $p<0.05$).

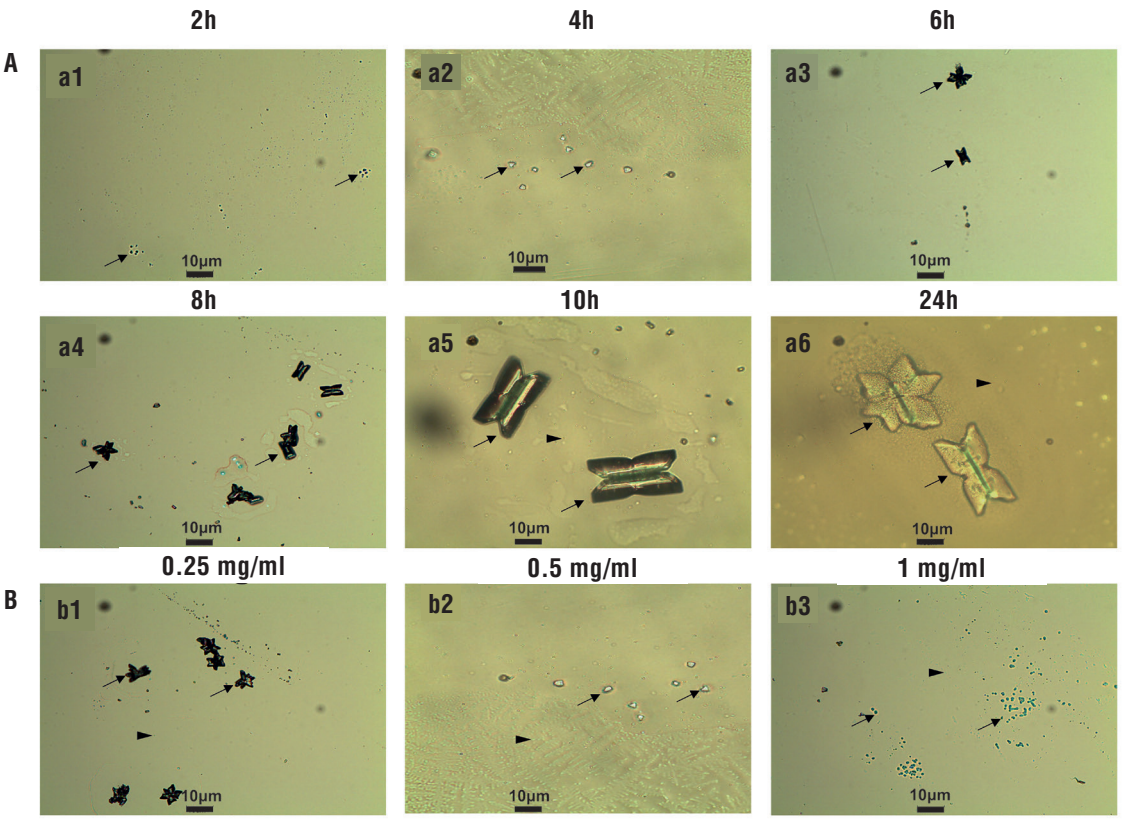
Effect of pH, Bacterial Viability and Dimensions of Struvite during Crystallization.

In the *P. aeruginosa* infected synthetic urine, a progressive change in the pH was observed during the initial 24h, from pH 5.65 to 8.9. In particular, in the presence of vitamin C, urine pH increased slower compared with the control (Figure-3A). Interestingly, crystal formation presence of vitamin C in the gel was in the higher depth compared to control. This may be possibly due to the penetration of vitamin C into the upper region of the gel. The depth at which crystals formed increased with the concentration of vitamin C (Figure-3B). The size and weight of the harvested crystals showed concentration dependent changes with vitamin C treatment (Figure-4).

Structural and Morphological Characterization of Struvite Crystals

Struvite crystals showed characteristic IR spectrum at 1010cm^{-1} due to the absorption of PO_4^{3-} (v_3). Peaks at 1469, 1435 and 1400 cm^{-1} attributed to (v_4) NH_4^+ bending and the peaks at 892 and 761cm^{-1} correspond to the ammonium-water H bonding and water-water H bonding respectively. The P-O bend (v_4) and the PO_4^{3-} (v_2) modes were represented by peaks at 572 and 462cm^{-1} represent. In the presence of vitamin C, the band at 1263cm^{-1} was absent and a new peak at 2385cm^{-1}

Figure 1 - Struvite crystals grown in artificial urine infected with *Pseudomonas aeruginosa* (a). Temporal growth pattern in control (a1-a6) (b). Inhibition of struvite crystals due to vitamin C treatment 24 h (b1:0.25 mg/mL, b2:0.5 mg/mL and b3:1 mg/mL). "Arrow" shows crystals and "arrowhead" shows bacteria.



was found (Figure-5A). XRD patterns for the crystals obtained with and without vitamin C treatment are shown in Figure-5B. The struvite crystallizes were in the orthorhombic Pmn21 space group (cell parameters a=6.955 Å, b=11.2 Å, c=6.142 Å). Vitamin C induced struvite exhibited increased peak intensity corresponding to (021) and decreased intensity of (020) and (040) planes as compared to the control. It shows that vitamin C interfere in the crystal growth and the preferred growth being at (111) plane. This indicates preferential adsorption and binding of vitamin C onto these faces and results in the prominent development in (111) face.

The struvite crystals exhibited porous nature with characteristic tubular pores in FE-SEM and the single struvite crystals had well-defined crystalline faces and multi-layered depositions. In addition, vitamin C induced struvite crystals had highly porous appearance compared to control (Figure-6). Moreover, the presence of Mg, N, P and O using X-ray spectroscopy confirmed the major elements present in struvite crystal.

DISCUSSION

The effect of vitamin C on *P. aeruginosa*-induced struvite crystallization was analysed in detail using microscopy, changes in pH, and bacterial viability during 24h incubation in synthetic urine. Struvite exhibited typical hemimorphic morphology in the presence of uropathogenic bacteria. These characteristic hemimorphic habit and morphology were reported previously in detail (9). And similar observations were also made for struvite crystallized in the presence of *Proteus mirabilis* (8). In the presence of vitamin C, crystal formation was delayed compared to control and X-shaped crystals were observed. These charac-

Figure 2 - Photograph of the struvite crystals grown in gel medium in artificial urine infected with *Pseudomonas aeruginosa* and treated with different concentrations of vitamin C at different time points (0th, 1st, 7th and 15th day). (a) In the absence of vitamin C (b & c) In the presence of 0.5 mg/mL and 1 mg/mL vitamin C respectively.

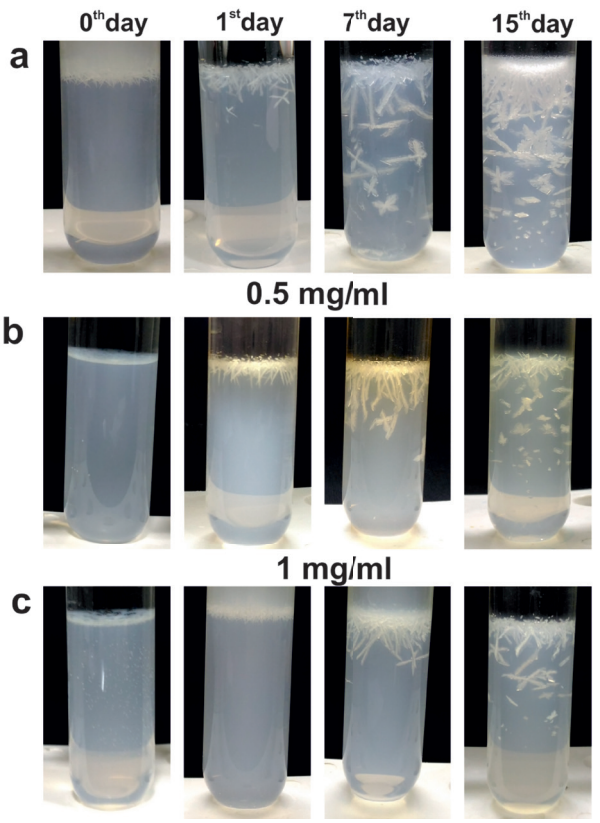
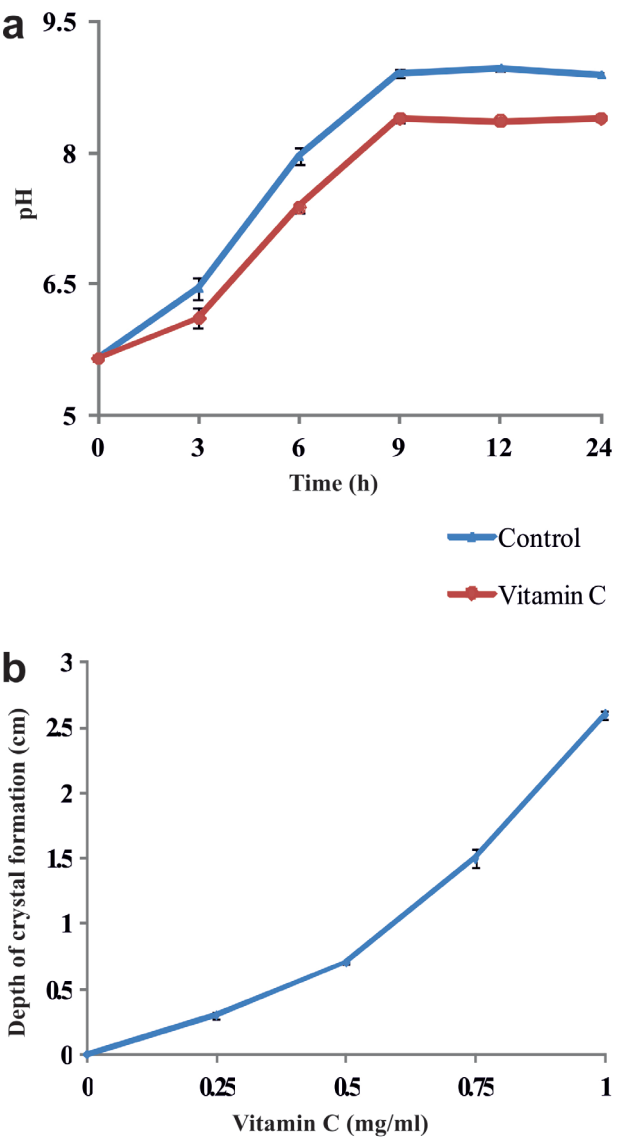


Figure 3 - a) Kinetics of pH of the artificial urine infected with *Pseudomonas aeruginosa* without (control) and with vitamin C. b) Depth of struvite crystals formed in the gel media in different concentration of vitamin C.



teristic X-shapes have been reported in bacteria--induced struvite crystallization (8, 21).

The induction time for crystallization in the presence of vitamin C was delayed. Similar observation was also found with other inhibitory compounds during bacteria induced struvite crystallization in artificial urine (11). Moreover, the size and weight of the harvested crystals showed concentration dependent changes with vitamin C treatment. Such inhibitory reduction was observed in struvite crystallization under polyaspartic acid treatment of different concentrations (9). In FTIR spectra, shift in the bands to lower frequencies were seen, which may be due to the vibrations related to stretching Mg-O modes. Such changes in the structure of the struvite crystals were also reported earlier (22, 23). Vitamin C interferes in the crystal growth and the preferred growth being at (111) plane and the XRD pattern of struvite crystals grown in the gel medium are identical with the reported literature (7, 24). The struvite crystals exhibited porous nature and the multi-layered depositions. These multi-layered depositions are commonly observed during the formation of struvite crystals *in vitro* in the presence of natural compounds (24). The internal porous nature

Figure 4 - Relationship between vitamin C concentrations and (a) length and (b) weight of struvite crystals obtained in gel growth technique.

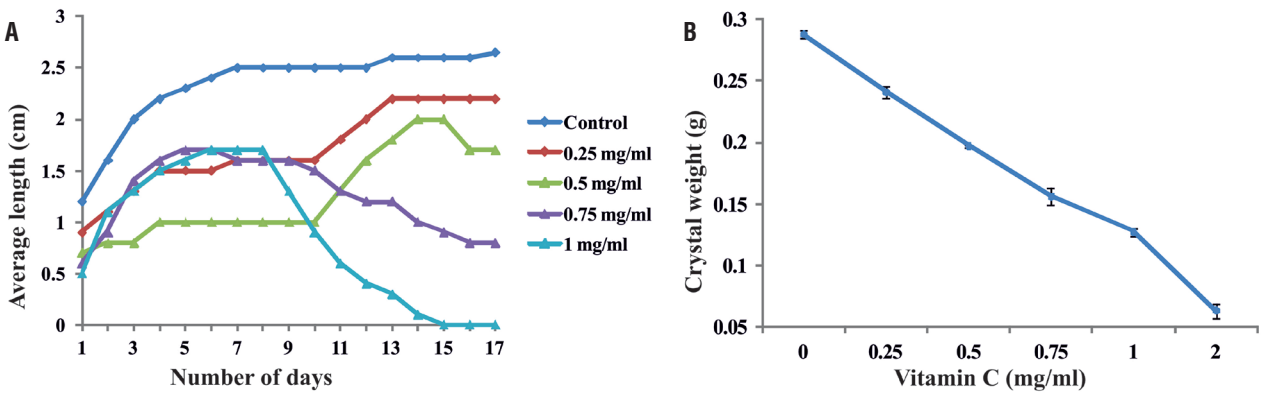


Figure 5 - Representative (a) FTIR and (b) XRD patterns of the of the grown struvite crystal. Arrow indicates differences in the peak.

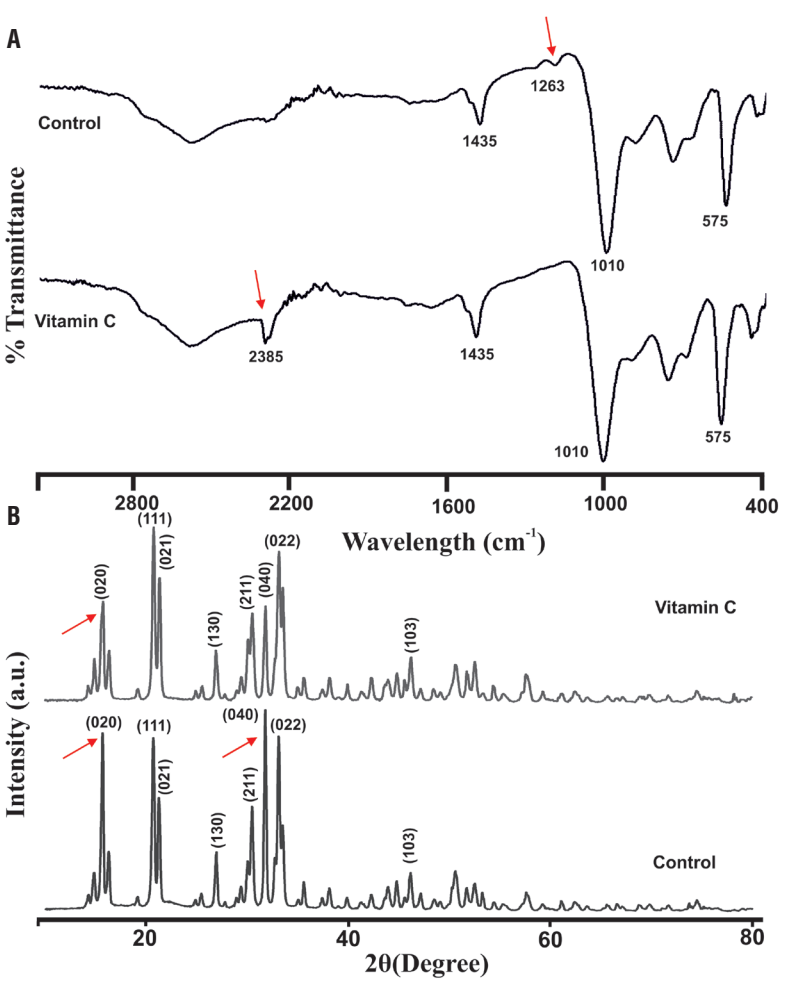
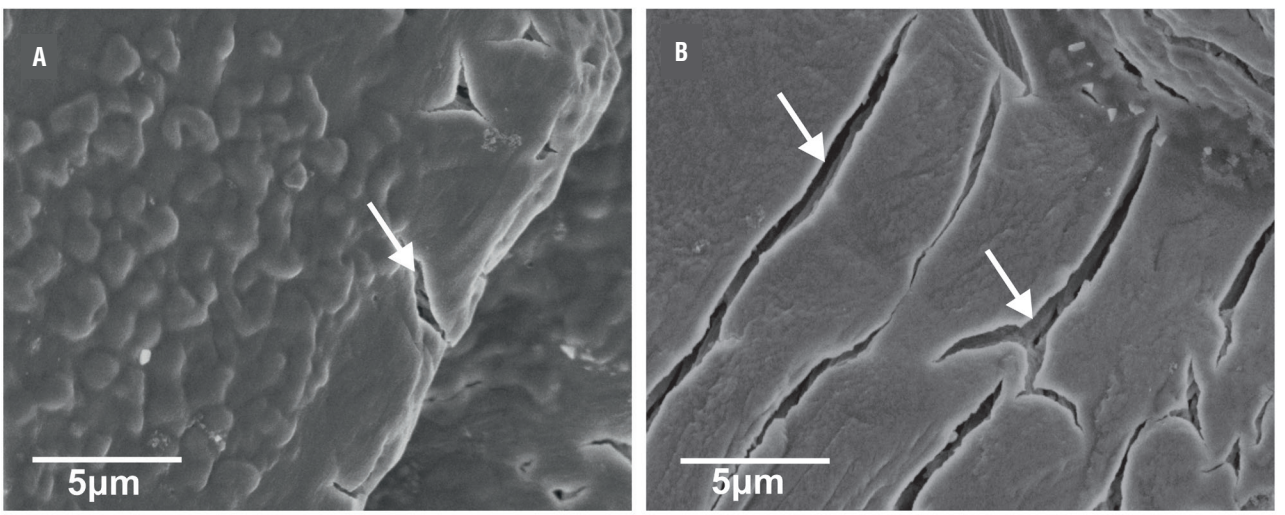


Figure 6 - FE-SEM micrographs of struvite crystals grown in artificial urine infected with *Pseudomonas aeruginosa* revealing the porous structure and mesoscopic arrangement (a) control (b) in presence of vitamin C (1 mg/mL). Arrow indicates porous morphology.



with characteristic tubular pores of struvite crystal during *P. mirabilis* induced crystallization was well explained (8).

Possible Mode of Action for the Struvite Inhibition by Vitamin C

The results obtained with respect to *in vitro* inhibition of struvite showed a decrease of crystal size and weight in the presence of vitamin C in a concentration dependent manner. pH of supernatant solution was acidic in nature, indicating that vitamin C can constantly bring down the pH thereby preventing the crystallization in the synthetic urine. Vitamin C can lower the pH and also inhibit the urease activity due the altered pH. In addition, these characteristics delay the nucleation and appearance of struvite crystals in the presence of vitamin C. Citrus fruits containing vitamin C had similar interaction with struvite minerals and inhibited crystal growth *in vitro* (20).

Blood levels of vitamin C can have a significant role in preventing struvite stone formation. Vitamin C has been shown to play a significant role in preventing infection progression through increased reactive oxygen species production against bacteria (25). Current treatment of infection stones remains challenging, and management of the struvite calculi requires a comprehensive approach. Dietary manipulation, antibiotic therapy along with acidification therapy with vitamin C may improve the clinical outcome of the patients. Furthermore, understanding the mechanism of vitamin C interaction with urinary tract bacteria can be studied to understand its role in the prevention of bacteria-induced struvite. The effect of vitamin C on struvite stone formation needs to be evaluated with other urease-producing bacteria. The effect of vitamin C on struvite stone formation needs to be further evaluated using *in vivo* models to establish the findings of the present study to support vitamin C as a potential choice for prevention of struvite stones.

CONCLUSIONS

Struvite crystals displayed well-defined faces and dendritic morphology in biogenic systems. Vitamin C has inhibitory activity on bacterially induced struvite crystal formation.

ABBREVIATIONS

ADP = Ammonium di-hydrogenphosphate
ATR-FTIR = Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy

FE-SEM = Field Emission Scanning Electron Microscope
SMS = Sodium metasilicate
TSA = Trypticase soy agar
TSB = Tryptic soy broth
XRD = X-ray diffraction

Compliance with Ethical Standards

Disclosure of potential conflicts of interest: All authors have nothing to declare and no competing financial interests in relation to the work described.

Research involving Human Participants and/or Animals: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: Not applicable

ACKNOWLEDGEMENTS

M.A.P.M. acknowledge Yenepoya University research fellowship (YU-SRF). Authors acknowledge Dr. K.K. Achary and Mrs. Megha Nair for their assistance in data analysis.

Surya Ram Duwal contributed similarly as first author.

CONFLICT OF INTEREST

None declared.

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Correspondence address:
Punchappady-Devasya Rekha, Ph.D
Yenepoya Research Centre
Yenepoya University Mangalore
Karnataka-575018, India
FAX: + 91 824 220-4667
E-mail: rekhapd@hotmail.com

ORIGINAL ARTICLE

Vol. 44 (6): 1243-1251, November - December, 2018
doi: 10.1590/S1677-5538.IBJU.2018.0232

Beneficial effects of Oltipraz, nuclear factor – erythroid – 2 – related factor 2 (Nrf2), on renal damage in unilateral ureteral obstruction rat model

Emre Can Polat [1], Huseyin Besiroglu [2], Levent Ozcan [3], Alper Otunctemur [1], Ahmet Tugrul Eruyar [4], Adnan Somay [5], Nurver Ozbay [5], Mustafa Cekmen [6], Ceyla Eraldemir [7], Emin Ozbek [8]

[1] Department of Urology, Okmeydani Training and Research Hospital, University of Health Sciences, Istanbul, Turkey; [2] Department of Urology, Catalca Ilyas Cokay State Hospital, Istanbul, Turkey; [3] Department of Urology, Derince Training and Research Hospital, University of Health Sciences, Kocaeli, Turkey; [4] Department of Pathology, Derince Training and Research Hospital, University of Health Sciences, Kocaeli, Turkey; [5] Department of Pathology, Fatih Sultan Mehmet Training and Research Hospital, University of Health Sciences, Istanbul, Turkey; [6] Department of Biochemistry, Istanbul Medeniyet University, Istanbul, Turkey; [7] Department of Biochemistry, Kocaeli University, Kocaeli, Turkey; [8] Department of Urology, Cerrahpasa Medical School, Istanbul University, Istanbul, Turkey

ABSTRACT

Introduction: We investigated whether Oltipraz (OPZ) attenuated renal fibrosis in a unilateral ureteral obstruction (UUO) rat model.

Materials and Methods: We randomly divided 32 rats into four groups, each consisting of eight animals as follows: Rats in group 1 underwent a sham operation and received no treatment. Rats in group 2 underwent a sham operation and received OPZ. Rats in group 3 underwent unilateral ureteral ligation and received no treatment. Group 4 rats were subjected to unilateral ureteral ligation plus OPZ administration. Transforming growth factor beta-1 (TGF-β1), E-cadherin, nitric oxide (NO) and hydroxyproline levels were measured. Histopathological and immunohistochemical examinations were carried out.

Results: TGF-β1, NO and E-cadherin levels in the UUO group were significantly higher than the sham group and these values were significantly different in treated groups compared to the UUO group. In rats treated with UUO + OPZ, despite the presence of mild tubular degeneration and less severe tubular necrosis, glomeruli maintained a better morphology when compared to the UUO group. Expressions of α-SMA in immunohistochemistry showed that the staining positivity decreased in the tubules of the OPZ-treated group.

Conclusions: While the precise mechanism of action remains unknown, our results demonstrated that OPZ exerted a protective role in the UUO-mediated renal fibrosis rat model highlighting a promising therapeutic potency of Nrf2-activators for alleviating the detrimental effects of unilateral obstruction in kidneys.

ARTICLE INFO

Keywords:
Oltipraz [Supplementary Concept]; Renal Insufficiency; Ureter

Int Braz J Urol. 2018; 44: 1243-51

Submitted for publication:
March 28, 2018

Accepted after revision:
July 16, 2018

Published as Ahead of Print:
August 05, 2018

INTRODUCTION

Ureteral obstruction occurs at any stage of life from fetal development to adulthood in any segment of the ureter between ureteral orifices and the renal pelvis. The histopathologic changes are characterized by tubular dilatation or atrophy, inflammatory cell infiltration, fibroblast activation and proliferation, increases in matrix proteins, and progressive tubulointerstitial fibrosis. These histopathologic processes might eventually result in the loss of renal parenchyma leading to permanent renal function deterioration.

One of the most prevalent molecular mechanisms of tubulointerstitial fibrosis due to ureteral obstruction is epithelial - to - mesenchymal transition. Numerous studies have proposed that under pathologic conditions renal tubular epithelial cells may undergo a phenotypic transformation into matrix - producing myofibroblasts by an epithelial - mesenchymal transition (EMT) process (1-3). Activated by several growth factors such as transforming growth factor β (TGFβ), myofibroblasts function as the primary source for producing extracellular matrix (ECM), including collagen and fibronectin. Furthermore, the expression of intercellular epithelial adhesion molecules such as E - cadherin decrease and mesenchymal cell markers such as α - smooth muscle actin (α - SMA), N - cadherin and vimentin increase. For this reason, EMT is recognized as a molecular component of renal fibrosis (4-6).

Reactive oxygen species (ROS) have a major role in the development of renal fibrosis inducing epithelial - mesenchymal transition (EMT) in the presence of UUO. Nitric oxide (NO), a vasodilator, has been implicated in late renal hemodynamic changes and observed upregulated in the kidney during the UUO process. Even though the excessive amounts of nitric oxide (NO) are oxidized into ROS, they have proven to be useful in interrupting signaling and controlling inflammation (7, 8). In addition, renal hydroxyproline (Hyp), which is commonly used to measure the collagen content in biological tissue values, has been used to assess fibrosis (9).

Oltipraz (OPZ), 5 - (2 - pyrazynyl) - 4 - methyl - 1, 2 - dithiole - 3 - thione, is a synthetic dithiolethione that targets Nrf2 (nuclear factor - erythroid - 2 - related factor 2), an agent that plays a pivotal role in cellular defense against oxidative stress by promoting the transcription of various antioxidant genes (10). Several agents have been used to prevent UUO - induced renal injury in animal models but there is no experiment for the role of oltipraz (OPZ) in the literature. A randomized, double-blind, placebo - controlled phase II trial demonstrated that reductions in inflammation, oxidative stress and fibrosis could be achieved using oltipraz (OPZ), nuclear factor - erythroid - 2 - related factor 2 (Nrf2) activator in patients with liver fibrosis or cirrhosis (11).

Based on these findings, we investigated the potential effect of OPZ in attenuating renal fibrosis induced by UUO in rats.

MATERIALS AND METHODS

Animals

Male Wistar albino rats, weighing 200 to 250 g and six to seven weeks old, were housed in clean plastic cages in a temperature - and humidity - controlled facility under constant 12 - hour light / 12 - hour dark photoperiods with free access to food and water. The Institutional Animal Care and Use Committee approved the use of animals and the experimental protocol and animals were treated according to the Guide for the Care and Use of Laboratory Animals of Research Council.

Treatment and experimental protocols

One week after acclimatization, UUO was induced. Briefly, after induction of general anesthesia by intraperitoneal injection of thiopental (100 mg / kg), the abdominal cavity was exposed via a midline incision and the left ureter was ligated at two points with 3 - 0 silk. The sham - operated rats had their ureters manipulated but not ligated. All rats were given amikacin sulfate (6 mg / kg, intramuscular route) before the operation (12). After a quarantine period of seven days, 32 rats were randomly divided into four groups, each consisting of eight animals as follows: Rats in group 1 underwent a sham operation and received no treatment. Rats in group 2 underwent a sham operation and received OPZ (Sigma Chemical Co.,

St. Louis, MO) (p.o. 30 mg / kg body weight / day). Rats in group 3 underwent unilateral ureteral ligation and received no treatment. Group 4 rats were subjected to unilateral ureteral ligation and received OPZ. The OPZ dose was based on the previous studies (13).

At 14 days after UUO, all rats were sacrificed by high - dose ketamine. Kidneys were reached with an abdominal midline incision. The left kidney was immediately excised and separated from the surrounding tissues, washed twice with cold saline, and stored at - 800 C to determine the markers of renal fibrosis, EMT and tubular injury.

A portion of the left renal tissue was stored in formol solution for the histopathologic and immunohistochemical examinations. Paraffinized tissue samples were examined for leukocyte infiltration and renal fibrosis.

Measurement of transforming growth factor beta - 1, E - cadherin, nitric oxide and hydroxyproline levels.

The TGFβ - 1 ELISA kit (ref MB100B, R &t D Systems) was used to measure TGFβ - 1 in renal tissue 50 microns of total proteins from each tissue were assayed. This assay detects activated TGFβ-1 with a sensitivity of 4.6 pg / mL. The coefficient of variation intra-assay is < 4%, and the coefficient inter - assay is < 8%.

The concentration of soluble E - cadherin was measured with a commercially available sandwich enzyme - linked immunosorbent assay kit based on monoclonal antibodies (Zymed Laboratories Inc., CA). The coefficient of variation intra - assay is < 5%, and the coefficient inter - assay is 7%. The sensitivity is 2.0 ng / mL.

The nitrate concentrations in samples were assayed by enzymatically reducing nitrate. 50 microL of samples were incubated with the same volume of reductase buffer (0.1M) potassium adenine dinucleotide and four units of nitrate curve were obtained by incubating sodium nitrate (10 - 200 µM) with the buffer. The total amount of nitrite and the amount of nitrite in the samples was then determined using the Griess method (14). The samples were incubated with the same volume of Griess reagent (1% sulphanilamide and 0.1% naphthyl ethylenediamine dihydrochloride in 5% phosphoric acid). The absorbance at 550 nm was determined using a multiwell plate reader. The results were reported as the concentration of nitrate plus nitrite (microM NO3 + NO2) for samples of nitrite for supernatants.

Renal tissue fragments were homogenized in saline 0.9%, frozen and lyophilized. The assay was performed with 40 mg of the lyophilized tissue that was subjected to alkaline hydrolysis in 300 micros plus 75 microLNaOH 10 moL / L at 120 degrees C for 20 minutes. An aliquot of 50 microL of the hydrolyzed tissue was added to 450 microL of chloramine T oxidizing reagent (Chloramine T 0.056 moL / L, n - propanol 10% in acetate / citrate buffer pH 6.5) and allowed to react for 20 minutes. A hydroxyproline standard curve with the highest concentration of 400 microns was prepared in a similar fashion. The color was developed by the addition of 500 microL of the Ehrlich reagent (p - dimethylamine - benzaldehyde, 1 moL / L) diluted in n - propanol / perchloric acid, 2: 1 supernatant was transferred to 96 - well plates, and the absorbance was read at 550 nm.

Histopathological and immunohistochemical examination

Histopathological evaluation was carried out on kidney tissues. Paraffin - embedded specimens cut into 6 - µm thickness sections were processed with hematoxylin and eosin stain for examination under the light microscope using a conventional protocol (15) (BH - 2; Olympus, Tokyo, Japan). Semi - quantitative evaluation of renal tissues was performed by scoring the degree of severity according to previously published criteria (16). All sections of kidney samples were examined for tubular necrosis. Briefly, a minimum of 50 proximal tubules associated with 50 glomeruli were examined for each slide and an average score was obtained. The severity of lesion was graded from 0 to 3 according to the percentage of tubular involvement. Slides were examined and assigned for severity of changes using scores on a scale in which (0) denotes no change, grade (1) changes affecting < 25% tubular damage (mild), grade (2) changes affecting 25 - 50% of tubules (moderate) and grade (3) changes affecting > 50% of tubules (severe).

The histopathological and immunohistochemical evaluation were performed on left kidney tissues. Paraffin - embedded specimens were cut into 5 - mm thick sections and stained with hematoxylin and eosin, Masson's trichrome and α - smooth muscle actin (α - SMA) were used for examination under the light microscope (BH - 2; Olympus, Tokyo, Japan).

To evaluate leukocyte infiltration, the widening of interstitial spaces with focal leukocyte infiltration was assessed in five randomly chosen sections prepared from each kidney sample. For each section, the average number of leukocytes per 0.28 mm^2 was calculated from these leukocyte - infiltrated foci using a high - power microscopic field (x 400).

To estimate the grade of interstitial fibrosis, the interstitial area that was stained green with Masson's trichrome was evaluated as a percentage of the total examined area in five randomly chosen sections prepared from each kidney sample using an image analyzer (Leica; Leica Micros Imaging Solutions, Cambridge, UK). For each section, interstitial space widening with focal leukocyte infiltration and interstitial fibrosis was assessed in high - power fields (x 400) to quantify the results. The Banff classification of kidney pathology was used to score the degree of mononuclear cell infiltration and interstitial fibrosis. The score was graded from 0 to 3, depending on the severity of histological characteristics (17).

Statistical analyses

Continuous variables of all groups were demonstrated as mean values ± standard deviation (SD). Statistical analyses of the histopathologic evaluation of the groups were carried out by the Chi - square test, and biochemical data among four groups were analyzed by Kruskal - Wallis test. The p - value of < 0.05 was accepted as statistically significant. In case a statistical significant was observed between three or four groups in Kruskal - Wallis test, Mann - Whitney U test was utilized for the detection of the significance between two groups.

RESULTS

Transforming growth factor beta - 1, E - cadherin, nitric oxide and hydroxyproline levels TGF - β1 level in sham, UUO and UUO + OPZ groups were 2.5 ± 0.6; 7.2 ± 1.5 and 3.6 ± 0.9 respectively. NO level in sham, UUO and UUO + OPZ groups were 20.6 ± 3.5; 62.3 ± 19.8 and 37.4 ± 15.1 respectively and E - cadherin level in those groups were 3.7 ± 0.5; 7.4 ± 1.2 and 3.9 ± 1.8 respectively. TGF - β1, NO, and E - cadherin levels were significantly higher in the UUO group than the sham group. UUO + OPZ group had lower levels of these markers compared to the UUO group. Hydroxyproline level was 423.5 ± 63.8 in UUO group, whereas the remaining three groups had lower levels; but this difference did not reach a statistical significance. The details are shown in Table-1.

Histopathologic and immunohistochemical examination results

Histopathologic examination of kidneys showed no pathologic findings in the sham and sham + OPZ groups (Figures 1A and 1B). Mild and severe tubular necrosis in the proximal tubu-

Table 1 - TGF-β1, E-cadherin, NO and Hydroxyproline levels in kidney.

Parameters	Sham	Sham + OPZ	UUO	UUO+OPZ
TGF-β1(pg/ mL)	2.5 ± 0.6	2.7 ± 0.5	7.2 ± 1.5	3.6 ± 0.9*
E-cadherin(ng/ mL)	3.7 ± 0.5	3.5 ± 0.6	7.4 ± 1.2	3.9 ± 1.8*
NO(μmoL/g)	20.6 ± 3.5	19.5 ± 2.9	62.3 ± 19.8	37.4 ± 15.1*
Hydroxyproline (pg/mL)	357.5 ± 84.9	368.1 ± 51.8	423.5 ± 63.8	386.8 ± 62.1

TGF = transforming growth factor β1; **NO =** nitric oxide.
Values are expressed as mean ± SD for eight rats in each group.
* Significantly different from UUO group ($p < 0.05$).
TGF-β1 = NO and E-cadherin levels in UUO group were significantly higher than Sham group, in treated groups these values were significantly different from UUO group. Hydroxyproline levels were higher in UUO group, but results were not significant among other groups.

les was found in rats with UUO compared to the sham group (Figure-1C). Despite the presence of mild tubular degeneration and less severe tubular necrosis in rats treated with UUO + OPZ, glomeruli maintained a better morphology compared to the UUO group (Figure-1D).

Histopathologic examination was normal in rats with the sham operation (group 1). Severe leukocyte infiltration was observed in the periglomerular and peritubular interstitium of the rat kidneys in group 3 with UUO (Figures 2A and B). Quantitative analysis of the focal leukocyte infiltration area in the interstitium showed that leukocyte infiltration was significantly reduced in rats administered with UUO + OPZ (group 4) (Figure-2C).

UUO caused significant interstitial fibrosis in rats that received no treatment (group 3). The percentage of the area of interstitial fibrosis in the rats with UUO that received no treatment was significantly greater than that of rats with UUO that received OPZ (group 4) (Figures 3A-C). These changes are summarized in Table-2. Expressions of α - SMA in immunohistochemistry showed that the staining positivity decreased in the tubules of the OPZ - treated group (Figures 4A-C).

DISCUSSION

In this study, we analyzed the protective effect of OPZ against renal fibrosis in a rat UUO model, a well - established in vivo model of renal fibrosis. Our study confirmed the protective role of OPZ through a quantitative examination of renal tissue damage after the induction of UUO in rats. To

Figure 1 - A) Normal tubulus and glomeruli in kidney cortex H&Ex100 (sham group). B) Normal tubulus and glomeruli in kidney cortex H&Ex100(sham+OPZ group). C) Severe tubular total necrosis, tubular degeneration and epithelial vacuolization in the proximal tubules H&Ex400(UUO group). D) Mild epithelial vacuolization in the proximal tubules and normal glomeruli H&Ex100 (UUO+OPZ treated group).

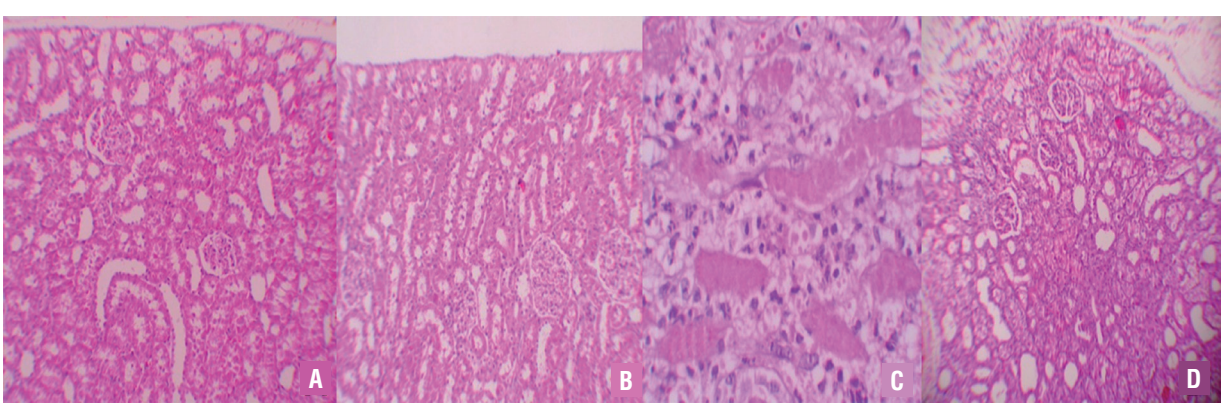


Figure 2 - A) Normal kidney morphology in a sham group. B) Leukocyte infiltration was observed in the peritubular interstitium of the UUO. C) Leukocyte infiltration was reduced in the OPZ-treated group (hematoxylin&eosin,*400).

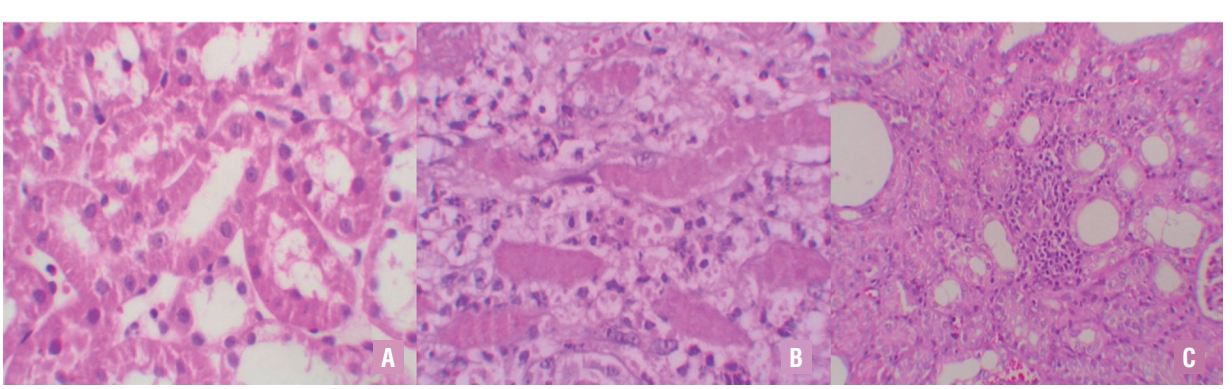


Figure 3 - A) Normal kidney morphology in a sham group. B) Severe fibrosis was observed in the peritubular interstitium of the UUO. C) Mild fibrosis was reduced in the OPZ-treated group (Masson&Trichrome, *400).

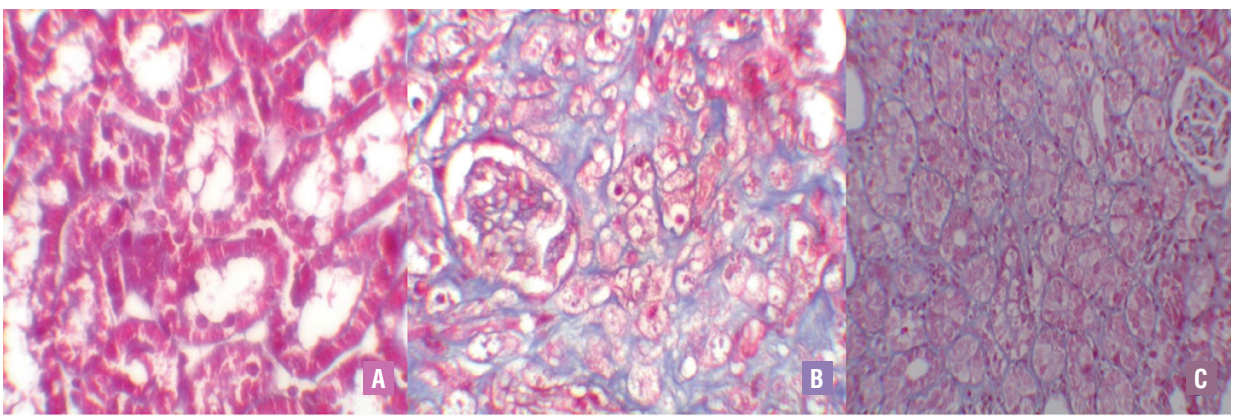
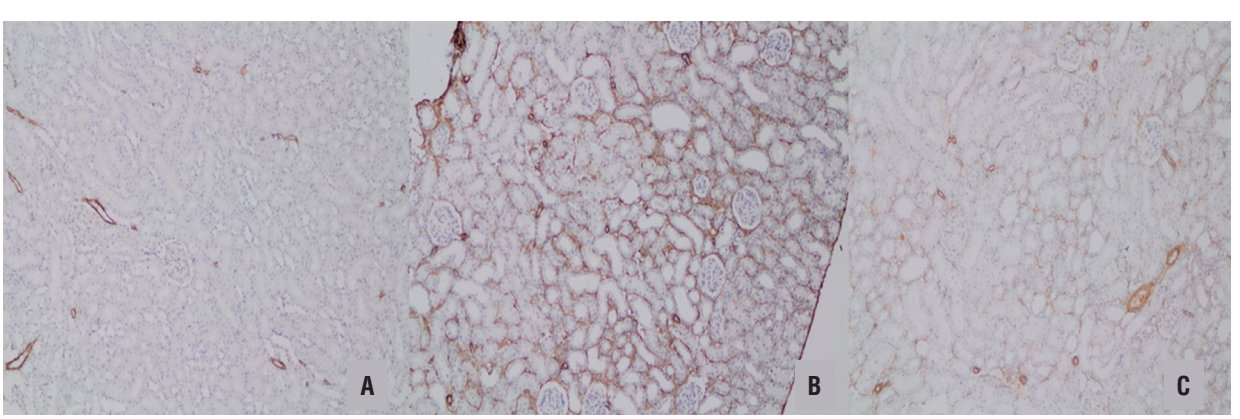


Table 2 - Scoring of tubular necrosis.

	Tubular necrosis					Interstitial fibrosis				Mononuclear cell infiltration			
	n	0	1	2	3	0	1	2	3	0	1	2	3
Sham	8	8	0	0	0	8	0	0	0	8	0	0	0
Sham+OP	8	8	0	0	0	8	0	0	0	8	0	0	0
UUO	8	0	1	3	4	0	1	4	3	0	1	2	5
UUO+OPZ*	8	0	6	2	0	2	5	1	0	1	5	0	2

Score 0 = no degeneration; **1 =** mild degeneration; **2 =** moderate degeneration; **3 =** severe degeneration
* Statistical significant difference from the UUO group and $P < 0.05$.

Figure 4 - A) α-SMA staining was not observed in the tubules of the sham group. B) Moderate αSMA positivity was observed in more than 50% of the tubules in the UUO group. C) mild αSMA positivity was observed in 25-50% of the tubules in the OPZ-treated group (αSMAx100).



the best of our knowledge, this report is the first to show that OPZ has a preventive effect on functional, histological kidney injury caused by UUO.

The molecular mechanisms of tubulointerstitial fibrosis owing to UUO include several components such as inflammatory cell infiltration, fibroblasts and extracellular matrix production, and epithelial to mesenchymal transition. Among these, EMT has been of great interest to researchers during recent decades. EMT has been asserted as a functional and phenotypic change of epithelial cells that is reminiscent of mesenchymal cells reflecting a global process affecting adjacent cells (18). During the EMT process, expression of epithelial adhesion molecules such as E - cadherin is decreased whereas mesenchymal marker proteins such as α - smooth muscle actin (α - SMA) and N - cadherin are up - regulated. The EMT process has been viewed as a pathological predicator of renal fibrosis as myofibroblasts transformed from epithelial cells act as the main origin of ECM production (19). Moreover, the phenotype transformation of renal epithelial cells can cause dysfunction of the kidney, eventually resulting in glomerulosclerosis. Furthermore, accumulating lines of evidence support the concept that ROS affects EMT changes. Higher levels of ROS may aid the EMT process of epithelial cells and lead to fibrosis in the context of transcription factors and the multifunctional role of ROS in cellular signaling pathways (20, 21).

Nuclear factor - erythroid - 2 - related factor 2 (Nrf2) is a vital molecule of the endogenous antioxidant system that plays a central role in stimulating expression of various antioxidant - associated genes in cellular defense against oxidative stress. In the absence of oxidative stress, Nrf2 resides in the cytoplasm together with its repressor kelch - like ECH - associated protein 1 (KEAP1). Many agree that the upregulating in the production of antioxidant enzymes, which follows the detaching of Nrf2 from KEAP1 and subsequent movement into the nucleus, is a result of ROS overproduction or results from responses to electrophilic reagent treatment (22). Debates continue as to whether the EMT process is a real and direct contributor to renal fibrosis pathology in vivo (23, 24).

Based on the results showing the linkage between Nrf2 and TGFβ1 signaling, we hypothesized that Nrf2 could have a potential role in protecting the kidney in unilateral ureteral obstruction. It is believed that oltipraz, a prototype dithiolethione, might support the joining of the Nrf2 to the antioxidant response element (25).

We measured renal cortical E - cadherin, TGF - β1, hydroxyproline and NO levels biochemically as evidence of EMT. Except for the hydroxyproline level, all these markers were heightened in UUO rats and OPZ attenuated their levels. These results suggest that OPZ ameliorates the renal fibrosis by inhibiting these markers, which are the indicators of renal fibrosis. The possible explanation for the level of hydroxyproline not being significant among the groups might be the fact that the duration of UUO may be insufficient for observing the exact fibrotic changes in the kidney. It is probable that hydroxyproline, being a component of collagen, would be detected as increased in UUO kidney rats if they were examined one month or later after the obstruction date.

Additionally, pooled urine in UUO is markedly hypotonic (26). In theory, hypotonic cell growths could be caused by the hypotonic nature of pooled UUO urine. Increases in E - cadherin expressions and the induction of connective tissue growth factors (CTGF) were instigated through hypotonic media. It has been proposed that CTGF is an element of renal EMT via evidence of upregulation in UUO (27, 28).

We think that E - cadherin can be simultaneously upregulated in response to hypotonic stretch, suggesting that profibrotic activation of tubular cells might not require a 'classical' EMT process.

In this study, the histopathologic examination of kidneys showed severe and extensive damage in UUO rats with tubular necrosis and edema. This could be due to the formation of highly reactive radicals as a consequence of oxidative stress caused by UUO. The kidneys of the sham group showed normal histological features, but the UUO group revealed more extensive and marked tubular necrosis. On the other hand, the tubules from rats of the UUO + OPZ group were nearly normal in histological appearance except

for slight desquamation and atrophy of the tubular epithelial cells. Also, expressions of α - SMA in immunohistochemistry showed that the staining positivity decreased in the tubules of the OPZ - treated group. Scientific evidence from the various field of diseases including pulmonary hypertension, liver ischemia / reperfusion injury yielded promising results on the potential effect of OPZ (29, 30). In a cell culture study, Atilano - Roque A et al. demonstrated that Nrf2 activating agent, OPZ had a beneficial effect on the viability of human kidney cells and expression of antioxidant and efflux transporter genes in proximal tubules which was exposed to cisplatin. Our results have similar findings compared to these studies. Given the similar pathophysiologic mechanisms, we may propose that OPZ is a promising agent for alleviating kidney injury in the presence of the ureteral obstruction.

We should address some potential limitations of this study. Firstly, we measured NO levels and reported that rats with UUO had increased levels of NO. However, our study lacks the measurement of NO isoforms including neuronal, endothelial, and inducible NOS. Secondly, although we exerted the conventional methods including the detection of the levels of molecules as mentioned earlier and histopathologic examination of renal tissue, we did not use quantitative RT - PCR, western blot, and immunofluorescence analysis to analyze further and demonstrate the expression of cell markers. Thirdly, although UUO is a well - accepted animal renal fibrosis model, it is criticized for the fact that the contralateral kidney may compensate for the deteriorated function of the obstructed kidney precluding meaningful renal function measurements (31). Lastly, whether the sensitivity and specificity of the markers used for EMT in our study are higher than some other EMT markers such as vimentin, cytokeratin, and β catenin is not known. Further studies on these markers will probably yield more insights to this debate.

CONCLUSIONS

Taken all together, while the mechanism of actions remains unknown, our results show that OPZ plays a protective role in UUO - mediated renal fibrosis. However, further well - designed animal and clinical studies are needed to confirm our results.

CONFLICT OF INTEREST

None declared.

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Correspondence address:
Emre Can Polat, MD, FEBU
Department of Urology
Okmeydani Training & Research Hospital
University of Health Sciences,
34384, Sisli, Istanbul, Turkey
Fax: + 90 212 314-5503
E-mail: dremrecan@hotmail.com

CHALLENGING CLINICAL CASES

Vol. 44 (6): 1252-1255, November - December, 2018
doi: 10.1590/S1677-5538.IBJU.2017.0588

Encrusted cystitis caused by corynebacterium urealyticum: a case report with novel treatment strategy of intravesical dimethyl sulfoxide

Tayyar Alp Ozkan [1], Mustafa Savas Yalcin [2], Ozdal Dillioglugil [2], Ibrahim Cevik [3]

[1] Department of Urology, Kocaeli Derince Traning and Research Hospital, Kocaeli, Tukey; [2] Department of Urology, Kocaeli University, School of Medicine, Kocaeli, Tukey; [3] Okan University, School of Medicine, Department of Urology, Istanbul, Tukey

ABSTRACT

Encrusted cystitis (EC) was first described as chronic cystitis with mucosal calcification in 1914 (1). It is a very rare chronic inflammatory disease presenting with dysuria, pelvic pain and gross hematuria. Voided urine contains mucus or calcified mucopurulent stone like particles. Urinalysis always reveals alkaline pH. It may be present in healthy individuals with no predisposing etiological factors (2-4). Etiologically, previous urological diseases, immunosuppression, urinary infection with urea splitting bacteria, or urological interventions resulting in bladder mucosa trauma may also be present (5, 6). In the present case report, we describe a novel treatment for EC with intravesical dimethyl sulfoxide.

ARTICLE INFO

Keywords:
Corynebacterium; Cystitis; Dimethyl Sulfoxide

Int Braz J Urol. 2018; 44: 1252-5

Submitted for publication:
November 01, 2017

Accepted after revision:
March 26, 2018

Published as Ahead of Print:
April 15, 2018

CASE REPORT

We report a case of a 40-years old female with a history of transurethral bladder tumor resection (TUR-BT) in 2012. She was admitted to an outpatient clinic elsewhere in January 2015 with pelvic pain and recurrent urinary tract infection for the past 4 years. Diagnostic cystoscopy elsewhere revealed stone like particles covering the bladder mucosa. Later on, several TUR-BTs had been performed to remove these lesions in various hospitals elsewhere and histopathology reports revealed non-specific chronic cystitis without tumor. Patient had a re-TUR-BT in March 2015 again elsewhere for suspicious tumor, macroscopic hematuria and voiding stone like particles in urine. A necrotic bladder mucosa containing calcified encrustations with underlying inflammatory polymorphonuclear cell infiltration with abundant blood vessels was observed. Pathology result for this TUR-BT was encrusted cystitis (EC) (Figures 1A and B). Patient had a negative urinary tuberculosis screening, negative tuberculosis culture and PCR.

Patient was admitted to our clinic with severe pain, gross hematuria, and voiding stone like

particles, and she had lower urinary tract symptoms (LUTS) reminiscent of interstitial cystitis. Urinalysis showed alkaline urine with struvite crystals in the sediment. Her functional bladder capacity was 75 mL. Urine culture was sterile. An irregularity on the right bladder wall, and a moderate hydro-nephrosis at the right side was revealed with ultrasound. Filling defect at the dome and right bladder wall was also seen in magnetic resonance study (Figure-2). A diagnostic cystoscopy showed a calcified, hyperemic, fragile, edematous mucosa involving the whole bladder dome and right lateral wall covering right ureteral orifice. These lesions were completely removed with TUR, and part of the material and urine from bladder barbotage was sent for specific bacteriologic culture for Corynebacterium urealyticum, which was positive. Treatment was instituted according to antimicrobial susceptibility tests. For 2 weeks intravenous teicoplanin 400 mg/day (minimum inhibitory concentration 90% 0.5 micrograms/mL), was given, and weekly intravesical dimethylsulfoxide (DMSO) treatment was started for 6 weeks. A standard solution of 50 mL of 50% DMSO (Rimso-50®) in aqueous solution (each mL solution contains 0.54 gr of DMSO) was administrated intravesically with a 10 French catheter, weekly for 6 weeks. Patient was allowed to void after 1 hour. LUTS relieved immediately. She had a cystoscopy with normal signs of bladder at 6th month of follow-up. At 18th month follow-up she was free from any complaint and infection with a remarkably increased functional bladder capacity of 340 mL.

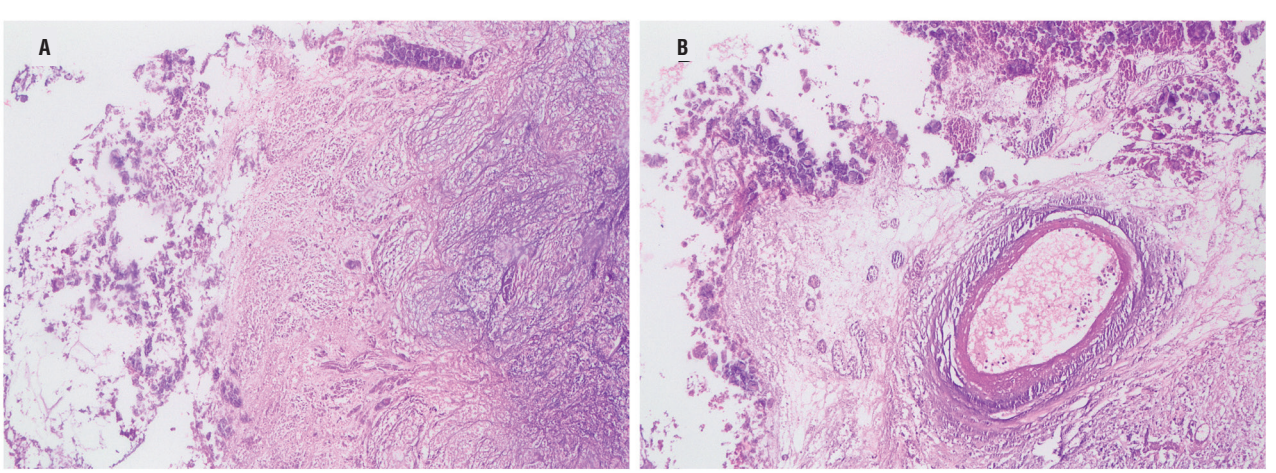
DISCUSSION AND FUTURE PERSPECTIVES

Urea splitting bacteria play a role in the EC etiology. A bacterial culture is essential for the bacterial confirmation of the condition. Though sterile urine culture should not rule out the diagnosis of EC, prolonged culture or tissue cultures should be performed. A prolonged 48 to 90 hours 5% carbon dioxide or sheep blood agar urine culture may be helpful when EC is suspected in the presence of sterile pyuria (3, 7).

Risk factors such as intravesical chemotherapy or BCG installations, urinary trauma, or bacterial urease activity results in ammonia release, which in turn damages the glycosaminoglycan layer of the bladder mucosa (2). As a consequence of this, characteristic encrustations composed of calcified plaques in the interstitium of the bladder mucosa occur.

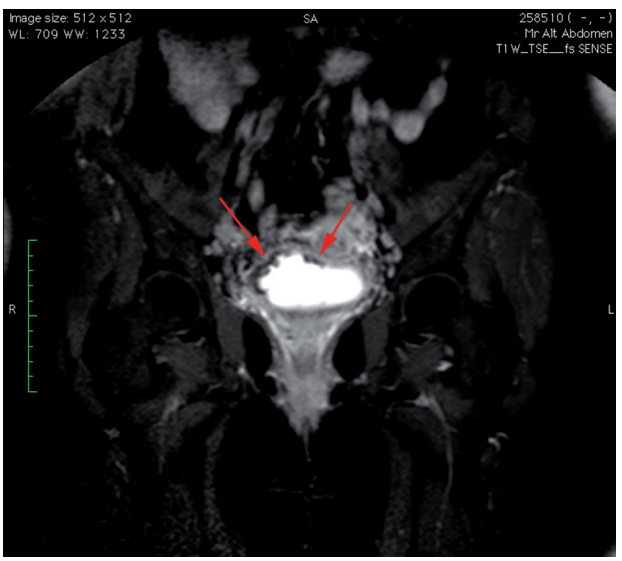
Having a positive urine culture for C urealyticum does not necessarily means that patient has calcified encrusted particles in the urothelial mucosa. EC incidence was reported as 15.6% (n=18/115) for patients with positive C urealyticum urine culture (8). In this study, patients were

Figures 1 A and B - Bladder transurethral biopsy of encrusted areas 200xHE



The deposits of calcium on the mucosa and chronic inflammatory infiltrate in the lamina propria.

Figure 2 - Coronal magnetic resonance image of bladder demonstrating filling defects (arrows) of right bladder wall.



treated with bladder instillation, and/or surgery in combination with antibiotics. The bladder had been irrigated with acidifying solutions such as, G-suby, Thomas, and N-acetylcysteine + acetohydroxamic acid + aztreonam combination. They reported that acidifying with these solutions was not completely effective to calcified particles. Alkaline urine is a product of a urea splitting bacteria. In our experience, we use DMSO in combination with TUR-B and antibiotics to resolve symptoms related to EC, though promising results were obtained even with a relatively high pH solution.

In our present case, the patient had a TUR--BT history, and she presented with LUTS, gross hematuria and micturition of sandy grits. Urine culture was sterile, and ultrasound revealed stone like particles. Patient had a re-TUR-BT and part of the material and urine from bladder barbotage was sent for C urealyticum culture, which was positive. Treatment with teicoplanin 400 mg/day, and weekly intravesical DMSO was started.

C urealyticum is the most commonly reported cause of EC, and it has multiple antibiotic resistance (9). Teicoplanin, vancomycin and glycopeptides are the antibiotics of choice in the first line treatment of C urealyticum. However, EC usually does not respond to antibiotic treatment alone. The bacteria contained in calcified plaques prevent antibiotic penetration. Multimodal treatment consists of plaque resection, urinary acidification and antibiotic treatment (3).

To the best of our knowledge, DMSO has never been reported as treatment agent for encrusted cystitis. DMSO does not act as an acid or base with a pKa=35. It is an important polar aprotic solvent that dissolves both polar and non--polar compounds. It is miscible in a wide range of organic solvents as well as water. DMSO is a FDA approved drug mainly used in interstitial cystitis/painful bladder syndrome. DMSO plays a role in replenishing the damaged glycosaminoglycan layer and it has anti-inflammatory activity on injured bladder urothelium (10). The presence of osteocalcin and ostonectin in EC urothelium has hypothesized that EC may be related with systemic inflammation (11). DMSO is a weak acid with pH of 6.7. It is an aprotic solvent that replenish glycosaminoglycan layer and has anti-inflammatory effect on urothelium. We can hypothesize that anti-inflammatory treatment enhancing urothelial recovery may improve patient's symptoms and treat EC. In our patient, no discomfort except sensation of bladder burning was observed. Quite contrary to acidic solutions, pain and swelling due to DMSO treatment were caused by bladder inflammation or irritation.

In conclusion, rare pathologies such as EC are limited to case reports and case series. Although urine acidification has been reported to be a component of the treatment, bladder irrigation with an aprotic solvent was effective in our case, and has not been reported previously. Further studies are needed to confirm our treatment effect and results.

CONFLICT OF INTEREST

None declared.

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Correspondence address:
Tayyar Alp Ozkan, MD
Department of Urology,
Kocaeli Derince Traning and Research Hospital
Ibni Sina Blv 1, 41200 Derince, Kocaeli, Turkey
Fax: + 90 262 317-8070
E-mail: tayyaralp.ozkan@saglik.gov.tr

RADIOLOGY PAGE

Vol. 44 (6): 1256-1257, November - December, 2018
doi: 10.1590/S1677-5538.IBJU.2017.0199

Neurofibromas of the bladder in a child with neurofibromatosis type 1

Gulec Mert Dogan [1], Ahmet Sığirci [1], Leyla Karaca [1]

[1] *Department of Radiology Pediatric, Inonu University Malatya, Turkey*

CASE DESCRIPTION

A 17-year-old boy diagnosed with neurofibromatosis type 1 (NF1) presented with a six-month history of hematuria, dysuria, and urinary frequency. Ultrasonography (USG) revealed diffuse thickening of the anterosuperior and posterior walls of the bladder with round, <5mm nodular echogenities in the thickened walls (Figure-1). Magnetic resonance imaging (MRI) of the pelvis revealed a nodular lesion with low signal intensity on T1 and fat suppressed T1 weighted (T1-W) images; and nodular lesions with a 'target sign' on T2 weighted (T2-W) images. This consisted of low signal intensity fibrosis surrounded by high signal intensity stroma at the posterior of the bladder wall (Figures 2 and 3). The patient's symp-

Figure 1 - Ultrasound imaging; Diffuse thickening of the anterosuperior and posterior walls of the bladder and multiple round, <5mm nodular echogenities in the thickened walls marked with arrows.

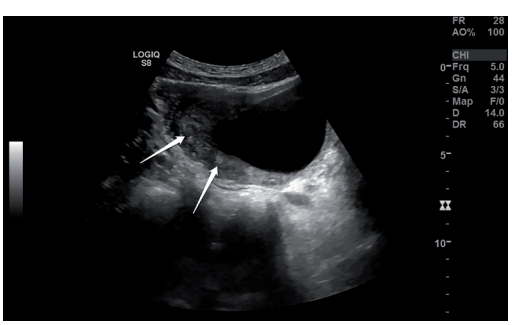


Figure 2 - MRI axial images; nodular lesions marked with arrows. A. T1 weighted B. post contrast T1 weighted fat suppressed C. T2 weighted fat suppressed image.

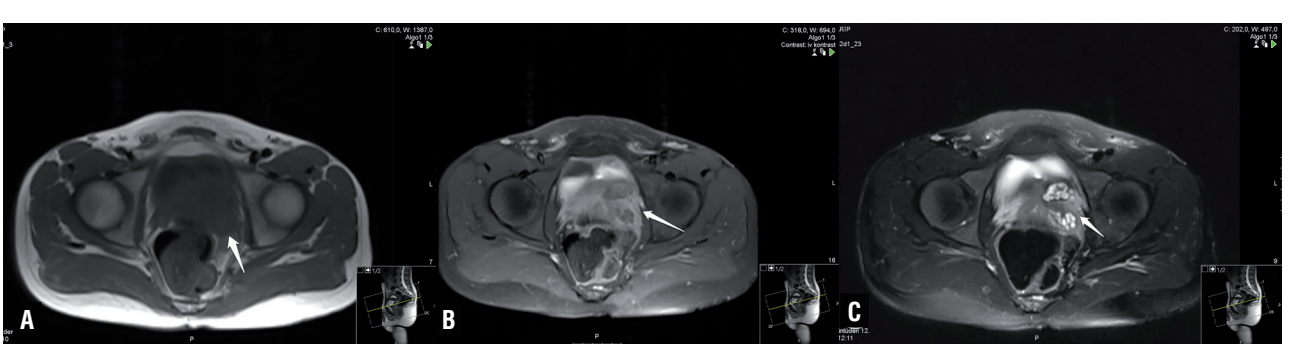
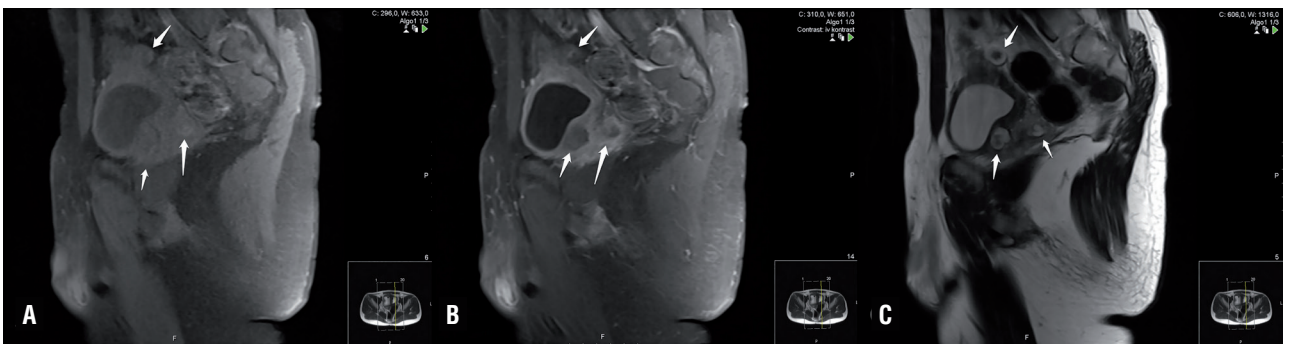


Figure 3 - MRI sagittal images; nodular lesions marked with arrows A. T1 weighted fat suppressed B. Post contrast T1 weighted fat suppressed C. T2 weighted image.



toms were relieved after antibiotic treatment and he has had no serious complaints since then. He is now monitored by the urology outpatient clinic.

Children with NF1 should always be evaluated for neurofibromatosis of the genitourinary system (1). Bladder involvement of neurofibromatosis is rare and presenting features include irritative voiding symptoms and hematuria due to recurrent urinary tract infections (2). On USG, bladder involvement of neurofibromas can manifest as a focal mass or as diffuse bladder wall thickening. On MRI, neurofibromas display low-signal intensity on T1-W images and non-homogeneous high-signal intensity with a 'target sign' on T2-W images (3). Differential diagnosis includes rhabdomyosarcoma, ganglioneuroma, and retroperitoneal fibrosis (4). In a patient with NF1, the primary consideration should be neurofibroma. Generally, management of patients with NF1 and bladder involvement is conservative. If there are intractable symptoms such as hydronephrosis, bladder volume loss and suspicion for malignant degeneration, surgical treatment may be needed (3).

In conclusion, conventional MRI and ultrasound are important imaging modalities for the evaluation of genitourinary involvement of neurofibromatosis disease type 1.

CONFLICT OF INTEREST

None declared.

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Correspondence address:
Gulec Mert Dogan, MD
Department of Radiology Pediatric
Inonu University Malatya
Malatya 44280, Turkey
E-mail: dr_gulecmert@hotmail.com

ARTICLE INFO

Int Braz J Urol. 2018; 44: 1256-7

Submitted for publication:
April 05, 2017

Accepted after revision:
December 27, 2017

Published as Ahead of Print:
February 22, 2018

RADIOLOGY PAGE

Vol. 44 (6): 1258-1260, November - December, 2018
doi: 10.1590/S1677-5538.IBJU.2017.0468

Recto-urethral fistula presenting as fever of unknown origin: a rare complication of prostatic abscess

Sun Hwa Lee [1], Seong Jong Yun [2], Seokyong Ryu [1]

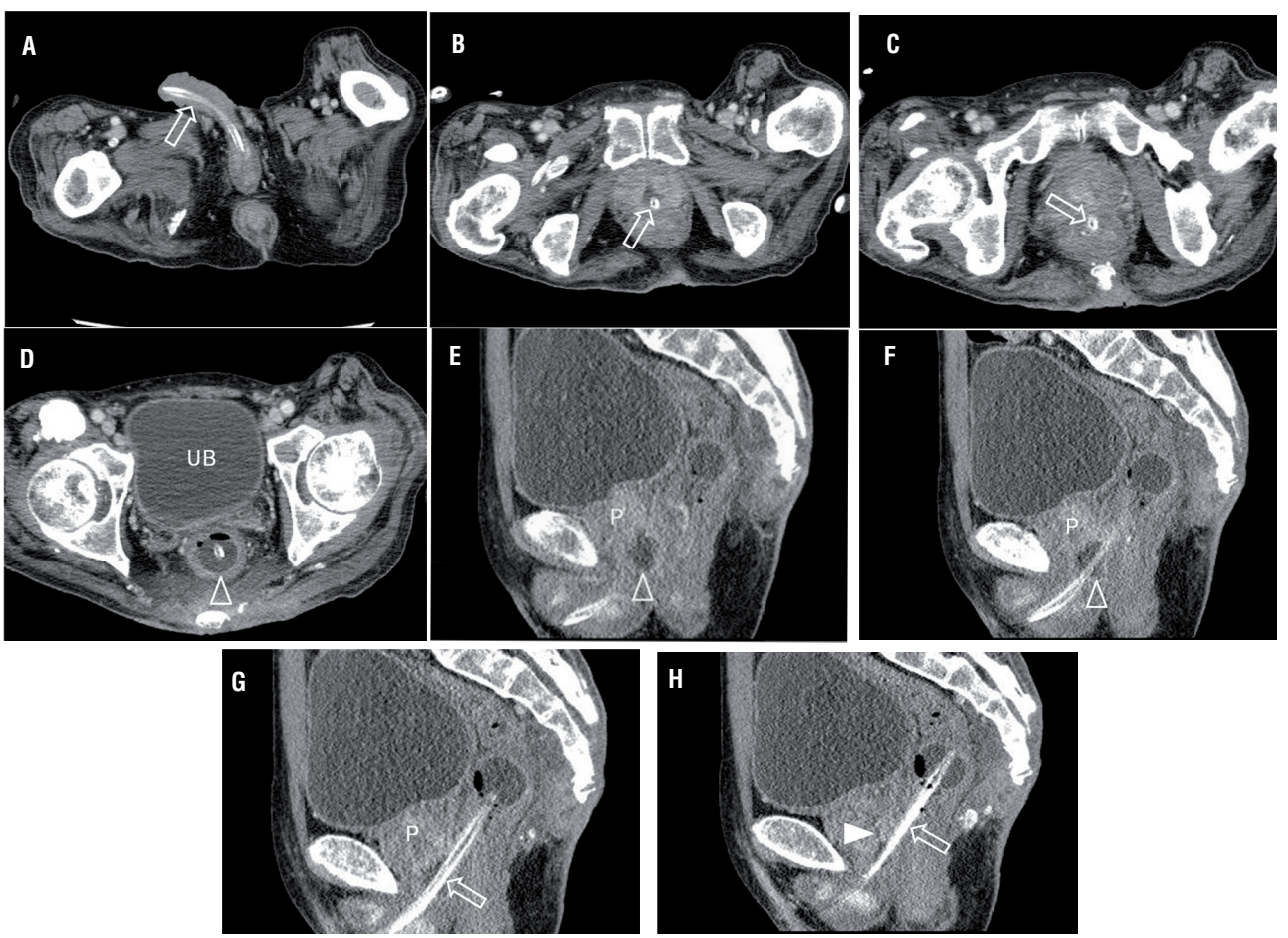
[1] Department of Emergency Medicine, Sanggye Paik Hospital, Inje University College of Medicine, Seoul, Republic of Korea; [2] Department of Radiology, Kyung Hee University Hospital at Gangdong, Kyung Hee University School of Medicine, Seoul, Republic of Korea

CASE DESCRIPTION

A 76-year-old man was admitted in the emergency department complaining of fever of unknown origin for 1 month. His medical history was only significant for stroke, but there was no history of neoplasm, trauma, chemo-radiation, or other surgeries. He was admitted with a long-term Foley catheter in situ, which was inserted one year prior due to dysuria and changed regularly every 4-5 days. The present Foley catheter was inserted 4 days prior and the patient's urine color gradually changed and was dark green on presentation to hospital. Laboratory tests showed elevated white blood cell count (17.900/μL) and C-reactive protein (5.70mg/dL). In urine analysis, pyuria was seen. Abdominopelvic computed tomography (APCT) revealed there was no evidence of urinary tract infection or acute pyelonephritis. However, malposition of the Foley catheter was seen. It was located along the urethra-prostate--rectum (Figures 1A-1D). Also, a prostatic abscess between the prostatic urethra and rectum was bulging and abutting to the anterior wall of the rectum (Figures 1E-1H); thus, we diagnosed the recto-urethral fistula (RUF) caused by prostatic abscess. The patient was treated with intravenous antibiotics and percutaneous nephrostomy for urine diversion. Fecal diversion was not performed because fecaluria was not seen. The patient improved after three months of conservative treatment (pyelostomies) and he was discharged with Foley catheter reinsertion.

RUF is an abnormal connection between the rectum and urethra that is a rare complication of pelvic surgery, radiation, trauma, or infection/inflammation. The incidence of RUF has been on the rise due to an increase in the number of surgeries and pelvic irradiation performed for genitourinary neoplasm (1, 2). The early diagnosis of RUF using APCT in the emergency setting is important to not only confirm the diagnosis and initiate appropriate medical management, but also ensure pre-operative localization in patients that require surgery (3-5). In general, conservative management can be attempted by using urinary/fecal diversion for small (<2cm), simple RUF in non-irradiated patients who do not have sepsis. In contrast, large (≥2cm), complex RUF in irradiated patients may require surgical management (1, 2). Our case underscores physicians need to consider the possibility of RUF for early diagnosis and management in patients with risk factor for prostatic abscess or with history of recent low urinary tract procedure.

Figure 1 - (A–D) Serial axial images of abdominal computed tomography shows malposition of the Foley catheter. It is located along the urethra (arrows, A) -prostate (arrow, B) -rectum (arrow, C), indicating presence of the recto-urethral fistula. The balloon (arrowhead, D) of the Foley catheter is located in the rectum, and not in the urinary bladder (UB). (E-H) Serial sagittal images of abdominal computed tomography reveals the penetration (arrows, G and H) of the prostate (P) with loculated fluid collection with air bubbles in the postero-inferior aspect of the prostate (open arrowhead, E and F), indicating a prostatic abscess. This abscess is bulging and abutting to the anterior wall of the rectum. Normal prostatic urethra is also seen (arrowhead, H). Additionally, there is soft tissue tumefaction involving the presacral space, indicating inflammation. However, bone window setting did not demonstrate bone involvement (no evidence of osteomyelitis).



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Correspondence address:
Seong Jong Yun, MD
Department of Radiology
Kyung Hee University Hospital at Gangdong,
Kyung Hee University School of Medicine,
892 Dongnam-ro, Gangdong-gu,
Seoul 05278, Republic of Korea
Fax: + 82 2 440 6932
E-mail: zoomknight@naver.com

ARTICLE INFO

Int Braz J Urol. 2018; 44: 1258-60

Submitted for publication:
August 18, 2017

Accepted after revision:
January 10, 2018

Published as Ahead of Print:
March 18, 2018

VIDEO SECTION

Vol. 44 (6): 1261-1261, November - December, 2018
doi: 10.1590/S1677-5538.IBJU.2017.0384

Robotic excision of complex adrenal mass with retrocaval extension and encasement of renal hilum with renal preservation

Vishnu Raveendran [1], Ramaprasad Manasseri Koduveli [1], Kishore Thekke Adiyat [1]

[1] *Aster Medcity, Kochi, Kerala, India*

ABSTRACT

Objective: The purpose of this video is to present robotic excision of a complex adrenal mass with retrocaval extension and encasement of renal hilum in a 16 year old boy. Biochemical screening was negative for metabolically active component. Computerized tomographic scan with contrast revealed a homogenous mass of approximately 10.8 cm x 6.2 cm x 4.2 cm in the suprarenal area on right side that was extend-ing behind inferior vena cava and encasing renal hilar vessels. Imaging findings were that of a classical ganglioneuroma.
Material and methods: Robot assisted laparoscopic adrenalectomy with sparing of renal hilar vasculature was performed. With patient in lateral position, five ports were used, including one for liver retraction. Da Vinci® system with four arms was docked from over the right shoulder. The displaced renal hilar structures were identified by opening Gerota's fascia. Mass was dissected completely and removed through Pfan-nensteil incision.
Results: Duration of procedure was 345 minutes and console time was 290 minutes. Blood loss was 250 mL. Post-operative renal doppler showed normal blood flow. He was discharged on post-operative day three. Histopathologic examination of specimen revealed ganglioneuroma arising from adrenal gland.
Conclusion: Ganglioneuroma is a rare adrenal tumor with good prognosis on surgical removal. The advent of robotic surgery has made complex surgical procedures involving vital structures like inferior vena cava be performed using minimally invasive techniques without compromising oncologic principles.

CONFLICT OF INTEREST

None declared.

ARTICLE INFO

Available at: http://www.intbrazjurol.com.br/video-section/20170384_Raveendran_et_al
Int Braz J Urol. 2018; 44 (Video #18): 1261-1

Submitted for publication:
July 05, 2017

Accepted after revision:
March 14, 2018

Published as Ahead of Print:
April 5, 2018

Correspondence address:
Vishnu Raveendran, MS
Department of Urology
Aster Medcity
Kochi, Kerala, India
E-mail: drvishnu@outlook.com

VIDEO SECTION

Vol. 44 (6): 1262-1262, November - December, 2018
doi: 10.1590/S1677-5538.IBJU.2018.0105

The Lithocatch™ by Boston Scientific: how to use it and how to solve a common problem

Giuseppe Giusti [1], Marco Lucci Chiarissi [1], Antonello De Lisa [1]

[1] *Department of Urology, University of Cagliari, Cagliari, Italy*

ABSTRACT

Introduction: The Lithocatch™ basket is a immobilization device commercialized by Boston Scientific. It allows to collect multiple stone fragments from the ureter. The ability of the basket to capture a large number of stone fragments, is however responsible for a problem connected to its usage: the entrapment of the basket inside the ureter. In this video we explain how to use it and how to solve this problem.
Material and Methods: After positioning the Lithocatch™ over the fragments, the basket is opened and it is rotated through a special handle to collect stones. One frequent problem occurs when too many fragments are collected at once, preventing the extraction of the device. We research our archives to extrapolate the total number of procedures carried out with the Lithocatch™ in the last two years and the total number of complications occurred.
Results: We experienced the above mentioned complication in 16 procedures (14% of the total) of 114 surgeries performed. The way described to solve this complication was efficient and did not produce any damage to the ureter or to the basket.
Conclusion: The Lithocatch™ has an excellent ability to capture small stones so it allows to reduce the length of the procedure. Paying attention to limit the amount of fragments collected, it is possible to avoid the entrapment of the basket. If this complication occurs, the problem can be solved by reducing the size of the stone fragments. The preferable type of energy is the ballistic one.

CONFLICT OF INTEREST

None declared.

ARTICLE INFO

Available at: http://www.intbrazjurol.com.br/video-section/20180105_Giusti_et_al
Int Braz J Urol. 2018; 44 (Video #19): 1262-2

Submitted for publication:
February 16, 2018

Accepted after revision:
March 26, 2018

Published as Ahead of Print:
April 5, 2018

Correspondence address:
Giuseppe Giusti, MD
Department of Urology
University of Cagliari
Via Is Mirrionis 92
09121, Cagliari, Italy
Fax: +70 280-773
E-mail: giuseppegiusti.med@gmail.com

VIDEO SECTION

Vol. 44 (6): 1263-1264, November - December, 2018
doi: 10.1590/S1677-5538.IBJU.2018.0037

Addressing the challenges of reoperative robotic-assisted sacrocolpopexy

Wilson Lin [1], Nitya Abraham [2]

[1] Yeshiva University Albert Einstein College of Medicine, NY, USA ; [2] Department of Urology, Montefiore Medical Center, NY, USA

ABSTRACT

Sacrocolpopexy is the gold-standard repair for apical pelvic organ prolapse (POP). However, over half of women with POP who undergo the surgery experience recurrence, particularly those with higher preoperative stage, younger age, and greater body weight. We address the challenges of repairing recurrent POP in a patient with a prior transabdominal mesh sacrohysteropexy.

INTRODUCTION

A 50-year-old woman complaining of vaginal pressure presented with Stage II prolapse. She had three previous abdominal surgeries including an open sacrohysteropexy with retropubic sling placement. Given her young age and desire to maintain vaginal length, we opted for robotic supracervical hysterectomy and sacrocolpopexy.

RESULTS

As a result of the patient's prior surgeries, bowel and bladder were adherent to the uterus and required dissecting off prior to hysterectomy. Mesh was scarred into the peritoneum overlying the uterus and thus left in situ as the uterus was amputated. Due to insufficient peritoneum to cover the new mesh, a flap was created from the anterior abdominal wall. Seven months later, the patient's symptoms had resolved and her POP-Quantification measurements were improved.

DISCUSSION

Managing recurrent POP after prior sacrocolpopexy is complex due to scarring and concern for secondary repair durability. Repeat robotic mesh colpopexy is an option, but a vaginal approach may be easier. Preoperative cystoscopy, urodynamics, and upper urinary tract imaging should be considered. Intraoperative ureteral stent placement can help identify the right ureter. Cystoscopy should be performed at the end of the surgery to check for bladder or urethral injury. Ultimately, the surgical method should be individualized.

CONFLICT OF INTEREST

None declared.

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Correspondence address:
Wilson Lin, MD
1935 Eastchester Road #15F
Bronx, NY 10461, USA
Telephone: +1 91 7930-1815
E-mail: wlin1@mail.einstein.yu.edu

ARTICLE INFO

Available at: http://www.intbrazjurol.com.br/video-section/20180037_Lin_et_al
Int Braz J Urol. 2018; 44 (Video #20): 1263-4

Submitted for publication:
January 18, 2018

Accepted after revision:
July 09, 2018

Published as Ahead of Print:
August 05, 2018

VIDEO SECTION

Vol. 44 (6): 1265-1265, November - December, 2018
doi: 10.1590/S1677-5538.IBJU.2017.0444

A martius flap in the treatment of iatrogenic distal urogenital fistula

Ivan Ignjatovic [1, 2], Dragoslav Basic [1, 2], Milan Potic [1, 2], Ljubomir Dinic [1], Aleksandar Skakic [1]

[1] *Clinical Center Nis, Serbia;* [2] *University in Nis, Faculty of Medicine Nis, Serbia*

ABSTRACT

Introduction: Distal urogenital fistulas (DUF) are usually iatrogenic and are uncommon in Europe. They occur in the urethra or near the bladder neck, and can be caused by vaginal hysterectomy, para-urethral cyst surgery, or erosion of the bladder or urethra from tension-free slings or meshes. The psychological and physical health consequences of DUF are devastating because most patients consider themselves "healthy" before surgery. Incontinence can appear after successful DUF closure due to previously occult incontinence or urethral incompetence. Additional surgery for incontinence is sometimes necessary to achieve satisfactory outcome.
Materials and Methods: A Martius flap was used in 23 patients between 2000 and 2015. Patient age range was 38-75 years (mean, 58.7). DUF was due to gynecologic surgery for benign disease (15 / 23; 65.2%), mesh / sling erosion (2 / 23; 8.7%), and malignancy (6 / 23; 26.1%). The follow-up period was one year.
Results: DUF was closed in 22 patients (95.6%). Satisfaction and complete dryness was achieved in 16 patients (69.6%) after the first procedure. Postoperative complications were: postoperative hematoma in 1 (4.4%), primary failure in 1 (4.4%), overactive bladder (OAB) syndrome in 3 (13.2%) and postoperative incontinence in 6 (26.4%) patients. A fascial sling was placed in patients with incontinence. All patients were dry after the secondary surgery. Anticholinergics were used for the treatment of OAB syndrome. Discomfort at the flap harvesting site was of minor importance. Finally, 22 out of 23 patients (95.6%) were satisfied.
Conclusion: A Martius flap and additional fascial sling could be successfully used to optimize DUF treatment.

ARTICLE INFO

Available at: http://www.intbrazjurol.com.br/video-section/20170444_Ignjatovic_et_al
Int Braz J Urol. 2018; 44 (Video #21): 1265-5

Submitted for publication:
July 31, 2017

Accepted after revision:
July 09, 2018

Published as Ahead of Print:
August 01, 2018

Correspondence address:
Ivan Ignjatovic, MD
Clinig of Urology, Clinical Center Nis
Bulevar Zorana Djindjica 46
Nis 18000, Serbia
E-mail: ivanig@live.com

LETTER TO THE EDITOR

Vol. 44 (6): 1266-1271, November - December, 2018
doi: 10.1590/S1677-5538.IBJU.2017.0282

The present and future enhanced recovery after surgery for bladder cancer

Michael A. Poch [1], Sephalie Patel [2], Rosemarie Garcia-Getting [2]

[1] Department of Genito-Urinary Oncology, Moffitt Cancer Center, Tampa, Florida, United States; [2] Department of Anesthesia, Moffitt Cancer Center, Tampa, Florida, United States

To the editor,

Enhanced Recovery After Surgery (ERAS) protocols have been increasingly used in radical cystectomy over the past few years. While the principles were started in colorectal surgery, they can be easily applied to any surgery. The evidence is overwhelmingly in favor of these principles due to improved outcomes such as length of stay, complications, and cost (Table-1) (1-5). Development and implementation of an ERAS protocol requires multidisciplinary involvement, strong participation of everyone involved, and analysis of outcomes. We discuss our experience in development of ERAS for radical cystectomy, barriers to implementation, results at our institution, and future directions.

Role of Multidisciplinary Approach

We believe the multidisciplinary approach to implementation of an ERAS protocol is crucial for success in patients undergoing radical cystectomy. Prior to starting multidisciplinary ERAS, surgical components of ERAS were already in place with administration of alvimopan, limited use of nasogastric tube, early feeding, and early ambulation. Involvement of our anesthesia colleagues allowed inclusion of anesthetic related ERAS techniques such as multimodal analgesia, limited fasting state, epidural use, and goal directed fluid therapy (GDFT). Our adapted approach to fluid management can be seen in Figure-1. This change translated to involvement with the Pre-Admission Testing clinic, preoperative holding staff, recovery room staff, and floor nursing. Identification of leaders from each discipline is critical to successful implementation of ERAS (6).

Surgical and anesthesia partnership is crucial to ensure the proper functioning of an ERAS protocol. Anesthetic components have been consistently proven to have long-term effects on patient outcomes. This has been recently demonstrated by Jaeger et al. who showed that anesthesiologist experience with radical cystectomy is directly correlated with readmission rate for this procedure (7). Anesthesiologist involvement and accountability is needed in order to include the best evidence based practices within the specialty and to standardize the techniques amongst anesthesia providers.

Limitations of ERAS

Study Design

One of the challenges of identifying meaningful differences in clinically important endpoints is lack of using standard criteria. Almost all of the Genitourinary Enhance Recovery studies use length of stay as an endpoint. While this is easy to capture it does not give an adequate representation of clinical recovery. As one could imagine, absolute discharge date is dependent on multiple factors including de-

Table 1 - Enhanced recovery after surgery – radical cystectomy series.

Author	Type of Study	Comparison	Number of patients	Length of Stay	Complication	Location
Maffezzini (1)	Retrospective	ERAS	71	15	26.7% - 30 day	Italy
	Historical Control	pre-ERAS	40	22	22.50%	
Smith (2)						
1	Retrospective	ERAS 2	27	7	55.6% - 90 day	UK
2		ERAS 1	37	10	76.7% - 90 day	
	Historical Control	pre-ERAS	69	14	72.5% - 90 day	
Pruthi (3)	Prospective	ERAS	40	5.2	not documented	US
	Historical Control-	pre-ERAS	30	10		
Arumainayagam (4)	Retrospective	ERAS	56	13	30/90 - day not documented	UK
	Historical Control	pre-ERAS	56	17		
Daneshmand (5)	Prospective Observational	ERAS	110	4 Days	65% - 30 day	US
	Historical Control - Matched	pre-ERAS				

mographic logistics, pharmacy readiness and even day of operation (8). The concept of "readiness for discharge" rather than absolute length of stay is likely more relevant and meaningful as a clinical endpoint. Wong-Lun-Hing et al. described 5 specific criteria for discharge or readiness for discharge in patients undergoing ERAS for hepatic surgery (9). These thresholds included regular diet, lack of IV fluid support, oral medication only for pain control, full mobilization, and improving laboratory values; once met, patients were considered "ready for discharge". In addition to objective criteria for readiness for discharge having independent reviewers to assess whether these criteria are met can remove investigator bias (10).

Use, efficacy, & perception

While ERAS protocols for radical cystectomy have demonstrated significant improvement in both recovery from surgery and decreasing morbidity, limitations of the protocols and previous studies should be recognized. ERAS studies were initially established for patients undergoing colorectal surgery; modifications such as early feeding were important for patients undergoing large bowel surgery but not necessarily for patients undergoing small bowel surgery, where risk of ileus is substantially lower. It also should be noted that while a majority of the ERAS protocol initiatives implement each element simultaneously, individual element use and adherence is often

not reported in the study. We have previously shown that after FDA approval of alvimopan, 13% of patients who were eligible did not receive it (11). This can be seen in the original alvimopan study as well, where only 83% of patients received the medication (12). In addition, in our experience epidural analgesia was not utilized in 100% of patients. Studies evaluating ERAS implementation should be analyzed as both an "intention-to-treat" and "as treated" analysis. Unlike in randomized control trials where-in a single intervention is used, a multi-faceted ERAS program has many moving parts. It's important to distinguish what drives outcomes and how they can be measured. For example, is GDFT more or less important for patients with higher ASA scores or limited ejection fraction? The majority of ERAS studies do not document individual element use (13-15). It should also be noted that while many surgeons and anesthesiologists believe they are using ERAS programs, the reality is far from that. Kukreja et al. reported that only a fraction of the ERAS elements are used among physicians who self-report as ERAS users (16). Among the urologists queried 1, 2 or 3 of the elements were omitted by 13%, 25%, and 23% of the respondents, respectively.

FUTURE DIRECTIONS

Preoperative Optimization

After successfully implementing a multidisciplinary ERAS protocol for radical cystectomy, we believe the future lies in providing a more comprehensive approach to perioperative care; as such, we have turned our efforts to expanding the preoperative components of ERAS. The overall goal is to ensure patients are optimized prior to radical cystectomy in order to achieve better postoperative outcomes. This is accomplished by risk stratifying patients and targeting preoperative interventions at modifiable risk factors, such as malnutrition, anemia, and frailty.

The association between poor preoperative nutritional status and increased morbidity and mortality after gastrointestinal surgery is well established (17). Similarly, in urologic surgery malnourished patients undergoing RC have shown higher overall morbidity and 90-day mortality (18). Patients at risk for malnourishment can be identified by various tools, such as the nutritional risk score (NRS). The incidence of malnutrition in patients undergoing RC has been reported up to 19%, making this a promising target for optimization before surgery (18, 19). Providing nutritional support preoperatively to malnourished patients has been shown to reduce incidence of postoperative complications. Immuno-nutrition is a newer nutritional supplement which consists of a mixture of arginine, glutamine, omega 3 fatty acids, and nucleotides taken orally for five days prior to surgery. Though evidence in patients undergoing RC specifically is limited, two pilot studies have shown that preoperative immune-nutrition was associated with fewer postoperative complications, including infections and ileus (20, 21). Munbauhal et al. concluded that immune-nutrition should be considered for malnourished patients undergoing RC beginning 1 week before surgery (22).

Preoperative anemia (PA) is another common surgical risk factor and can be easily diagnosed with routine preoperative laboratory analyses. In patients undergoing radical cystectomy, the prevalence of PA was 40% and associated with worse oncologic outcomes (23). Thus diagnosis and treatment of anemia in the preoperative period is recommended. Optimal treatment in this setting has not been established, however treatment of iron-deficiency anemia with iron infusion may be considered when prompt response is required, as with many oncologic surgeries (24).

Frailty is another reliable predictor of postoperative complications and adverse outcomes, including increased length of stay, discharge to rehabilitation facility, and mortality (25). Though a universal clinical definition is lacking, there are numerous validated frailty assessment tools available. Frailty can be described as an age-related decline in physiologic function and resulting vulnerability to stressors across physical, cognitive, and psychosocial domains, and is estimated to affect 42% of geriatric cancer patients (26-28). Given that two-thirds of urologic surgeries are performed in those >65 years of age, frailty syndrome is likely common in patients undergoing urologic cancer surgery (29). Various interventions have been studied and appear beneficial in reducing

frailty and its complications, including physical rehabilitation before surgery (30). Identifying patients at risk for frailty and attempting to optimize functional status before surgery should be considered in patients undergoing RC.

Minimally Invasive Surgery

Over the past three decades, many studies have demonstrated an improvement in clinical recovery with the incorporation of minimally invasive techniques. These improvements have been demonstrated in post-operative pain scores, length of stay and metabolic stress response to surgery. While the use of minimally and robot-assisted techniques for bladder cancer are still evolving there is certainly potential that perioperative benefits may be seen. There is currently a paucity of data regarding incorporation of ERAS programs for robotic radical cystectomy, however those published demonstrated improvements in length of stay. Unfortunately, those studies evaluating ERAS for robotic cystectomy had varying number of elements use (31). The EAU robotic urology section scientific working group consensus also highlighted the need for "core teams" for operating room staffing. Presence of a "core team" has been shown to improve operating room efficiency and thereby potentially improving outcomes (32). While not unique to robotic surgery, the concept of a "core team" may have higher value in robotics where the primary surgeon is not at bedside. As a general concept, minimally invasive surgery including robotic cystectomy should be seen as a potential additional element to be incorporated into an ERAS program not to be used in lieu of.

Perioperative Surgical Home

Endorsed by the ASA and American Academy of Orthopedic Surgeons (AAOS), the Perioperative Surgical Home (PSH) is a health care delivery model that has recently gained the attention of the American Urological Association (AUA), which hosted a webinar on the subject in early 2017. The PSH is focused on patient-centered, physician-led, coordinated care from the decision for surgery until the patient has recovered as fully as expected after surgery (33, 34). This model includes anesthesiologist participation in patient care from preoperative optimization to postoperative medical management. While the PSH contains many elements of ERAS, additional emphasis is placed on preoperative interventions to risk stratify and optimize patients, post-discharge follow--up tailored to reduce readmissions, and improved coordination between phases of care. We have learned through design and implementation of ERAS in radical cystectomy at our institution the importance of interdisciplinary collaboration. This has become paramount as we look to expand our care to encompass the full perioperative spectrum, into areas that are not traditionally managed by a single team, or by surgeons and anesthesiologists.

CONCLUSIONS

Patients undergoing RC benefit from ERAS techniques, as seen in our institution after implementation of a multidisciplinary ERAS protocol with participation from all stakeholders. As we look to further improve clinical outcomes after RC, expansion of preoperative risk assessment and implementation of evidence-based interventions aimed at optimizing high-risk patients are logical next steps. In addition, identifying what ERAS elements provide the highest clinical and financial value, standardized reporting methodologies are paramount. Ultimately, the PSH care model of comprehensive, coordinated care may prove to yield the best clinical results, particularly for complex surgeries such as RC.

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Correspondence address:
Michael A. Poch, MD
Department of Genito-Urinary Oncology
Moffitt Cancer Center
12902 Magnolia Drive
Tampa, Florida 33612, United States
Telephone: +1 813 745-2226
E-mail: michael.poch@moffitt.org

Submitted for publication:
December 05, 2017

Accepted after revision:
January 25, 2018

Published as Ahead of Print:
April 02, 2018

ARTICLE INFO

Int Braz J Urol. 2018; 44: 1266-71

LETTER TO THE EDITOR

Vol. 44 (6): 1272-1274, November - December, 2018
doi: 10.1590/S1677-5538.IBJU.2018.0484

The role and importance of SBRT in prostate cancer

Yasemin Cihan [1]

[1] *Department of Radiation Oncology, Kayseri Egitim ve Arastirma Hastanesi, Hastane cad Kayseri, Kayseri, Turkey*

To the editor,

Practical applications in radiotherapy have developed considerably over the past 60 years. Medical imaging, immobilization techniques and advances in computer soft ware programs enable the use of broad-based stereotactic body radiotherapy (SBRT). SBRT is a special type of external radiation therapy that is irradiated with a high radiation dose of 1 to 5 fractions with smaller safety margins than the target conventional irradiation. Although different from traditional radiobiological concepts, SBRT is a promising treatment model with a high local control rate, low normal tissue toxicity, and short treatment duration compared with conventional radiotherapy. Stereotactic radiotherapy can be used in various parts of the body due to noninvasive stabilization methods and the availability of taking target images during treatment. It has been started to be used in primer lung cancers and lung metastases, primary liver cancers and liver metastases, pancreatic cancers, prostate cancer, recurrent head and neck cancer, recurrent gynecologic cancers and many other types of cancer and in different regions (1-5).

Hypofractional radiotherapy to the prostate is based on modern radiobiology knowledge and advances in SBRT. Many trials of hypofractional administration in prostate cancer have demonstrated that hypofractionation treatment provides the same or better tumor control than normal fraction therapy, while late and early toxicity remains unchanged in normal healthy tissue. According to clinical data, large fraction doses are biologically superior in prostate cancer to small fraction doses. Due to the low α/β ratio (1.4-3 Gy) of tumor cells in prostate cancer, high doses can be achieved. Therapeutic rate is also increased with hypofractionational application (2, 3, 5-6). Recently, SBRT has been started to be used in the treatment of prostate cancer in curative, salvage and boost treatments in the literature. Studies presenting the specific complications and success rates of all these applications have begun to be published. According to the results of the studies on the efficacy of SBRT treatment for prostate cancer treatment, biochemical relapse free survival of 90-100% with a median follow up of 5 years or more was reported (1-4). In a study conducted by Katz, in low-risk prostate cancer SBRT was 35 Gy-36.25 (equivalent dose of 90-95 Gy at 1.8 Gy per fraction, or 200-212 Gy BED). It has been shown that Gy dose is an effective and low toxic treatment in the early period. Again, in this study it was stated that dosing above 35 Gy resulted in more toxicity than clinical benefit (6). Koskela et al. investigated the efficacy of SBRT in high-risk group. They emphasized that genitourinary or rectal toxicity wasn't found at acute grade 3

and above. The rates of intermediate-term grade 3 genitourinary, rectal and infectious toxicity were low. They stated that PSA control was better in the low- and moderate-risk group (7). Janowski et al reviewed the efficacy of SBRT in patients with high prostate volume. This study investigated the efficacy and toxicity profile of SBRT in 57 prostate cancer patients with low, moderate, and high risk groups and prostate volume ≥50 cm^3. SBRT Cyberknife (Accuray) device was used with doses of 35-36.25 Gy in 5 fractions. The 2-year actuarial incidence rates of genitourinary and gastrointestinal toxicity ≥ grade 2 were 49.1% and 1.8%, respectively. They reported that SBRT is more reliable and effective than brachytherapy and conventional radiotherapy (RT). Another feature of this study is the evaluation of symptom flare of late gastrointestinal toxicity due to SBRT and the improvement with conservative treatment (1). Mbeutcha et al compared high-dose brachytherapy in post radiation salvage therapy in patients with prostate cancer previously treated with RT with SBRT. They emphasized that both treatments were effective in the treatment of salvage (2). Fuller et al followed-up recurrent prostate cancer patients treated with SBRT in 34 Gy / 5 fraction for median 24 months. They reported 2-year biochemical-free survival of 82% with only 7% of grade 3-4 urinary toxicity and no severe digestive toxicity (3). Paydar et al investigated whether boost therapy with SBRT (19.5Gy in three fractions) was as effective and reliable as brachytherapy in patients who received intense-modulated radiation therapy (IMRT) (45-50.4 Gy). Inpatients they followed up median 4.2years, cumulative late ≥ grade 2 and ≥ grade 3 genitourinus toxicities were observed respectively in 40 and 6% of the patients. Overall modest rates of gastrointestinal toxicity were with a 12% cumulative incidence of late ≥grade 2GI toxicity, 7% late ≥ grade 2 rectal bleeding, and 1% late grade 3 bleeding. They emphasized that the side effect of SBRT boost treatment after IMRT is less and more reliable (8).

In conclusion, the data that do not have sufficient maturity and follow-up period suggest that today a gold standard for effective treatment of SBRT in prostate cancer treatment is not yet available. However, because of the characteristics of SBRT, it is seen that it can be used as the prostate therapy and as an alternative to effective treatment methods. There is a need for studies to be conducted in this regard.

CONFLICT OF INTEREST

None declared.

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Submitted for publication:
July 16, 2018

Accepted after revision:
July 22, 2018

Published as Ahead of Print:
September 15, 2018

Correspondence address:
Yasemin Cihan, MD
Department of Radiation Oncology
Kayseri Egitim ve Arastirma Hastanesi
Hastane cad Kayseri
Kayseri, 38010, Turkey
E-mail: cihany@erciyes.edu.tr

ARTICLE INFO

Int Braz J Urol. 2018; 44: 1272-4

LETTER TO THE EDITOR

Vol. 44 (6): 1275-1276, November - December, 2018
doi: 10.1590/S1677-5538.IBJU.2018.0459

Comment on 'polygamy, sexual behavior in a population under risk for prostate cancer diagnostic: an observational study from the black sea region in Turkey'

Mete Özkidik [1], Anar İbrahimov [2]

[1] Department of Urology, Sanliurfa Egitim ve Arastirma Hastanesi, Karşıyaka, Şanlıurfa, Turkey; [2] Ankara university, Ankara, Turkey

To the editor,

I read the article by abdullah cırakoglu et al. (1) with great interest but i have some points of concern with this article.

Firstly, it is stated that all patients participated in the study have similar lifestyle and nutritional habits. However, the details of lifestyle and nutritional habits such as having office or night - shift work, consumption of processed meat or alcohol and smoking habits were not mentioned in the article. Also using hormonally active medications would be a predisposing factor for prostate cancer. Testosterone supplements (2) or 5 - alpha - reductase inhibitors (5 - ARIs) (3) are common examples currently under research whether leading to prostate cancer. The article does not reveal any details about the routine medications of the participants. On the other hand, I think, it would be rarely possible to say that all of 317 patients who were assessed in the study have similar lifestyles and nutritional habits even they used to live in the same geographical region.

Secondly, the increased number of sexual partners is under consideration in the article that it might be liable for having prostate cancer. I think it would be an important predisposing factor for prostate cancer due to causing chronic prostatitis which leads to prostatic intraepithelial atrophy (PIA) and than cancer in the long - term by recurrent sexually transmitted infections (STIs) (4). But the history of urethritis or prostatitis of the participants were not mentioned in the article. Although serologic testing could not be performed, questioning the history of urethritis would be beneficial. So, I think that considering only the number of sexual partners of the participants through their whole life would not be appropriate to determine the risk of prostate cancer unless the number or severity of attacks of prostatitis is considered.

Finally, sexual intercourse frequency is assessed in the study population as a risk factor for prostate cancer. Sexual intercourse frequency per month is compared between the two groups only for the youth and current period. But the limits of age for these periods are not determined in the article. In other words, it is not clear which decade is referred as considering "youth" or "current". In addition, there is conflicting data about the association of ejaculation frequency (5) and prostate cancer in the current urologic literature. For some authors, the age of 30 is a critical point that ejaculation frequency in younger ages and older ages have different impacts on development of prostate cancer. So I believe that the period of age should also be mentioned in the article as well as the frequency of sexual intercourse as considering the risk of prostate cancer.

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ARTICLE INFO

Int Braz J Urol. 2018; 44: 1275-6

Submitted for publication:
July 09, 2018

Accepted after revision:
July 22, 2018

Published as Ahead of Print:
September 20, 2018

Correspondence address:
Mete Özkıdık, MD
Department of Urology,
Sanliurfa Egitim ve Arastirma Hastanesi
498, Street 5A, 15 Karşıyaka
Şanlıurfa, Sanliurfa, 63850, Turkey
E-mail: meteozkd@gmail.com

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