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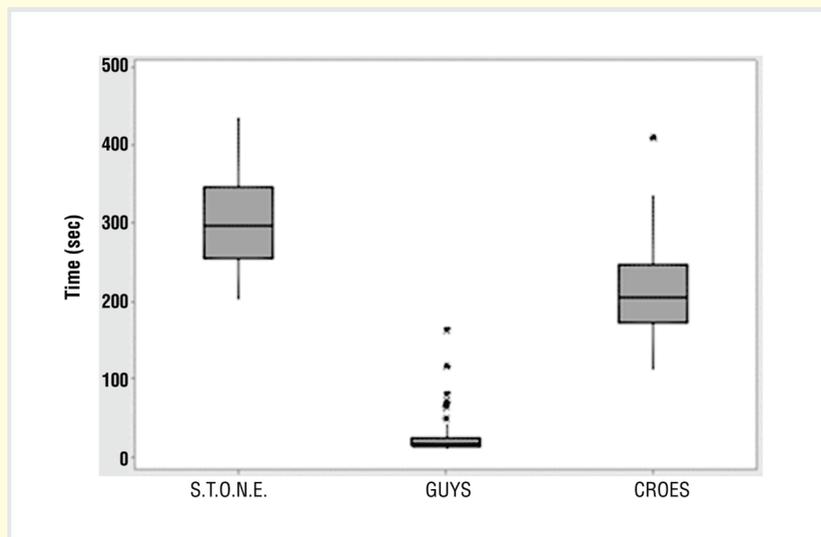


Figure 1 – Time of application of the nomograms (in seconds). (Page 1106)



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Editorial: Anthony J. Thomas Jr.

In November, 5th, 2017, on the day Dr. Anthony J. Thomas Jr. would turn 74, he passed away. He was an internationally recognized reputed urologist, that popularized microsurgery in the urological community.

Tony was a great friend of Brazilian Urology. He opened the doors of the Cleveland Clinic Foundation to Brazilian urologists. When he introduced me to his colleagues during meetings, he always stressed that I was his first fellow at Cleveland Clinic; he joined the Clinic in 1982, and worked there for more than 30 years. During that period, many other Brazilians attended fellowship under his guidance.

He always supported SBU and many times helped us bring several American experts to our Congresses without costs. He himself attended several Brazilian Congresses of Urology, and many national courses on Infertility.

But Tony's main quality was that he was a Family man. He involved his heart in all his actions. His patients firstly were persons that deserved his greater respect. He was a very thoughtful person, always with a nice and pleasant word for all. His concern with his fellows was touching. My car was very old, and he always would catch me when we had meetings together, since he was afraid that I would not make it due to mechanical problems. And I lived in the other side of the city! Probably he was the most honest, ethical and professional man that I have ever met.

In one occasion, we attended an Arabian prince with azoospermia due to epididymal obstruction, that had been submitted to two surgical unsuccessful procedures. The year was 1983, without the possibility of testicular or epididymal aspiration and ICSI. The boy's testicles looked like rocks, and Tony, very sadly, informed that it was impossible to reconstruct the tract satisfactorily. The very upset patient retorted that he would pay any amount of money for Tony to perform the surgery without assurance of success; Tony declined, because he could not offer any hope. When we left the office, the patient was crying and Tony's eyes were tearful. One week later, Tony received a golden watch with a card that simply stated: thank you for your honesty.

Talking to Tony was a pleasure; he always made you feel like the most important person in the world! I lived daily with him for almost one year and he had a huge influence in my career and in my way of being.

Humanity needed more people like Tony! What a pity!

Rest in Peace!

Sidney Glina, MD, PhD

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Focal therapy will be the next step on prostate cancer management? | *Opinion: Yes*

Stênio de Cássio Zequi^{1,2}

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In the last decades, the main goal of the treatment of several solid malignancies was the maintenance of high cure rates, along with morbidity reduction. As occurred with the drastic reduction of radical mastectomies for breast cancer and popularization of nephron sparing surgeries for kidney cancer, winds for reducing the radicality of prostate cancer (PC) treatment are blowing the candles.

Despite of recent recommendations against PC screening, the high rates of overdiagnosis and overtreatment of PC patients are still observed and relevant. Nowadays, many of PC cases are diagnosed in early stages, comprising no more than 5 or 10% of the gland, and much of them are low or intermediate risk PC (1-3). However, for decades, the accepted treatments for all localized PC cases have not changed and were based in whole gland therapies (WGT), for example: radical prostatectomy, or radical external beam radiotherapy, brachytherapy, cryotherapy, HIFU or androgen deprivation (1-3).

Historically, all WGT result in excellent cancer control and high survival rates, however, these procedures are associated with high prevalence of urinary, sexual and intestinal side effects. These WGT's complications can negatively affect patients and their relatives' quality of life. In many cases, these side effects are severe, and additional high costs treatments are required.

Many PC new cases are indolent and/or clinically insignificant, and do not require active therapeutic intervention. For those patients, active surveillance (AS) and delayed intervention, proposed around 15 years ago, are well established as competitive, secure and ethical options, resulting in few urinary, sexual and intestinal side effects in short or mid-term follow-up (4). However, AS protocols can also generate inconveniences. They require patients adherence, multiple serum tests, medical visits, periodic MRI scans etc. that can result in psychological distress, and economic costs. Anxiety or depression due of a "non-treated cancer" can affect some individuals, or their partners or family. Additionally, against AS, there are the odds of tumor clinical/pathological understaging, and the risks of urinary tract infection, and lethal or fatal urosepsis associated with repeated prostate biopsies (1-4).

In summary, at present, for patients with low risk PC, it is absolutely ethical in one extreme to offer a WGT (with its side effects) or, in the other extreme, it is absolutely ethical to offer no intervention (AS). Philosophically, why not adopt a "middle term", in which we could treat focally the cancer that affects the small percentage of the prostate (eliminating the cancer,

as with WGT) and, at the same time, maintain under surveillance the rest of the gland (similarly to AS)? Could we with this approach reach high rates of cure as with WGT and preserve sexual, urinary and intestinal functions as with AS, without the concerns of an untreated cancer? These are the goals that are moving the researchers in favor to PC Focal Therapy (FT).

FT rationale has emerged with skepticism and critics, since PC is usually multifocal and heterogeneous, and only 17-40% of patients have unifocal tumor. On a first glance, FT can be administered only for this minority of patients. But the concept has expanded, and today the most accept rationale is to treat Index lesions (IL), and preserve the surrounding glandular tissue, that can be healthy, or with secondary lesions (SL) that can be submitted to surveillance, as in AS protocols.

Genetic sequencing studies of primary tumor and its respective metastases, performed after necropsies of patients who died due to advanced castration refractory PC, and several pathologic investigations, defined Index lesion (IL) as that with lethal clone of cells, that can originate the most aggressive metastases and are responsible for tumor aggressiveness and oncological outcomes (5, 6). Usually, IL hosts the highest histological grade of malignant cells, and is the largest lesion in the prostate (>0.5cc-1.3cc), being detectable by Multiparametric Magnetic Resonance Image (mpMRI). On the other hand, "secondary lesions" (SL) can co-exist in parallel with IL, but usually are microscopic or small lesions (undetectable at mpMRI), constituted by low grade tumors cells (Gleason \leq 6), corresponding to "clinically insignificant tumors" and without aggressive cell clones, with minimal metastatic potential. In the majority of the cases, SL are not responsible for disease evolution/progression, being suitable for surveillance, without treatment (3, 5, 6).

FT was proposed almost 20 years ago, using Focal HIFU (high intensity focused ultrasound), and Focal Cryotherapy. Initially, protocols were proposed for unilateral or single lesions, and included prostatic hemi-ablations; other authors proposed hemi-ablations plus contralateral apex or bases ("hockey stick fashion"), or even ablation of the whole gland, but sparing the neurovascular bundles, uni or bilaterally. With the progress of the devices, the side effects for adjacent organs have progressively reduced (as thermic damages in urethra or rectum that reduced dramatically with modern HIFU or Cryotherapy machines).

After treating only low grade tumors, the authors have expanded the indications for some intermediate risk PC (Gleason 7 tumor) and a few high grade (Gleason 8) tumors, but with favorable location. Since most patients with low risk tumors are suitable for AS, the more recent recommendations of experts (7) confirm that FT can be used in patients with localized intermediate risk PC (2). Patients with small focus of Gleason 7 or that with a large amount of Gleason 6 tumors are also fit for FT.

In a review in 2014 of 2350 men undergoing FT, 36% were classified as intermediate risk patients and 8% were high risk ones. Gleason 7 and 8 were found in 25% and in 4% of the patients, respectively (8).

A seminal question is the safety of margin around the tumor, that could configure a limitation of FT indication in apical or anterior lesions (particularly in lesions close to the capsule).

Beyond the scope of treatment of primary tumors, FT has been applied as salvage therapy for recurrent PC after external beam radiotherapy or brachytherapy. With the use of salvage FT, the side effects may be minimized in comparison with those of whole gland salvage methods. Wenske et al. have shown similar disease specific survival rates in 55 patients underwent salvage partial cryotherapy versus 273 underwent whole gland salvage cryotherapy after failure of radiotherapy or primary cryotherapy (9).

New FT strategies have emerged due to the best knowledge of natural history of PC, aside from the concepts of IL and SL, and modern multiparametric magnetic resonance image (mpMRI) and other image technologies (that generate tridimensional reconstructed images of the IL (in fusion with, for example, real time ultrasound)) and new devices, that can deliver

energy in more specific and precise points of the gland. Based on these new technologies, it is possible to target only IL and around 25% of its surrounding tissue, in a security margin equivalent to 3–5mm, far from the “rims” of the IL. By the use of these procedures, other additional attractive possibilities include treatment of two IL lesions located in opposite regions of the prostate, with limited damage to non-tumor parenchyma.

According to a systematic review by Vallerio et al., in 2014, 49% of FT were performed with hemi-ablation or “hockey stick” ablation, 51% underwent focal ablation, and bilateral focal ablation was used in only 0.3% of patients. (8) There is an increasing tendency in favor of lesion ablation instead of hemi-ablation (7).

Reflecting the current approaches of the Scientific Community to FT, we can find in the web site *clinicaltrials.gov* (10) more than 40 trials investigating several aspects of FT, as in low volume localized disease, as a salvage therapy in United States. Similarly, researchers from United Kingdom and France are studying more than 10 trials on FT for PC. In the *pubmed.gov* web site, (11) when we insert the mesh term “prostate cancer focal therapy” we find 1952 articles, with 812 of those published in the last 5 years.

The more used surrogates to define biochemical control of FT are based on ASTRO or Phoenix criteria used for external beam radiotherapy and brachytherapy. In a recent review (2), the ranges of biochemical recurrence free survival (BRFS) for Focal Cryotherapy, Focal HIFU, Focal Lasertherapy and focal brachytherapy were respectively: 71–98%; 72–90%, 88% and 92%. The presence of cancer in treated sites ranged from 5% to 30%, in the majority of these series, but only around 10–15% of these cases had clinically significant tumors. Secondary treatments were used in around 25% of the patients (2).

In the largest cohort of Brachytherapy, with 1160 patients submitted to focal cryotherapy, the 3-years BRFS (ASTRO criteria) was 75% (12); urinary incontinence was detected in less than 4% of patients and sexual dysfunction in 42% (13).

In 2012, Ahmed et al. reported preliminary results of a prospective trial using focal HIFU in 20 patients (median PSA of 7.3ng/mL, and 75% of intermediate risk). After 3 months, 80% of the patients presented significant PSA reductions. Negative biopsy was verified in 18 of 19 patients, erectile function was preserved in 95% of the patients, and only 5% of the individuals were using pads for non-severe urinary incontinence. At 12 months of follow-up, 89% achieved the “trifecta”. In a larger population, the same authors reported no incidence of cancer in 30 of 39 patients biopsied after 6 months. And 92% of these patients were free of clinically significant PC. Four patients were re-treated. After 6 months, 100% of the patients were classified as “pad free” or “leak free” continence. Erections sufficient for penetration during sexual intercourse was present in 89%, intake of inhibitors of 5 phosphodiesterase drugs was reported in 45%. Trifecta was scored in 84% of these men (14).

Researchers, industry and their investors are concentrating efforts in developing new FT therapy devices, reinforcing the search for lesser aggressive PC treatment modalities.

At present, there are several FT equipment’s as: Focal HIFU, Focal cryotherapy, laser ablation, interstitial laser thermotherapy, photodynamic therapy, irreversible electroporation, focal brachytherapy, focal radiotherapy, nanoparticles thermotherapy, interstitial thermal microwave therapy and interstitial radiofrequency ablation.

In relation to surgeons, new propositions of FT in prostate cancer surgery are also evolving. Recently, Villers et al. reported, for the first time, early results and complications rates of robotic partial prostatectomy, in the treatment of anterior apical tumors (15, 16) that are not suitable for the above mentioned FT modalities. Although we must have to wait for more data in this field, they confirm that FT will be one of the next steps for the treatment of primary low/intermediate risk, low volume, or recurrent localized PC.

Several concerns regarding FT have been questioned as: How to identify precisely the target area and its safe margins? How to deliver more precisely the energy? How to follow-up

patients after therapy? What would be the best PSA level after treatment (since there are normal parenchyma that continues to produce this marker)? How to detect therapeutic failures? What would be the success and of salvage treatments applied for FT recurrences? What about the costs of FT?. It is necessary to differentiate recurrences from new lesions, and recurrences from new treatments etc.

As in almost all other aspects of prostate cancer, open questions still remain. Despite of these, new conservative treatment of this malignance is warranted and FT will be better understood with time. In this new era, for small low risk tumors, AS will continue to be the best approach; for locally advanced and high risk tumors, WGT (with or without multidisciplinary approach) will continue to be the preferred options. Between these two groups, there is a large amount of men with PC that will benefit from focal treatment of their tumors.

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Focal therapy will be the next step on prostate cancer management? | *Opinion: No*

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Several procedures are being described to treat prostate cancer (PCa) using minimally invasive methods (MIM), in order to achieve total cure of the disease, lower side effects and preservation of quality of life. However, we must pay maximum attention to existing scientific studies, verifying follow-up time, number of patients treated and well-designed comparative studies.

In spite of all technological advances, curative surgery is still the most important treatment for localized PCa. Alternatives to radical prostatectomy include active surveillance (for minimum volume or indolent tumors), radiotherapy and focal treatment.

Evidences obtained by randomized controlled studies show that there are very few differences among active surveillance, surgery and radiotherapy, regarding global and specific survival of low risk localized PCa in a medium follow up of 10 years. Choice of treatment by patients many times is related to urinary and rectal side effect rates presented (1-5), and cultural, economic, psychological and emotional aspects.

Focal therapies (FT) are part of the available MIM treatments for low risk PCa. Several techniques are available, including brachytherapy, cryotherapy, high intensity focal ultrasound (HIFU), interstitial laser, radiofrequency and photodynamic vascular therapy (PVT) (6). Those methods use focal energy to treat tumors obtained with low dose of radioactive substances (brachytherapy), freezing (cryotherapy), ultrasonic waves (HIFU), photothermal (interstitial laser) and action of photo-reagent drugs (PVT-Tookad) (6).

Also, FT technique may “theoretically” preserve surrounding tissues of healthy prostate, as well as neuro-vascular and sphincter structures responsible for potency and urinary continence, respectively (7). “Preliminary” results of current studies show good acceptance, low side effects and good oncologic results.

The bigger question of FT is related to the bad quality of scientific studies published: most include preliminary analysis, with low casuistic, short follow up and inadequate methodology (6).

In a systematic review (SR) recently published on FT, 43 retrospective studies were included, with low level of evidence and none randomized. In that SR, it was included 6 studies involving cryotherapy, 12 HIFU, 1 photodynamic therapy, 3 photothermal therapy, 1 radiofrequency, 1 brachytherapy guided by magnetic resonance image, and 1 with several ablation te-

chniques, with a medium follow-up of 6 years, comprising 25 studies with 2,332 treated patients (8). Although it may seem that the number of studies and treated patients is adequate, several FT methods were used, characterizing heterogeneous groups, with short follow up period of time.

Most studies selected only patients with tumors with minimum volume, with PSA <10ng/mL, absence of Gleason 4 and 5, and low volume of disease demonstrated by histologic evaluation. This means that FT was offered to patients with very low risk tumors as an alternative to active surveillance (9).

It is fundamental to detect correctly the localization of the prostate tumor in order to perform FT. Nowadays, there is no image method totally reliable for that. Previous analysis showed that transrectal biopsy guided by ultrasound (USTR) is inaccurate to identify FT candidates and correct localization of PC. Transperineal template guided biopsy is the most recommended method to localize the disease for FT treatment, but it is an invasive method (10).

Multiparametric MR presents the needed characteristics to locate clinically significant areas of PCa. This method associated to biopsies was frequently used in FT studies, to select patients and to therapeutic planning (11, 12).

Since PCa is considered a multifocal disease in 80% of patients (13), the use of FT in only specific sites is debatable. It may not treat other significant neoplastic areas surrounding the main lesion, different from surgery and external radiotherapy, that treat the whole gland.

The concept of *index tumor* is related to the theory that only the dominant main lesion may cause progression of PCa, and distant metastasis (14). Later, this concept was modified, dividing the tumors in clinically significant lesions, with impact on longevity and quality of life, and clinically insignificant (15-17).

However, *index tumor* studies are still incipient and the theory that satellite lesions include only insignificant tumors still need more clinical evidences. Very few authors recommend treatment of *index tumor* and of clinically significant lesions.

Another great challenge for focal therapy is the definition of therapeutic success, that usually is referred as global and disease-free survival. This difficulty is observed with any treatment of PCa, since it is a disease with slow progression and with many possible subsequent treatments.

One substitute and "extrapolation" of success rates is PSA kinetics. At surgery, with complete gland removal, the ideal expected value is <0.2ng/mL. At radiotherapy, the cure and recurrence criteria are different, and PSA must be lower than 2.0ng + Nadir (lower PSA value after RT), according to *Phoenix or American Society of Radiology and Oncology (ASTRO)* criteria (18).

None of the above criteria are valid or adequate for FT, what adds confusion to disease control scenario. There is remaining prostatic tissue and the mechanism of cellular death is different in radiotherapy and immediate ablation. Therefore, PSA kinetics for FT must be different and some authors proposed the Stuttgart criteria developed for HIFU in the treatment of the whole gland: PSA value must be lower than 1.2ng/mL + Nadir following FT (19). Associated to the Stuttgart criteria, as used for brachytherapy in previous studies, it may also be considered as success the presence of velocity of elevation of PSA<0.75ng/mL per year (20).

Another controversial aspect is the evaluation of oncologic control of FT. Progression to metastatic disease is not informed in most studies, since follow-up is usually short to identify patients that develop metastasis. Cancer specific survival rates were high in published studies for the same reason. Mortality rates were also lower due to short follow up and inclusion of low risk patients (8).

One way to evaluate that aspect, also used in radiotherapy, is prostate biopsy, to verify the presence of residual disease. The results are very hard to interpret, whether with unilateral or whole gland biopsies, in clinically significant tumors or not. Positive biopsy rates were very heterogeneous due to previously cited criteria, from 0 to 17% for significant tumors, and 13-71% for all kinds of tumors (8).

Lastly, we must consider the results regarding quality of life (QL). Previous studies stressed the difficulty to perform this evaluation, since it is directly related to used question forms: quality and validation of questionnaires, data collection subjectivity and information provided by patients.

Most frequent complications of FT include urinary retention, urethral stenosis and urinary infection, rates varying from 0-17%, 0-5% and 0-17%, respectively, in five studies that reported these complications (21-25).

In the main systematic review article (SR) on FT, functional results showed a rate of 95%-100% of urinary continence, without the use of pads, and little losses in 83-100%, using only validated question forms, and very few collateral effects related to urinary continence (8).

In that same SR, erectile function evaluation was reported in 10 studies using validated question forms, showing rates of enough erectile function for penetration by 54-100% of patients, with or without the use of 5-phosphodiesterase inhibitors (5PDI). Regarding rectal toxicity, a frequent complication of RT, it was poorly described, with rates of rectal fistula varying from 0-1% , when described (8).

CONCLUSIONS

Radical prostatectomy is still the standard treatment with better cure rates for localized PCa. Radiotherapy or brachytherapy are good alternatives for selected patients. Very low risk tumors must be submitted to active surveillance as first option. Available studies regarding FT present low level of evidences due to small number of patients, inadequate methodology, retrospective analysis and short period of follow up. Since there are still unsolved controversies, such as the existence of “index tumor”, the best evaluation of location of lesions, how to follow up such patients and how to detect failure, most FT treatments must be still be considered as experimental.

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Prostate cancer in renal transplant recipients

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ABSTRACT

As patients with end-stage renal disease are receiving renal allografts at older ages, the number of male renal transplant recipients (RTRs) being diagnosed with prostate cancer (CaP) is increasing. Historically, the literature regarding the management of CaP in RTR's is limited to case reports and small case series. To date, there are no standardized guidelines for screening or management of CaP in these complex patients. To better understand the unique characteristics of CaP in the renal transplant population, we performed a literature review of PubMed, without date limitations, using a combination of search terms including prostate cancer, end stage renal disease, renal transplantation, prostate cancer screening, prostate specific antigen kinetics, immunosuppression, prostatectomy, and radiation therapy. Of special note, teams facilitating the care of these complex patients must carefully and meticulously consider the altered anatomy for surgical and radiotherapeutic planning. Active surveillance, though gaining popularity in the general low risk prostate cancer population, needs further study in this group, as does the management of advance disease. This review provides a comprehensive and contemporary understanding of the incidence, screening measures, risk stratification, and treatment options for CaP in RTRs.

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Keywords:

Prostate cancer, familial [Supplementary Concept]; Kidney Transplantation; Prostatectomy; Radiotherapy; Prostate-Specific Antigen

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INTRODUCTION

In 2013, nearly 30,000 patients underwent solid organ transplantation in the United States, of which 16,894 were renal allografts (1). It is widely acknowledged that patients are receiving grafts at older ages and are experiencing longer life expectancies with sustained renal function. Treating these patients for non-transplant related conditions, including prostate cancer (CaP), has become more frequent. In this review, we provide a comprehensive and contemporary assessment of CaP risk, screening, and treatment effectiveness in the renal transplant population.

MATERIALS AND METHODS

We performed a comprehensive literature review of articles published from January 1, 1989 through May 1, 2014 using PubMed/Medline and the Cochrane Collection. We utilized a pre-determined search strategy including the terms prostate cancer, end stage renal disease, renal transplantation, prostate cancer screening, prostate specific antigen (PSA) kinetics, immunosuppression, prostatectomy, and radiation therapy. All studies included were performed in adult human beings (>18 years old), written in English, and had full text obtainable for review.

RESULTS

Incidence

Compared to age-matched controls in the general population, transplant recipients are at an increased risk for a variety of malignancies. Overall, the 5-year incidence of cancer in solid organ transplant recipients is 4.4%, although hazard ratios vary based on age and organ transplanted (2). Among RTRs, genitourinary malignancies are the third most common malignancy behind de novo skin malignancies and post transplant lymphoproliferative disorder (3, 4). Of the genitourinary malignancies, CaP is the most common (5).

It remains a point of controversy as to whether RTRs are truly at increased risk of developing CaP. Recently reported standardized incidence ratios for CaP in solid organ transplant recipients are

variable, ranging from 0.88-1.70 (6-10) (Table-1). Data from the 1980's and 1990's suggested that transplant patients were not at increased risk for CaP (3, 11). However, many theorize that CaP has become more frequent in the RTR population due to increased allograft survival, increasing recipient age, and more rigorous screening. Variability in reported incidence may also be attributed to differences in study design, geography, screening practices, reporting criteria, sample size, and the immunosuppressive regimen used (3, 6, 11-18).

More recent data indicates that renal transplant recipients do indeed have a higher incidence of CaP. Current U.S. Medicare data reveals a 3-year CaP incidence of 1.74%, which is significantly higher than age-matched controls in the general population (13). Similarly, data from 22 transplant centers in France has revealed a similar two-fold

Table 1 - Standardized Incidence Ratio (95% Confidence Interval) of Malignancies in Renal Transplant Recipients (6 – 10).

	Collett 2010 (6)	Cheung 2012 (7)	Vajdic 2006 (8)	Piselli 2013 (9)	Tessari 2013 (10)
Prostate	1.1 (0.9-1.4)	0.88 (0.39-1.95)	0.95 (0.68-1.29)	1.7 (1.2-2.3)	1.3 (0.8-2.1)
Lip	65.6 (49.9-84.6)	-	47.08 (41.75-52.89)	9.4 (3.1-22.0)	-
Esophagus	1.8 (1.3-2.5)	1.12 (0.28-4.49)	3.82 (2.26-6.03)	1.2 (0.3-3.6)	-
Stomach	2.0 (1.4-2.6)	2.85 (1.62-5.02)	1.84 (1.07-2.94)	1.4 (0.8-3.3)	1.1 (0.5-2.4)
Colorectal	1.8 (1.6-2.1)	1.75 (1.22-2.52)	2.36 (1.87-2.92)	0.8 (0.5-1.2)	1.2 (0.7-1.9)
Pancreatic	1.5 (1.0-2.1)	1.56 (0.41-4.87)	1.21 (0.56-2.30)	0.9 (0.3-2.0)	0.4 (0.2-1.8)
Liver	2.4 (1.5-3.8)	2.53 (1.63-3.91)	3.19 (1.53-5.87)	0.4 (0.1-1.1)	1.2 (0.5-2.7)
Melanoma	2.6 (2.0-3.3)	9.09 (2.27-36.34)	2.53 (2.08-3.05)	1.8 (0.9-3.3)	1.0 (0.4-3.0)
Non-Melanoma Skin Cancer	16.6 (15.9-17.3)	7.38 (4.86-11.21)	-	-	29.3 (26.0-33.1)
Kaposi sarcoma	17.1 (8.9-30.0)	-	207.90 (113.66-348.82)	135 (106-169)	84.9 (56.9-126.7)
Renal	7.9 (6.7-9.3)	12.5 (8.51-18.36)	7.30 (5.67-9.22)	4.9 (3.4-6.8)	7.0 (5.0-9.8)
Bladder	2.4 (1.9-3.0)	8.22 (4.67-14.47)	3.33 (2.40-4.50)	1.1 (0.7-1.7)	1.4 (0.8-2.5)
Cervical	2.3 (1.4-3.5)	7.19 (3.87-13.37)	2.49 (1.33-4.27)	-	8.9 (4.4-17.7)
Uterine	1.0 (0.6-1.7)	1.44 (0.47-4.47)	1.74 (0.92-2.97)	1.3 (0.5-2.9)	1.1 (0.3-3.3)
Breast	1.0 (0.8-1.2)	1.66 (1.0-2.75)	1.03 (0.78-1.34)	0.8 (0.5-1.2)	1.2 (0.8-1.8)
Hodgkin's Lymphoma	7.2 (5.3-10.2)	-	3.75 (1.51-7.73)	2.3 (0.5-6.8)	1.0 (0.1-7.1)
Non-Hodgkin's lymphoma	12.5 (11.2-13.8)	15.79 (11.9-20.95)	9.86 (8.37-11.54)	4.5 (3.2-6.1)	7.9 (6.0-10.5)

increase incidence of CaP (1.74%) in RTRs. Multiple studies have also verified that CaP is diagnosed earlier in RTRs (~62.3 years) versus the general population (70 years) (15, 18, 19).

Race may also play a role in CaP risk among RTRs. Hall et al. recently used data from the Transplant Cancer Match study to compare CaP risk in Caucasian, African American, and Hispanic RTRs. Much like the general population, African American RTRs have an increased risk of CaP, with a 2.14 incidence ratio compared to the Caucasian population (2).

CaP Screening in renal transplant recipients

Best recommendations for CaP screening remain a point of contention in both the general and renal transplant populations. To date, there are no standard CaP screening regimens or established guidelines regarding prostate specific antigen (PSA) testing or cut-offs in pre or post-transplant patients. The American Society of Transplantation and the European Expert Group on Renal Transplantation do encourage annual CaP screening with digital rectal exam (DRE) and PSA measurement in all male RTRs who are older than age 50 and have a life expectancy of at least 10 years (13, 20). However, the validity of these recommendations has not been evaluated in a randomized controlled manner.

In centers that mandate strict CaP screening with a PSA threshold of >4ng/mL prior to biopsy, positive biopsy rates have been reported to be significantly higher than the general population, prompting some to suggest that lower PSA thresholds may be necessary in RTRs (12, 21).

It is unclear whether CaP screening is of benefit in the pre-transplant population, as the median survival for ESRD patients on hemodialysis is only 5 years and CaP is unlikely to cause significant mortality within that time period (22). However, post-transplant life expectancies now often reach well beyond 15 years, making the diagnosis and treatment of CaP in RTRs a realistic life-extending measure.

Yet, screening for CaP in RTRs may still be less cost effective and result in less overall benefit when compared to the general population. Kiberd et al. established computer simulation models to

estimate life lost due to prostate, breast, and colorectal cancer in RTRs. Compared to the general population, it is estimated that three times more RTRs over age 65 must be screened with annual DRE and PSA to save one life (number needed to screen of 96 vs. 306, respectively) (23).

PSA interpretation

An understanding of PSA kinetics is important when interpreting PSA values in ESRD and recently transplanted patients. Free PSA (f-PSA) is eliminated by the kidneys. Therefore, f-PSA and % f-PSA are elevated in ESRD patients. Serum f-PSA levels are significantly higher in hemodialysis patients, but are not significantly elevated in patients undergoing continuous ambulatory peritoneal dialysis. In contrast, % f-PSA is persistently elevated (by about 40%) in ESRD patients on any form of dialysis (24). After renal transplantation, f-PSA decreases significantly (by up to 60% within 6 days), especially in patients with better graft function as decline in f-PSA and % f-PSA correlate with decreases in serum creatinine (25, 26).

In contrast, total PSA (t-PSA) levels are more likely to reflect levels of complexed PSA (bound to α -1-antichymotrypsin or α -2-macroglobulin) which is metabolized by the liver. After renal transplantation, t-PSA levels are relatively unchanged. Therefore, t-PSA is the most reliable CaP marker in both pre-transplant patients on dialysis and in the early post-transplant population (24-26, 28-30). A summary of PSA kinetics and implications in RTRs can be found in Table-2.

Screening RTRs with t-PSA does seem to have important diagnostic implications; when t-PSA level is between 4-10ng/mL, there is only a 17% chance of CaP. If t-PSA levels exceed 10ng/mL, the risk of CaP increases to ~50% (29).

Although t-PSA does not seem to be significantly affected by renal transplantation itself, interpretation of PSA in RTRs may need to be adjusted based on immunosuppression regimen. Retrospective analysis by Chamie and colleagues has revealed that, among patients with similar PSA levels prior to renal transplant, post-transplant PSA was significantly lower in patients taking sirolimus (0.9ng/mL) versus tacrolimus (1.9ng/mL). It

Table 2 - PSA Kinetics in Renal Transplant Recipients (31-35).

Marker	Metabolism	Half-Life	Variation in ESRD Patients (compared to normal)	Expected Change after Renal Transplantation
t-PSA	Hepatic	1.9 - 3.4 days	No significant change	No significant change
f-PSA	Renal	69 - 110 minutes	Increased	Decreased (30% in 24 hours, up to 60% in 6 days)
%f-PSA	Renal	-	Increased	Decreased
f/T-PSA ratio	Renal/Hepatic	-	Increased	Normal

remains unclear whether sirolimus increases PSA elimination, decreases PSA production, or precludes the use of PSA as a CaP screening tool (36).

Use of Imaging in ESRD patients and RTRs

Regardless of immunosuppressive regimen, CaP screening in RTRs at most centers includes digital rectal exam and t-PSA. Subsequent transrectal ultrasound guided biopsy is performed if t-PSA is abnormally elevated (27). Although not specifically studied in RTRs, multiparametric magnetic resonance imaging (mp-MRI) is emerging as an accurate tool for identifying clinically relevant prostate tumors. Mp-MRI can be used to identify significant lesions prior to a target biopsy or as a cancer staging tool after a positive biopsy (37). MRI can also help rule out CaP metastases and aid in identifying the location of important structures (namely the transplant allograft and the transplant ureter) prior to CaP treatment (38).

Although a high prevalence of nephrogenic systemic fibrosis in ESRD patients exposed to gadolinium-based contrast agents has been reported (10%), nephrogenic systemic fibrosis is almost exclusively seen in patients with a glomerular filtration rate (GFR) less than 15mL/min/1.73m². Risk is minimal among patients with milder degrees of renal insufficiency (39, 40). To further minimize risk, it is generally recommended to avoid MRI contrast agents in patients with a GFR <30mL/min/1.73m².

Antibiotic prophylaxis for transrectal ultrasound (TRUS) guided biopsy in RTRs

American Urological Association guidelines recommend antibiotic prophylaxis in all pa-

tients undergoing TRUS-guided prostate biopsy. Multiple randomized controlled trials have shown a decrease in infectious complications of prostate biopsy with single-dose antibiotic prophylaxis. The antimicrobials of choice include fluoroquinolones or 1st/2nd/3rd generation and cephalosporins. Acceptable alternatives include trimethoprim/sulfamethoxazole or an aminoglycoside. Aztreonam can be used instead of an aminoglycoside in patients with renal insufficiency (41).

Although no specific guidelines exist for antibiotic prophylaxis in the undoubtedly higher risk transplant recipient, similar recommendations can be applied to this population. In small prospective studies, TRUS-guided prostate biopsy has been shown to be well tolerated in patients receiving immunosuppression, with no increased risk of infection compared to the general population (42). Nonetheless, careful consideration to culture directed prophylaxis may be crucial to minimizing the risk of infectious complications in RTRs exposed to multiple hospitalizations, previous exposure to multiple antibiotics, and concomitant immunosuppression (43).

When choosing a prophylactic regimen, it is also important to consider that one fourth of RTRs will develop a urinary tract infection within one year of transplantation. A urinalysis and culture should be checked and any active urinary tract infection should be fully treated prior to proceeding with prostate biopsy. Post-transplant TMP-SMZ prophylaxis is associated with a lower risk of UTIs in this population, but frequent use of TMP-SMZ in RTRs can lead to resistant organisms. Thus, in patients who are already on post-trans-

plant TMP-SMZ prophylaxis regimen, a different class of antibiotic should be used for TRUS-biopsy prophylaxis in RTRs (44). Because RTRs represent a population at high risk for harboring resistant organisms, they may also benefit from pre-biopsy rectal swabs for directed prophylaxis.

CaP Risk Stratification

The average stage at diagnosis of CaP in RTRs is no different than in the general population and ~84% of RTRs with CaP are diagnosed with localized disease. However, after diagnosis, CaP seems to progress more rapidly in RTRs and disease specific survival for stage II, III, or IV disease is significantly shorter compared to the general population (19, 45).

Risk stratification in RTRs with CaP may be heavily influenced by immunosuppression regimens. Use of immunosuppression has been linked to a variety of malignancies, but the association of immunosuppression and CaP is less clearly delineated. There are no large-scale comprehensive studies with adequate follow-up to assess whether immunosuppression truly increases risk of CaP occurrence, recurrence, or progression. Nonetheless, CaP is diagnosed at earlier ages in RTRs which may be due to more diligent screening or an actual increased predilection for CaP. Men with HIV on a variety of immunosuppression regimens also develop CaP at increased incidence and at earlier ages compared to the general population, indicating that immunosuppression may indeed be implicated (46, 47).

It has been postulated that a healthy immune system is essential for the inhibition of CaP cell growth. When CaP cell lines are exposed to a healthy conditioned media containing normal T-lymphocytes, they demonstrate decreased growth. Some experts propose that a suppressed immune system may disrupt the “normal” milieu of T-lymphocytes and lymphokines that are typically responsible for prevention of CaP development and progression (48).

CaP cells appear to respond differently based on the type of immunosuppressive agent introduced. In vitro studies and animal models suggest that calcineurin inhibitors (CNIs) increase aggressiveness and progression of CaP.

Cyclosporine has been shown to induce phenotypic changes that make various forms of adenocarcinoma more aggressive. In murine models of prostate adenocarcinoma, cyclosporine increases the size and number of lymph node and pulmonary metastases. The mechanism may be tissue growth factor beta (TGFB) mediated, as anti-TGFB antibodies have been shown to prevent these alterations (49, 50).

Azathioprine (AZA) has been strongly linked to an increase in skin malignancies and may also have a stimulatory influence on CaP cells. In 2008, a retrospective study of 19 French transplant centers evaluated immunosuppression in 62 RTRs with CaP. Patients on a CNI+AZA were more likely (45%) to be diagnosed with high stage (III and IV) CaP compared to CNI alone (15%) and had higher rates of metastatic disease. Use of AZA was the only independent risk factor for advanced disease in this cohort. However, other studies have shown no increased risk of CaP in association with AZA or mycophenolate (15, 51, 52).

Other immunosuppressive agents may be protective against CaP. In vivo, mammalian target of rapamycin (mTOR) inhibitors (sirolimus/rapamycin) have an inhibitory effect on the proliferation of CaP cell lines. As a downstream kinase in the Phosphatidylinositol-3/AKT signaling pathway, mTOR promotes cell survival and normal cell replication, and is often dysregulated in aggressive CaP cell lines. Therefore, inhibition of mTOR kinase and S6 kinase by mTOR inhibitors may prevent CaP cell cycle progression (53). Treatment of CaP cell lines with mTOR inhibitors has also been shown to increase the radiosensitivity of these cells by ~20% (54).

In 2005, Kauffman and colleagues evaluated data from 33,249 RTRs in the Organ Procurement and Transplant Network database and compared the identified occurrence of de novo malignancy in RTRs receiving either a mTOR inhibitor (sirolimus/everolimus), a CNI (cyclosporine/tacrolimus), or both. The incidence of de novo cancer was significantly lower in the mTOR inhibitor group compared to the CNI group (0.60% vs. 1.61%; p -value <0.001). The authors concluded that the use of mTOR inhibitors alone or in combination with other immunosuppressive agents may

reduce the incidence of CaP in RTRs. Combination therapy is often used to reduce the toxicity of individual agents (55).

In conclusion, current data suggests that cyclosporine, tacrolimus, and AZA are associated with a higher risk of malignancy, which may include CaP. In contrast, mTOR inhibitors are associated with a decreased incidence of malignancy, and should be considered for use in higher risk patients (56). Reduction of immunosuppression is frequently instituted after CaP diagnosis, but to date there is no data showing any benefit with regards to prognosis. It is postulated by some that the intensity of therapy, rather than choice of agent, is related to increased CaP risk. Therefore, the lowest possible dose of immunosuppression while maintaining rejection-free graft survival is advised (11).

Treatment

A variety of treatment options exist for CaP in the general population, and most have been applied to RTRs with CaP. There are a number of important considerations when deciding on treatment approach, mainly because of the proximity of the allograft and transplant ureter to the surgical or treatment field. Of utmost importance is to avoid any compromise to the allograft, as survival at 5 years is only 60% if renal allograft function is significantly impaired (57).

Most surgical approaches for CaP have been described in RTRs, including open radical retropubic prostatectomy (RRP), perineal radical prostatectomy (PRP), laparoscopic radical prostatectomy (LRP), robotic assisted laparoscopic radical prostatectomy (RARP), and extraperitoneal robotic assisted laparoscopic prostatectomy (ERARP). A summary of surgical series reporting prostatectomy approaches and outcomes in RTRs is shown in Table-3.

Regardless of approach, prostatectomy in transplant patients poses a number of unique challenges. Previous transplant surgery leads to distortion of normal anatomy and the renal allograft limits exposure within the pelvis. RTRs with a history of peritoneal dialysis are likely to have thickening of the peritoneum and obliteration of normal tissue planes. Performance of a bilateral

lymph node dissection in RTRs may be difficult, dangerous, or nearly impossible as most allografts are oriented along the iliac vessels. In many instances, it may only be possible to perform a contralateral lymphadenectomy. Doing so is not without future risk; extensive lymphadenectomy may render the contralateral iliac vessels unusable for future allograft implantation should the current graft fail at a later date.

Despite these challenges, the literature supports the safety and efficacy of prostatectomy in the post-transplant population. Disease specific and overall mortality after aggressive surgical therapy in transplant patients is comparable to that in the non-transplant population.

Active Surveillance

There is no data on active surveillance for prostate cancer in the renal transplant population. Conceptually, this may become an evolving arena in which the application of advanced biomarker evaluations and tissue genomics plays an increasing role in predicting who is a candidate for such an approach. In the future, multi-parametric magnetic resonance imaging (mpMRI) may shed some light on the presence or absence of prostate lesions of significance, provided the glomerular filtration rate permits the administration of gadolinium, which may help to guide decisions for intervention.

Radical Retropubic Prostatectomy (RRP)

RRP, the traditional standard approach for localized CaP in the general population, was first reported in a RTR by Manson in 1989. Inherent dangers of RRP in RTRs became evident, including the insertion of deep retractors that can damage the allograft and ureter (58). Since that time, the safety and efficacy of RRP in RTRs is well supported.

Regardless of surgeon experience, visualization during RRP in RTRs is likely to be limited due to the pelvic renal allograft. French surgeons have noted operative dissection during RRP to be “difficult” in 35% of RTRs versus 20% in control patients. Specifically, dissection to achieve bladder descent is challenging due to a tethering effect from the transplant ureteroneocystostomy (59).

Most urologists avoid lymph node dissection ipsilateral to the transplant kidney to avoid the risk of allograft injury. However, bilateral lymph node dissection can be completed if deemed necessary. Kinahan and colleagues reported bilateral ileo-obturator lymph node dissection by using modified placement of Wilkinson retractors to gain exposure while preventing pressure damage to the transplant kidney (17). Placement of

a transplant ureteral stent prior to prostatectomy may help with transplant ureteral identification if bilateral lymphadenectomy is to be performed.

In the early postoperative period, it is felt that immunosuppression does lead to delayed wound healing and may contribute to an increased risk of perioperative infection (60). French transplant centers have reported a significant increase in systemic (non-wound) perioperative infec-

Table 3 - Radical Prostatectomy in the Renal Transplant Recipient Population (15, 17, 60-71).

Radical Retropubic Prostatectomy (RRP)

Study	Surgery	Patients	Age	Pre-operative PSA (ng/mL)	Operative time (minutes)	Estimated Blood Loss(mL)	Length of Stay (days)	Graft injury/impairment	Complications
Kinahan 1991 (17)	RRP	3	60	-	133	1466	10	none	2a
Kleinclauss 2008 (15)	RRP	20	60.4	7.1	163	516	11.9	1	4b
Thompson 2008 (60)	RRP	17	59	4.8	161	500	3	none	6c
Antonopoulous 2008 (70)	RRP	8	59.6	4.5	183	656	5	none	2 d
Hoda 2010 (57)	RRP	16	61.8	4.7	108.3	211.1	10.1	none	2 e
Total	RRP	64	60.26	5.49	149.89	497.56	9.99	1	16

Perineal Radical Prostatectomy (PRP)

Study	Surgery	Patients	Age	Pre-operative PSA (ng/mL)	Operative time (minutes)	Estimated Blood Loss(mL)	Length of Stay (days)	Graft injury/impairment	Complications
Yiou 1999 (61)	PRP	2	62.5	15	-	-	-	none	none
Hafron 2005 (62)	PRP	7	62.3	8.0	92.7	492.9	2.6	none	1 f
Total	PRP	9	62.34	9.56	92.7	492.9	2.6	none	1

a. One patient with severe UTI; one patient with mild stress incontinence.

b. One transplant ureteral stricture with associated allograft failure; three patients developed urosepsis.; one patient developed medium-volume lymphocele requiring percutaneous drainage.

c. One patient with post-operative hemorrhage; one wound infection; one post-operative myocardial infarction; two patients had the late complication of incontinence at 1 year.

d. Two patients with perioperative blood loss requiring transfusion of 2 and 6 units of packed RBCs respectively.

e. One patient with prolonged hematuria requiring transfusion; one vesico-urethral anastomotic leak requiring prolonged Foley catheterization.

f. One patient had prolonged gross hematuria requiring continuous bladder irrigation and transfusion of 1 unit of packed RBCs.

tions (15% vs. 2.5% in nontransplant patients) (59). However, to date, no series of RRP in RTRs have shown a significant increase in wound infection.

Although RRP has been performed safely in RTRs with relatively efficient operative times, blood loss and hospital stays are significantly increased compared to laparoscopic and robotic assisted approaches (Table-3).

Perineal Radical Prostatectomy (PRP)

Although technically challenging, PRP offers a few potential advantages over RRP or laparoscopic approaches. The main benefit is minimal dissection near the renal allograft and transplant ureter. With the perineal approach, bladder descent is not impaired and vesicourethral anastomosis can be performed without tension. PRP also preserves access to the iliac fossa should the need for a second transplant arise in the future. Therefore, PRP should be considered in RTRs with higher risk of impending graft failure. Small series report operative times comparable to RRP, while blood loss and length of stay tend to be lower (61, 62). PRP is limited to patients with low grade disease that does not necessitate a lymph node dissection. Another potential downside of this approach is the resulting perineal wound that may be at increased risk of infection or breakdown in immunosuppressed patients. Although ideologically the “safest” technique from the standpoint of allograft protection, widespread feasibility is impractical as most urologic surgeons are more familiar with RRP or robotic assisted approaches.

Minimally Invasive Radical Prostatectomy: Laparoscopic (LRP) and Robotic (RARP)

The safety and efficacy of pure laparoscopic radical prostatectomy in RTRs has also been described (63, 64). Thomas and colleagues used a standard 6-port transperitoneal approach in three RTRs and successfully performed their dissection with harmonic scalpel and cold endoshears while using transrectal ultrasound to help guide the posterior prostatic dissection. They did not feel that the transplant ureteroneocystostomy affected their ability to complete the urethrovesical anastomosis or bladder neck transection. However, they did note some increased difficulty in dissecting and ligating the dorsal venous complex (64).

Laparoscopic prostatectomy has also been successfully performed with an extraperitoneal approach (ERARP). In 2009, Robert and colleagues described ERARP in nine consecutive RTRs. They used two 10mm ports and three 5mm ports. When compared to the general population, ERARP in RTRs resulted in similar operative times, estimated blood loss, and oncologic outcomes. However, they did report a heightened risk of rectal injury (2 of 9 patients) (65).

In current practice, robotic assisted radical prostatectomy has quickly become the most frequently utilized surgical approach for prostatectomy. RARP has been successfully performed in a growing number of RTRs and seems to result in the least blood loss and shortest length of stay versus other surgical modalities. Important technical nuances have been described to optimize the safety of RARP in RTRs. Polcari et al. reported seven RARPs in RTRs between 2004-2010. They used a 4-arm robotic platform in all patients. Port adjustments included placement of the robotic 4th arm ipsilateral to the allograft and placement of two assistant ports contralateral to the allograft. Lymphadenectomy was performed only on the contralateral side. They recommend initiating bladder mobilization and subsequent development of the space of Retzius on the contralateral side of the allograft. Upon crossing the midline and working toward the allograft, a “fibrotic veil” is encountered that will contain the transplant kidney and ureter. The authors emphasize that this area should be avoided; the transplant ureter need not be visualized for safe completion of the procedure (66). Others have described a variety of port site modifications for RARP in RTRs. Jhaveri et al. advocate for use of an extended length bariatric assistant port ipsilateral to the allograft, which allows instruments to safely bypass the allograft by entering directly at the level of the prostate (67).

Radiation Therapy

Radiation therapy has been used to treat CaP in RTRs, but is often avoided due to risks of allograft injury, ureteral injury, and urethral strictures. Mouzin et al. were the first to report the use

of modern conformal prostate radiation therapy (XRT) in RTRs. Eight patients were treated with 70Gy in 2G fractions with 10mm margins and a 9-field arrangement. At a mean follow-up of 28 months, there were two biochemical recurrences (25%) and over half of the patients experienced short term minor morbidity including diarrhea, rectal irritation, cystitis, or hematuria. Although post-XRT creatinine clearance was unimpaired, significant ureteral obstruction occurred in two patients. The radiation exposure to the ureteroneocystostomy has been estimated to be between 20Gy and 40Gy, and is likely variable based on transplant ureteral orifice location (72). The safety of XRT for CaP in RTRs remains in question, as ureteral obstruction may enhance the risk of allograft dysfunction and may require future surgical procedures for revision. Some experts now view renal transplantation as a contraindication to prostate XRT given the risk of injury to the allograft and transplant ureter. Others maintain that radiation therapy is a safe, viable option in RTRs as doses delivered to the graft are typically below thresholds reported to induce complications and risk of ureteral injury can be minimized by ensuring the bladder is full at the time of irradiation (73).

Brachytherapy

Compared to radical prostatectomy and EBRT, brachytherapy is thought to have less risk of damage to the allograft and transplant ureter. Although data is limited, these patients seem to have similar cancer control rates compared to the non-immunosuppressed population. Beydoun et al. reported successful brachytherapy in four RTRs with no PSA progression, morbidity, or altered allograft function at mean follow-up of 44 months (74). Similarly, Coombs et al. treated 17 immunosuppressed patients (including three RTRs) with brachytherapy and reported a long-term failure rate of 14.3%, which was not significantly different than age matched controls (15.8%) (75). Although data remains limited, current expert opinion supports the use of brachytherapy as a first line treatment option in RTRs with CaP who are over age 70 or are poor surgical candidates due to other comorbidities.

Other treatment modalities

To date, there is a paucity of data regarding the use of other CaP treatment modalities (proton beam therapy, cryotherapy, high intensity focused ultrasound, hormonal therapy, stereotactic guided radiation therapy) in RTRs. One study by Al Ekish et al. reported successful use of prostate cryotherapy in 30 nonsurgical candidates, including two kidney/pancreas transplant recipients. Neither transplant patient experienced intraoperative complications, postoperative complications, or CaP recurrence after 18 months of follow-up (76). Overall, it remains unclear whether or not these modalities can be used in RTRs with comparable risk profiles and results compared to the non-transplant population. Finally, in patients with advanced prostate cancer in the setting of a kidney transplant, there are no guidelines for the administration or sequencing of the now multiple agents available, nor is there safety data for the agents in these clinical stage groupings. As such, these patients are treated based on the clinical judgment of the treating oncologist.

CONCLUSIONS

CaP occurs with an increased incidence in RTRs and is diagnosed at an earlier age. As ESRD patients are being transplanted at older ages with improving allograft survival, the overall number of RTRs with CaP is increasing. The treatment team should pay special attention to PSA kinetics and immunosuppression in these patients. Older immunosuppressive agents (cyclosporine, AZA, tacrolimus) may increase risk of CaP, while newer agents (mTOR inhibitors) may decrease risk of CaP progression. Traditional prostate XRT should be used with caution in RTRs, as there is an increased risk of allograft and transplant ureteral injury. With special consideration and technical adjustments, surgical management of CaP in RTRs can be completed safely with similar morbidity and oncologic outcomes compared to the general population. In patients who are poor surgical candidates, prostate brachytherapy can also be performed safely with good oncologic outcomes. Further studies are needed to elucidate

the risks and benefits of other CaP treatment strategies in the post-transplant population.

CONFLICT OF INTEREST

None declared.

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Implantation of a biodegradable rectum balloon implant: Tips, Tricks and Pitfalls

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ABSTRACT

Introduction: A rectum balloon implant (RBI) is a new device to spare rectal structures during prostate cancer radiotherapy. The theoretical advantages of a RBI are to reduce the high radiation dose to the anterior rectum wall, the possibility of a post-implant correction, and their predetermined shape with consequent predictable position.

Objective: To describe, step-by-step, our mini-invasive technique for hands-free transperineal implantation of a RBI before start of radiotherapy treatment.

Materials and Methods: We provide step-by-step instructions for optimization of the transperineal implantation procedure performed by urologists and/or radiation oncologists experienced with prostate brachytherapy and the use of the real-time bi-plane transrectal ultrasonography (TRUS) probe. A RBI was performed in 15 patients with localised prostate cancer. Perioperative side-effects were reported.

Results: We provide 'tips and tricks' for optimizing the procedure and proper positioning of the RBI. Please watch the animation, see video in <https://vimeo.com/205852376/789df4fae4>.

The side-effects included mild discomfort to slight pain at the perineal region in 8 out of 15 patients. Seven patients (47%) had no complaints at all. Two patients developed redness of the skin, where prompt antibiotic regimen was started with no further sequelae. One patient revealed a temporary urine retention, which resolved in a few hours following conservative treatment. Further no perioperative complications occurred.

Conclusion: This paper describes in detail the implantation procedure for an RBI. It is a feasible, safe and very well-tolerated procedure.

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INTRODUCTION

Prostate cancer radiotherapy can develop limiting anorectal toxicity (1-3). It is therefore important to implement techniques to spare rectal structures (3).

Several devices have been established to spare anorectal structures by excluding them from high radiation dose exposure. Endo-rectal ballo-

ons are used to increase the distance from the dorsal rectal wall to the prostate (3), and implanted rectum spacers (IRS) are designed to separate the anterior rectal wall from the prostate by injecting a biodegradable material. Four types of IRS have been developed: hyaluronic acid (4), absorbable hydrogel (5), collagen implants (6), or a saline-filled balloon (7). In the past decade, research groups have investigated the use of a prostate IRS

(Figure-1), with hyaluronic acid and poly-ethylene-glycol (PEG)-based hydrogel (4, 5, 8-12). All reported a decrease of the rectal dose (Figure-2).

This paper describes in detail the implantation procedure for a (saline-filled) rectum balloon implant (RBI) (Figure-3). It provides step-by-step instructions, identifying the potential hazards and 'tips and tricks' for optimising the procedure as well as proper positioning of the RBI. Furthermore, we report the perioperative complications of the first 15 patients implanted in our institute.

MATERIALS AND METHODS

After approval by the local ethics committee and institutional review board, 15 consecutive patients with localised prostate cancer (cT1-2 N0) treated between June 2015 and December 2015 were included in this feasibility study. Gleason scores > 7 and high PSA-values were not exclusion criteria. Extended extra-prostatic disease extension (T3a/4) was an exclusion criterion, as were distant metastatic disease and previous pelvic EBRT. All patients signed

Figure 1 - A schematic illustration (www.bioprotect.co.il) (a) and an MRI image (Balanced Fast Field Echo- sequence) (b) of a biodegradable rectum balloon implant (RBI) between the anterior rectum wall (R) and the prostate (P), creating space between the two organs.

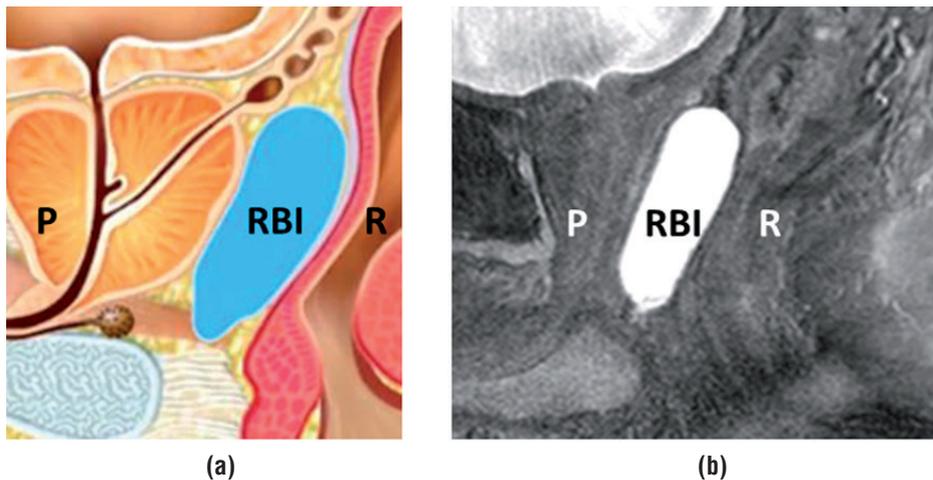


Figure 2 - Isodose distribution in an axial CT plane before (a) and after rectum balloon implant (RBI) implantation (b) in the same patient. The image on the left shows the high-dose region >80% (green) overlapping the entire part of the rectum (brown), whereas with the RBI *in situ* the high-dose region is not in the rectum. The 65% isodose contour (blue) overlaps the entire rectum in (a), whereas there is no overlap in the rectum in (b).

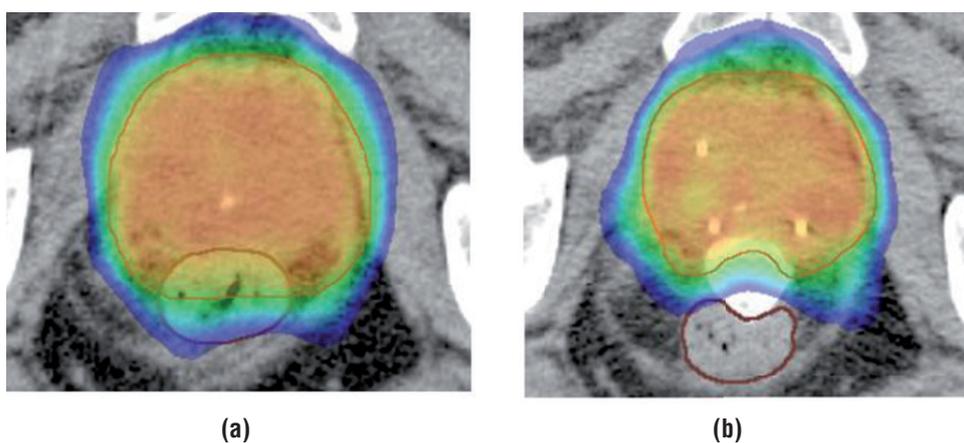
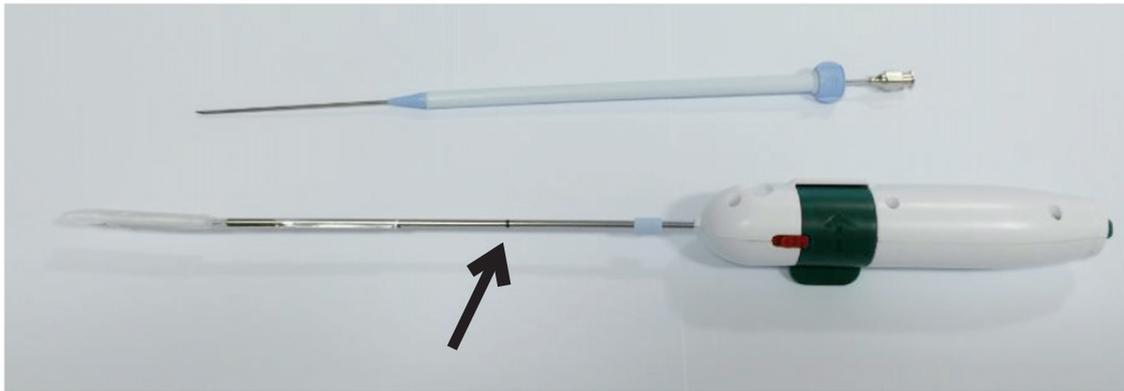


Figure 3 - The RBI kit, with the needle with the dilatator (blue) and sheath (white) above, and the rectum balloon implant (rolled up) deployer (RBID) below. Note the line on the RBID: when the RBID is inserted through the sheath up to this mark it means the tip of the deployer is at the end of the sheath. Retract the sheath while holding the RBID in place.



an informed consent document. The RBI (BioProtect Ltd, Israel) implantation was demonstrated in a video review to illustrate a clinically useful step-by-step technique see video in <https://vimeo.com/205852376/789df4fae4>. All patients were assessed immediately post-injection, 4 to 7 days after implantation. The possible complications were recorded in terms of Common Terminology Criteria for Adverse Events (Version 4.0) (13). Pain was scored 1 hour, 8 hours, and 24 hours after implantation using the visual analogue scale (VAS), ranging from 0 to 10.

RESULTS

Step-by-step description of application technique

Precautions - medications

Anticoagulation should be stopped before this minimally invasive procedure because bleeding can disturb transrectal ultrasound (TRUS) vision. The timing of therapy stop and re-initiation depends on the specific drug used. In contrast to transrectal biopsies, the transperineal RBI implantation yields a lower infection risk after careful skin preparation. Nevertheless, an antibiotic prophylaxis is recommended to reduce the risk of infection by the implant (12). A rectal enema will empty the rectum and improve the conditions for TRUS (14). We use oral ciprofloxacin (500 mg, bid, for three days) and Colex Klyisma (100mL, one hour before procedure).

Positioning - material

The RBI implantation is performed under TRUS guidance using the transperineal approach, with the patient placed in the dorsal lithotomy position (Figure-4). This setup is similar to the implantation procedure for prostate brachytherapy (9). A brachytherapy stepper unit is used to stabilise the TRUS probe and allows the operator to use both hands for the implantation.

A bi-plane TRUS probe (Pro Focus 2202 - BK Medical; transducer type 8848) is used with a US contrast gel-filled condom to improve visibility of the prostate, the Denonvilliers' fascia (DF) and the anterior rectal wall.

Anaesthesia

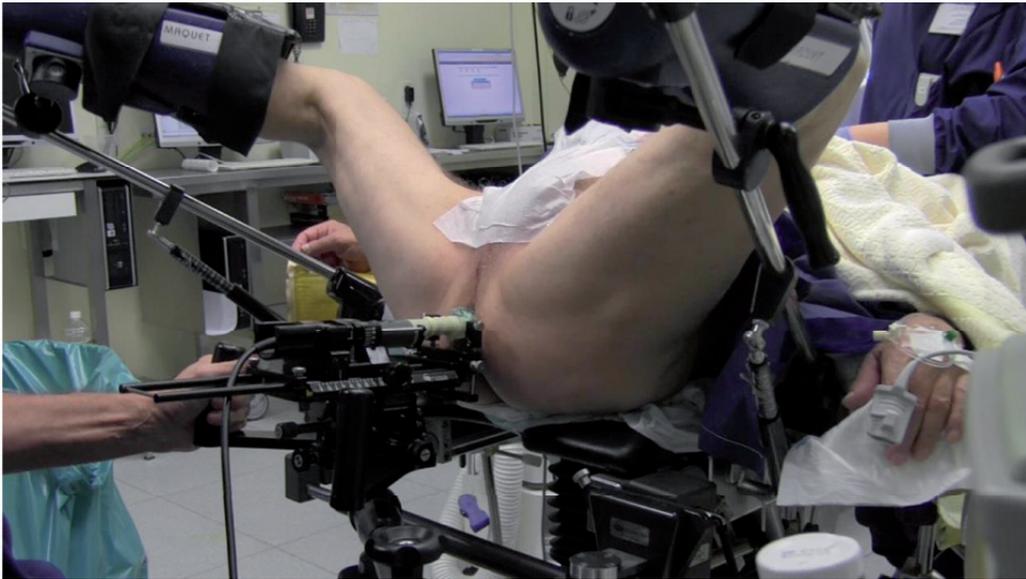
The implantation procedure can be performed under local, spinal or short general anaesthesia. A short general anaesthesia is preferred at the MAASTRO Clinic.

Procedure

First, a Foley balloon is inserted to empty the bladder so there is no resistance when the RBI is fully deployed, and to provide anatomical landmarks of the central plane, which consequently aids the central and effective positioning of the RBI.

Careful skin disinfection is performed with chlorhexidine solution (1%) 10mg/mL, and sterile drapes are used to cover the patient's legs.

Figure 4 - The setup: patient is placed in dorsal lithotomy position, with brachytherapy stepper unit and TRUS probe.



Fiducial intraprostatic markers are implanted for image-guided external beam prostate irradiation. These gold markers could pierce and deflate the RBI; to avoid this, we implant the fiducials via the transperineal (instead of transrectal) route simultaneous with the RBI implantations, as described by Gez et al. (15). We start implantation of the markers just before RBI implantation.

Hydrodissection

A hydrodissection using saline is performed to create tissue planes and facilitate correct placement of the RBI between the DF and the anterior rectal wall. A 20mL syringe is filled with saline. The needle is introduced through the perineum in the midline, ± 1.5 cm above the TRUS probe (little finger width) (Figure-5). This can easily be viewed

Figure 5 - Transperineal insertion of the needle, with the dilatator (blue) and the sheath (white).



on the axial TRUS view. Next, the needle must be introduced parallel to the probe (or slightly angled) into the prostate apex (switch to sagittal TRUS view). The hydrodissection is performed between the DF and the rectal fascia while advancing the needle within this space up to the prostate base. The DF is a fibromuscular structure, fused with the posterior prostate and seminal vesicles. Lowering the probe (dorsal) without pressure on the prostate (in contrast with brachytherapy procedure) before starting hydrodissection may help to open the space. The saline is injected slowly. As the space opens, the needle is advanced until it reaches mid-gland (4, 6, 7, 14). This manoeuvre must be monitored on axial and sagittal TRUS views. The three layers of the rectum (mucosa, muscle, fascia) must be visually inspected to ensure that no rectal fascia is caught by the needle (Figure-6).

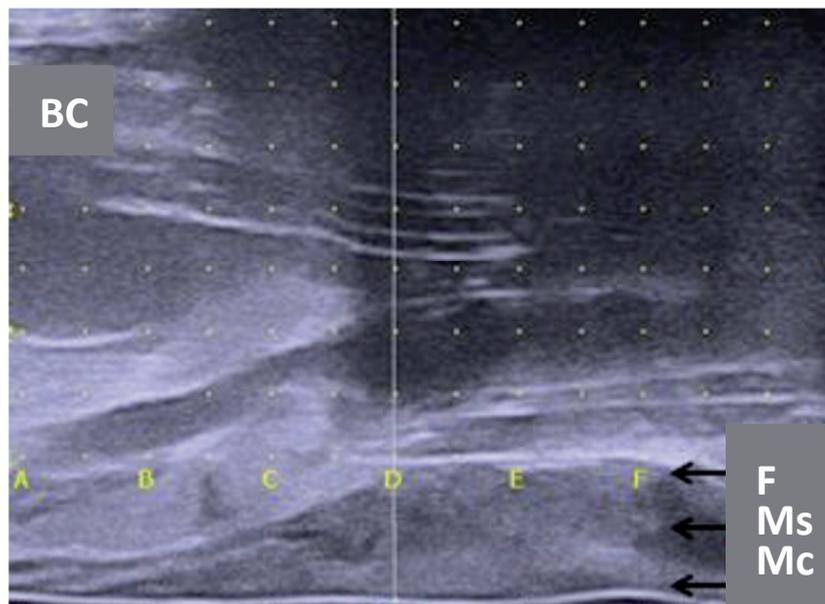
Balloon insertion

A 20mL syringe is filled with warm (35-40°C), bubble-free saline to fill the RBI. The saline is

combined with 1.5cc contrast iodine to visualise the RBI on the planning CT and cone-beam CT scans. If the patient is allergic to contrast iodine, the RBI should be filled with saline only. The saline should be at body temperature to ensure adequate RBI expansion. Just superior of the needle, 1.5cm above the anus, a longitudinal skin incision of the perineum is made with 1 cm in width and 1.5cm deep. The dilatator is advanced with a sheath to the tip of the needle. Axial view is used to check that the dilatator and the sheath are in the central plane ('D-line', or plane of the urethral catheter). A switch to sagittal view is then made to advance the dilatator and sheath over the needle. The needle is shifted to check that the rectum wall is free. If it is not clear, a palpation is performed to check and feel if the rectum wall is free. When the sheath has advanced to the prostate base, the needle and the dilatator are removed while the sheath is firmly held in place. The RBI deployer (RBID) is inserted through the sheath up to the line marked on the RBID: the tip of the RBID will now be at the end of the sheath.

Figure 6 - A hydrodissection is performed to separate the tissue planes with saline, helping to create space for the RBI between the Denonvilliers' fascia and the anterior rectal wall. Be mindful of the three layers of the rectum: fascia (F), muscle (Ms) and mucosa (Mc).

The vertical white line is the base of the prostate. Most of the prostate is not clearly visible because of the acoustic shadow of the needle. Note the Foley balloon catheter (BC) in the bladder and the catheter in the urethra, indicating that you are in the midline.



The sheath is retracted while the RBID is held in place. The RBI is exposed and slowly inflated to the specified (15–20cc) volume, approximately 3mL every 3–5 seconds, while the inflation of the RBI is carefully checked on axial and sagittal views (Figures 6 and 7).

Lowering the probe (dorsal) without placing pressure on the prostate may help to open the space and easily fill the RBI. The RBI must be in the midline between the prostate and rectum from base to apex. The three layers of the rectum (mucosa, muscle, fascia) must be visually inspected to ensure that no rectal fascia is caught by the needle (Figures 6 and 7). The TRUS probe is progressively moved down as far as possible, and a check is performed to verify that the rectum wall is free, in order to avoid rectum perforation. The RBID is detached from the RBI and left sealed *in situ* (7, 16). Axial and sagittal TRUS views are used to verify that the RBI is properly positioned (Figure-8). The rectal integrity and RBI position inflation are checked using rectal palpation. The skin incision is su-

tured using dissolvable stitches. Finally, the catheter is removed.

Perioperative side-effects

No grade 3 or 4 toxicities were reported in the week after implantation. The implantation procedure revealed no thrombosis and no perforation of bladder or rectum, and no anti-allergic shock reaction occurred. No penile bleeding was observed. One patient experienced a temporary urine retention, which resolved within a few hours following conservative treatment.

There was a slight increase of redness of the skin in two patients, where a prompt antibiotic regimen was started with no subsequent episodes of infection.

The major side effect included pain in the perineal region (range from 1–3, according to VAS) in 5 out of 15 patients, which was easily addressed with paracetamol or nonsteroidal anti-inflammatory drugs. Three additional patients felt slight discomfort. Dysuria occurred in five patients. Ecchymosis in the transperineal region and

Figure 7 - Axial ultrasound image: rectum balloon implant (RBI) being filled with saline between the prostate (P) and the rectum (R). Note the urinary catheter (C) in the central plane, or 'D-line'.

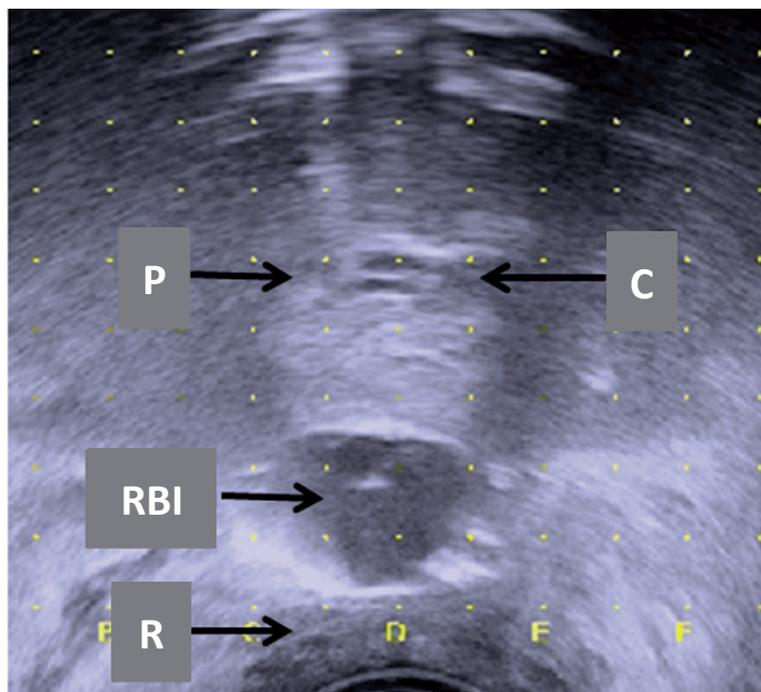
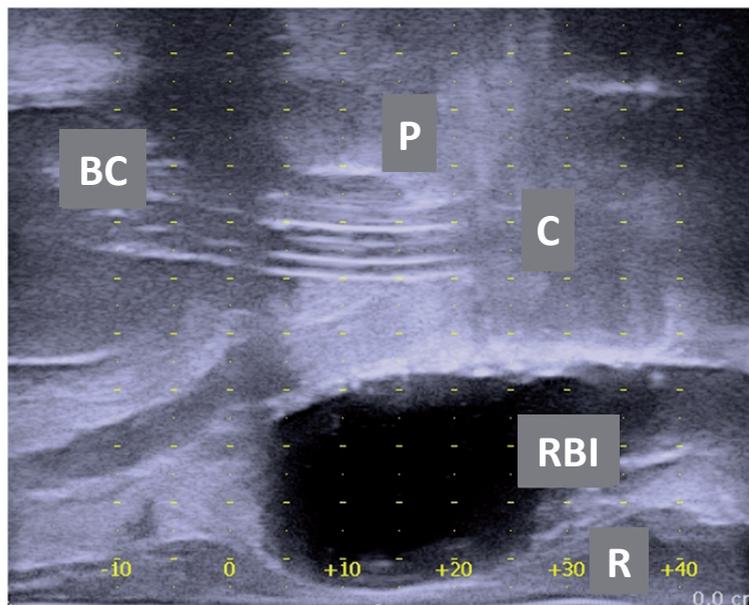


Figure 8 - Sagittal ultrasound image of patient with a rectum balloon implant (RBI) *in situ* between the prostate (P) and the rectum (R). The view is in the central plane with the urinary catheter (C) and the Foley balloon catheter (BC) visible in the bladder.



tenesmus occurred in two patients and one patient, respectively. Seven patients (47%) were free of complications.

DISCUSSION

The RBI separates the anterior rectal wall from the prostate, facilitating reduction of the high radiation dose to the anterior rectum wall. The potential failure modes, possible complications or pitfalls and corrective actions for this implantation procedure are described in Table-1.

Several types of spacers are available: hyaluronic acid, PEG-based hydrogel, human collagen, and biodegradable balloon. The advantage of the inflatable RBI system is that it allows for post-implant correction of the RBI position, whereas liquid spacers (hydrogels, hyaluronic acid, human collagen) do not permit any correction once injected (7). Furthermore, if such a liquid spacer is injected in the rectum wall, a rectum fistula can occur; this was recently mentioned by Habl et al., whereby they stopped using this promising technique (17). Next, the biodegradable RBI inflates to a predetermined and predictable shape, mea-

ning a learning curve is probably less important. Pinkawa et al. reported a learning curve of 64 implantations to fully implement and optimise rectum hydrogel spacer placement (18). Therefore, we choose to use the RBI. However, a possible disadvantage is early volume loss of the RBI before the end of the radiation treatment, as recently published by Wolf et al. (19). Further research is needed to evaluate and quantify this volume loss.

The implantation of rectum spacers is well tolerated. No severe grade 3-4 complications occurred in our series. In the literature, severe complications have been documented, but in very low numbers (8, 9). Perforation of the bladder or rectum are reported in 3 out of 23 cases (13%) in procedures performed without hands-free TRUS guidance (5, 8). According to the authors, these complications resolved with no further sequelae. After protocol modifications and introduction of TRUS guidance, no perforations or other severe complications have occurred, as in our series. We observed 1 out of 15 cases (7%) of temporary urinary retention, probably provoked by the use of general anaesthesia. The literature reported this in 1 out of 11 cases (9%) and 3 out of 26 cases (12%), respectively (6, 15).

Table 1 - List of hazards adapted to RBI implantation and corrective actions.

Potential failure mode	Corrective action
Bad TRUS view:	
Stool	Rectal cleansing
Bubbles	Wait a few minutes
Prostate calcifications	Not reliable
Hydrodissection:	
Needle is not advanced:	
in the midline	Check relation on TRUS axial view and the D-line/central plane with the urinary catheter
to the prostate base	Palpate with finger to check if rectum wall is free
Not performed in the proper plane	Check on TRUS axial view and perform again
Hydrodissection is not possible due to	
incorrect position of the needle, e.g. in the rectum wall or in the prostate	Reposition the needle
adhesions or patient anatomy	Lowering the probe before starting may help to open the space; if this is not possible, it is recommended to abort the procedure
Dilator:	
is difficult to insert	Make a deeper incision
is not advanced to the prostate base	Check on TRUS and reposition
Balloon:	
cannot be inflated	Remove the sheath sufficiently
is partially inflated and accidentally sealed	Push the balloon deeper, so it does not interfere with pelvic muscles
is inflated in a suboptimal position (wrong cleavage)	Remove RBI or detach it
is sealed and spontaneously deflates	Deflate RBI (percutaneous)
	Completely deflate RBI, be mindful of perforation
Post-procedure:	
Infection	Prophylactic antibiotic pre-procedure
	Quick start of antibiotic regimen
Bleeding	Stop antiplatelet therapy in advance
Urinary retention	Urinary catheter
Rectal perforation	Deflate RBI, suture, and post-operative antibiotics
Balloon is deflated	Implant transperineal fiducial markers before RBI implantation

Most of the current literature is limited to spacer implantation in patients with low-risk localised (intra-capsular) prostate cancer. So far, the role of spacers in locally advanced and high-risk prostate cancers is unclear (8). The possible negative influence of a spacer in cases with a dorsal prostate capsule rupture is yet unknown, as tumour cells could be displaced out of the high-dose region by the spacer (14). Studies are therefore needed to evaluate the advantages and possible disadvantages of spacers in these patients.

Each RBI is handmade and has a variable maximum volume of 15-20cc (BioProtect Ltd, Israel). The volume must not exceed the specific amount indicated on the individual balloon label in order to preserve RBI function and prevent bursting (with consequent loss of function). In practice, we correlate the volume of the RBI with the volume of the prostate: small prostates (<35cc) do not need the maximum RBI volume for sufficient space (at least 1cm). According to Pinkawa et al., a volume of 10mL is enough to ensure a distance of around 1cm (20).

Further clinical studies are required to define the place of an RBI in the treatment of prostate cancer radiotherapy. We believe that in the future, RBI should be prescribed on the basis of an individualised risk assessment with a validated predictive model and a decision support system to identify *a priori* whether individual patients will benefit from an RBI (21, 22). Prospective follow-up studies in independent patient cohorts are needed to assess the benefits of such an RBI.

CONCLUSIONS

This paper provides detailed step-by-step instructions for the safe implantation of an RBI. This procedure should be performed by urologists and/or radiation oncologists who are experienced in prostate brachytherapy and the use of TRUS. The RBI implantation is a safe and very well tolerated procedure with only slightly increased discomfort, and in some cases pain in the perineal region, which is easily addressed with mild pain medication. The theoretical advantages of RBI in-

clude reducing the high radiation dose to the anterior rectum wall, the possibility of a post-implant correction, and the implant's predetermined shape with consequent predictable position, meaning a learning curve is probably less important.

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CONFLICT OF INTEREST

None declared.

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Open radical prostatectomy reproducing robot-assisted radical prostatectomy: Involving antegrade nerve sparing and continuous anastomosis

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ABSTRACT

Purpose: To present modified RRP using the same method as RALP and compare its surgical outcomes with RALP.

Materials and Methods: Demographics, perioperative and functional outcomes of the 322 patients that underwent RRP (N=99) or RALP (N=223) at our institution from January 2011 through June 2013 were evaluated retrospectively. Postoperative incontinence and erectile dysfunction are involved functional outcomes. During the modified procedure, the bladder neck was dissected first as for RALP. After dissection of vas deference and seminal vesicle, the prostate was dissected in an antegrade fashion with bilateral nerve saving. Finally, the urethra was cut at the prostate apex. After a Rocco suture was applied, and then urethrovesical anastomosis was performed with continuous suture as for RALP.

Results: Perioperative characteristics and complication rates were similar in the RRP and RALP groups except for mean estimated blood loss ($p<0.001$) and operative time ($p<0.001$). Incontinence rates at 3 and 12 months after RRP decreased from 67.6% to 10.1 and after RALP decreased from 53.4% to 5.4%. Positive surgical margin rates were non-significantly different in the RRP and RALP groups (30.3% and 37.2%, respectively). Overall postoperative potency rate at 12 months was not significant different in RRP and RALP groups (34.3% and 43.0%).

Conclusions: RRP reproducing RALP was found to have surgical outcomes comparable to RALP. This technique might be adopted by experienced urologic surgeons as a standard procedure.

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INTRODUCTION

Prostate cancer is the second most commonly diagnosed cancer and the sixth leading cause of male death worldwide (1, 2). Radical retropubic prostatectomy (RRP) has been established as a standard surgical treatment in patients with localized prostate cancer over several decades (3) after it was introduced by Walsh et al. (4). More recently, several surgeons have refined the surgi-

cal procedure and reported excellent outcomes (5, 6). However, RRP still presents technical difficulties due to a narrow surgical field and complex anatomy. In addition, the aim of RRP is to maintain oncologic principles and retain functional outcomes, which include urinary continence and erectile function.

Robot assisted laparoscopic radical prostatectomy (RALP) represents the leading application of robotic surgery in the urologic field. RALP has

become the main treatment option for localized prostate cancer in worldwide and has been widely applied to improve operative outcomes (7-11). RALP involves advanced technologies that provide a 3-dimensional operative view, a laparoscopic instrument that mimics movements of the human wrist and hand, high-level resolution, enlarged images, and excellent lighting conditions. Therefore, RALP can be used to preserve neurovascular bundles more effectively and to enable the placement of anastomotic sutures in the narrow operative space without external loupes or a headlight. In addition, it has been reported that RALP is a feasible procedure that can enhance perioperative outcomes, by for example, reducing blood loss, hospital stays, and postoperative pain (12-14). Functional outcomes of RALP with respect to potency and continence have been evaluated in other studies and in terms of oncologic outcomes, RALP has replaced RRP for localized prostate cancer (15-17).

The widespread use of RALP has also contributed to the advancement of RRP. Numerous authors have compared the results of RALP and RRP in terms of surgical outcomes. However, the two techniques are quite different in terms of prostate dissection and urethrovesical anastomosis; RALP is conducted in an antegrade fashion using a continuous suture and RRP in a retrograde fashion using an interrupted suture. The widespread use of RALP has also contributed to advances of open prostatectomy. At our institution, we have been performing RRP using the method used for RALP, that is, using antegrade prostate dissection and urethrovesical anastomosis with a continuous suture. Here, we present the operative method of RRP and compare its pentafecta outcomes with those of RALP.

MATERIALS AND METHODS

Patients

We retrospectively analyzed the data of 322 consecutive patients that underwent RRP or RALP for prostate cancer between January 2011 and June 2013 (99 RRP and 223 RALP). This study was approved by the institutional review board and ethics committee of our hospital. The choice

of surgical procedure was based on patient's demand and surgeon preference. Study participants were followed for at least 1 year. Institutional review board approval was obtained prior to data retrieval and analysis. All 322 patients underwent radical prostatectomy performed by a single experienced surgeon who had performed over 300 RALP procedures and 500 RRP procedures. Demographic data, operative parameters, pathologic data, postoperative complications, postoperative incontinence (PPI), erection function recovery rates of the two study groups were compared for pentafecta outcomes of radical prostatectomy.

Surgical Technique

RALP was performed using a six-port transperitoneal approach using a four-arm da Vinci Si robotic system. In brief, patients were placed on the operating Table in the standard 30° Trendelenburg position. Laparoscopic adhe-nolysis was performed if required. The superficial dorsal vein was coagulated, divided, and pre-prostatic fat was removed. Both lateral sides of the bladder and prostate borderline were dissected first and then the bladder and prostate were divided using a Bovie knife along the bladder-prostate imaginary borderline until the prostatic urethra was exposed. The prostatic urethra was then incised and a previously placed Foley urinary catheter was observed, before continuing division of the bladder and prostate. When the prostate was completely divided, seminal vesicles and vas deferens were exposed and divided, vascular structures around them were ligated. Most vascular pedicles were ligated using 5mm Titanium Ligation clips (Aesculap, Melsungen, AG, Germany). Hem-o-lok clips (Teleex Medical, Durham, NC, USA) were used for large vessels unsuitable for ligation with titanium clips. Periprostatic tissue was then dissected antegrade fashion on each side by using scissors. Neurovascular bundles on both sides of the prostate gland were protected using an interfascial technique. The urethra was cut as distally as possible. The deep dorsal vein plexus was ligated by suturing after removing the prostate in an endo-pouch. After careful hemostasis, a Rocco suture

was applied at the time of urethrovesical anastomosis. Posterior muscle-fascia was sutured parallel from the apical portion to the bladder neck using two 3.0 Monocyn® (Aesculap, Melsungen, AG, Germany) strands tied together at their tails for the urethrovesical anastomosis. One strand of the running suture was directed right and the other directed left from 6:00 to 3:00 o'clock and from 6:00 to 9:00-o'clock, respectively. Anastomosis from 3:00 and 9:00 to 12:00 o'clock was performed while maintaining tension of the previous anastomosis using the third arm. At the end of this procedure a single tie was completed. The mucosa and serosa of the whole bladder neck were sutured divisively to prevent leakage and to tighten the anastomosis. A catheter was placed and the bladder was filled with 120mL of normal saline to check for leakage (Figures 1-3).

During RRP, patients were maintained in the standard 30° Trendelenburg position in common with RALP. The superficial dorsal vein and preprostatic fat were processed in the same manner as described for RALP. The bladder neck was dissected first after lateral bladder and prostate dissection and division at borderline as described for RALP. After dissection of vas deference and the seminal vesicle, the prostate was dissected in an antegrade fashion while preserving bilateral nerves. Finally, the urethra was cut at the prostate

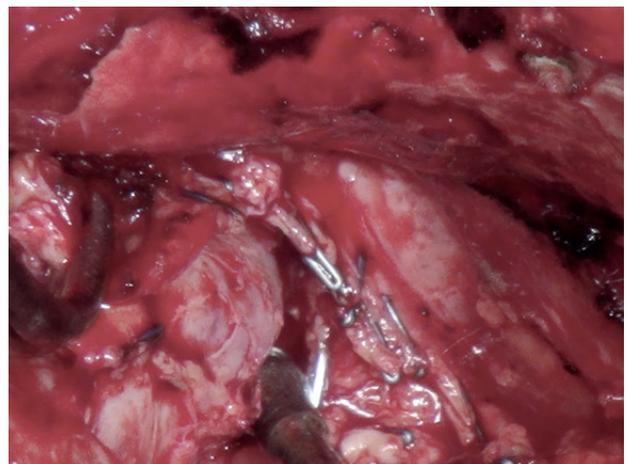
apex. After careful hemostasis, Rocco suture (3-0 monosyn) was applied at the same time as urethrovesical anastomosis. A two-strand running suture was placed in direction of both posterior sides first and then anastomosis was performed from 3:00 and 9:00 o'clock to 12:00-o'clock while maintaining tension at the posterior anastomosis site using a needle holder. A filling test was performed as described for RALP, and the Foley catheter used was removed on day 6 after surgery under cystographic control.

Figure 1 - Lateral bladder neck dissection (radical retropubic prostatectomy).



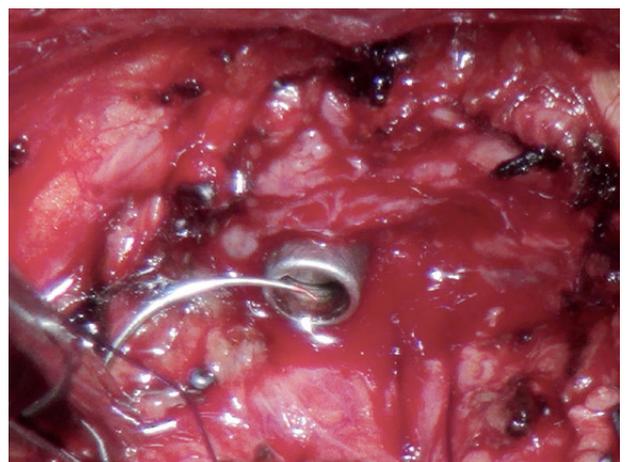
First dissection of both lateral sides of bladder and prostate borderline before anterior side dissection

Figure 2 - Antegrade prostate dissection including bilateral nerve sparing (radical retropubic prostatectomy).



Antegrade nerve sparing of right lateral side

Figure 3 - Continuous anastomosis (radical retropubic prostatectomy).



First running suture directed from 6:00 to 3:00 o'clock

Definition and assessment of continence

Continence was defined as using no pads and having no urine leakages, as determined by patient responses. Patients were asked the following question: "How many pads or adult diapers did you use per day to control leakage during the past 4 weeks?"

Recovery of continence was evaluated routinely at 1, 3, 6, and 12 months after surgery. In addition, we compared the severity of incontinence in the two groups using 0-1 pad use per day.

Definition of erection function recovery

Erection function recovery was defined as the ability to achieve penetration $\geq 50\%$ of the time and to maintain an erection significant enough for penetration $\geq 50\%$ of the time as per questions 2 and 3 of the International Index of Erectile Function (IIEF)-5 survey at 12 months after surgery. Our most patients were prescribed PDE-5 inhibitors for 3 months except patients with contraindication.

Definition of biochemical recurrence

Biochemical recurrence (BCR) was defined as a serum PSA >0.2 ng/mL on two consecutive measurements.

Follow-up evaluation

After hospital discharge, every patient was counseled to undergo a serum PSA test every 3 months for the first 2 years, every 6 months for the next 3 years, and then annually.

Statistical Analysis

Demographics and perioperative outcomes were analyzed using the Chi-square test and the Mann-Whitney test. The Chi-square test was used to analyze incontinence rates and erectile function recovery rates at the above mentioned times. The Kaplan-Meier method and the log rank test were used to assess biochemical recurrence-free survival rates. The analysis was performed using PASW® Statistics 18.0 (SPSS Inc., Chicago, IL, USA). For all comparisons, a p value of <0.05 was considered statistically significant.

RESULTS

No significant differences were found between the RRP and RALP groups into demographic data, such as, age, body mass index, prostate volume, or preoperative PSA (prostate specific antigen). However, some operative parameters were found to be significantly different. In particular, mean estimated blood loss (EBL) was significantly higher in the RRP group (253.4 mL vs. 192.6 mL, $p=0.001$), but mean operative time was significantly shorter (188.8 min vs. 244.6 min, $p=0.001$). Pelvic lymph node dissection (PLND) was performed in 22 (22.2%) and 36 (16.1%) members of the RRP and RALP groups, respectively, and a nerve sparing (NS) procedure was performed in 65 (65.7%) and 177 (79.1%), respectively. Whereas this difference in PLND was not significant ($p=0.190$), the NS difference was significant ($p<0.001$). The urine leakage as determined by cystography was similar in the RRP and RALP groups. (13.1% vs. 9.0%, $p=0.256$) (Table-1).

For the 322 study subjects, mean and median follow-up were 31.4 ± 10.3 months and 31 months (14-60), respectively. No intergroup difference was observed for pathologic stage or Gleason score. The positive surgical margin (PSM) rate was similar in the RRP group (30.3% vs. 37.2%, $p=0.230$). No intergroup difference was observed between PSM ranges ($p=0.219$). BCR occurred in 2 (2.0%) and 7 (3.2%) patients in the RRP and RALP groups, respectively. ($p=0.574$) (Table-2). Overall 3-year biochemical recurrence-free survival rates were 93.6% and 94.3% in the RRP and RALP groups (Figure-4).

Complication rates did not differ statistically in the RRP and RALP groups (5.1% vs. 2.7%, $p=0.282$). According to the Clavien classification (18), all complications were grade I or II and all cases were managed conservatively. In RRP group, 1 (1%) complication was grade I and 4 (4%) complications were grade II; in RALP group, 4 (1.8%) complications were grade I and 2 (0.9%) were grade II. One case of atelectasis, 2 cases of wound dehiscence and 2 cases of postoperative bleeding occurred in RRP group; 2 cases of atelectasis, 2 cases of ileus, 1 case of wound dehiscence and 1 case of pneumonia occurred in RALP group.

When continence was defined as no pad use per day, incontinence rates at 1, 3, 6, and 12 months decreased from 76.8% to 67.6%, 47.5%, and 10.1%, respectively, after RRP and from 70.0% to 53.4%, 39.5%, and 5.4% after RALP. The incon-

tinence rate at 3 months was significantly higher in the RRP group ($p=0.016$), but excepting the third month, incontinence rates were similar during the 12-month follow-up period. When continence was defined as no pad or a single secure pad per day,

Table 1 - Demographic data & Operative parameter.

	RRP (n=99)	RALP (n=223)	p value
Age (years)	65.5±5.6	65.0±6.5	0.530
Body mass index (kg/m ²)	23.6±2.5	24.0±2.8	0.243
Prostate volume (mL)	37.7±15.1	37.0±17.2	0.716
Preoperative PSA (ng/mL)			0.080
< 10	53 (53.5)	136 (61.0)	
10-20	25 (25.3)	61 (27.4)	
> 20	21 (21.2)	26 (11.7)	
PLND	22 (22.2)	36 (16.1)	0.190
NS	65 (65.7)	177 (79.1)	0.001
Mean operative time (min)	188.8±62.3	244.6±60.0	0.001
Estimated blood loss (mL)	253.4±155.5	192.6±112.5	0.001
Urine leak on cystogram*	13/99 (13.1)	20/223 (9.0)	0.256

* At postop 7 days

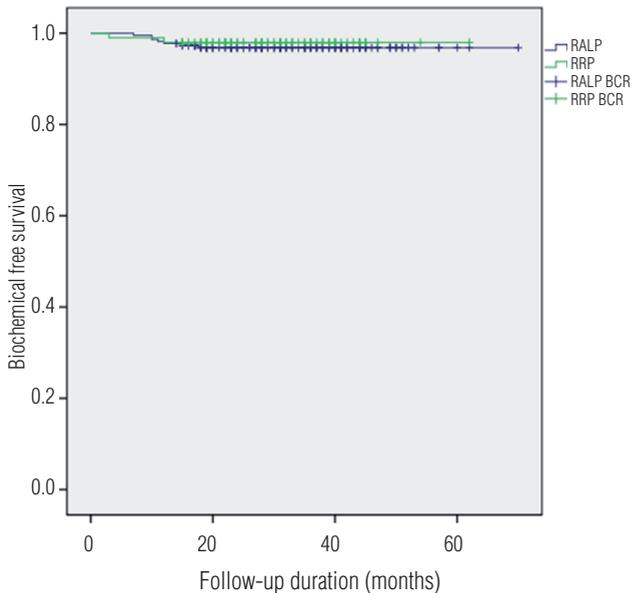
PLND = pelvic lymph node dissection; NS = nerve sparing procedure

Table 2 - Pathologic data.

	RRP (n=99)	RALP (n=223)	p value
Pathologic stage			0.634
T2	61(61.6)	129 (57.8)	
T3a	26 (26.3)	65 (29.1)	
T3b	12 (12.1)	26 (26.3)	
T4	0 (0)	3 (1.3)	
Pathologic Gleason score			0.744
6	33 (33.3)	86 (38.6)	
7	37 (37.4)	80 (35.9)	
8	24 (24.2)	42 (18.8)	
9	4 (4.0)	13 (5.8)	
10	1 (1.0)	2 (1.0)	
Positive surgical margin (%)	30/99 (30.3)	83/223 (37.2)	0.230
Focal/extensive	13/17	35/48	0.484
Biochemical recurrence (%)	7 (3.2)	2 (2.0)	0.574

Mean follow up duration: 31.4±10.3 months

Figure 4 - Overall 3-year biochemical recurrence-free survival rates.



the incontinence rates at 1, 3, 6, and 12 months decreased from 38.4% to 27.3%, 15.2%, and 4.0%, respectively after RRP to 22.9%, 16.1%, 9.4%, and 2.7% after RALP. Incontinence rates at 1 and 3 months were significantly higher in the RRP group.

With respect to erectile function, the overall postoperative potency rate at 12 months was 34.3% in the RRP group and 43.0% in the RALP group; however, this difference was no significant

(p=0.142). Postoperative potency rates at 12 months were 32.3% and 43.1% in the RRP and RALP groups for nerve sparing procedure, which was not a significant difference (p=0.975).

The prescription rates at 3 months were similar in both groups (90.6% vs. 85.9%, p=0.209). However, prescription rates at 12 months were significantly higher in RALP group (63.6% vs. 74.4%, p=0.048).

The overall pentafecta rate at 3 months was 11.1% and 14.3% in the RRP and RALP groups respectively. The pentafecta rate at 12 months was 21.2% and 29.6% for each group respectively. Pentafecta rate in both was not a significant difference. The potency rate was most common reason for not achieving the pentafecta (Table-3).

DISCUSSION

RRP is still the standard surgical treatment in terms of oncologic outcomes, but RRP is generally associated with significant decreases in quality of life as reflected by impotence and urinary incontinence rates. To address these problems, RALP has been widely introduced and has revolutionized prostate cancer surgery because of its associated magnified 3-D high-definition vision system and miniaturized wristed instruments, which allow microsurgery and respect of the most delicate anatomical structures. Furthermore, many recent technical and approach refinements during RALP have improved operative outcomes.

Table 3 - Pentafecta success rates between RRP and RALP at 6 and 12 months.

	3 months			12 months		
	RRP	RALP	p value	RRP	RALP	p value
No complication (%)	94.9	97.3	0.282	-	-	-
Negative PSM (%)	60.6	62.8	0.230	-	-	-
Continence (%)	32.4	40.6	0.016	89.9	94.6	0.121
Potent (%)	29.3	38.6	0.109	34.3	43.0	0.142
BCR (%)	1.0	0.0	0.133	2.0	2.1	0.900
Pentafecta (%)	11.1	14.3	0.430	21.2	29.6	0.118

PSM = Positive surgical margin; BCR = Biochemical recurrence; RRP = Retropubic radical prostatectomy; RALP = Robot assisted radical prostatectomy

Different centers have reported widely varying comparative results for RRP and RALP. Rocco et al. compared the early oncological perioperative and functional outcomes of RALP (n=120) and RRP (n=240), and found RRP seems to be the faster procedure and that RALP provides better results in terms of estimated blood loss, hospitalization, and functional results, such as, postoperative incontinence and erectile function. Furthermore, early oncological outcomes appeared to be equivalent in their two groups (19). Ficarra et al. performed a non-randomized prospective comparative study of all patients that underwent RALP or RRP, and concluded RALP offers better results in terms of urinary continence and erectile function recovery with similar positive surgical margin rates (20). Krambeck et al. retrospectively analyzed data obtained from RRP (n=588) and RALP (n=294) procedures, and observed no significant intergroup difference for overall early complications, long-term continence, or potency rates. Furthermore, early oncological outcomes were similar in their groups (21). However, in most previously reports, RRP was performed by retrograde nerve sparing dissection with interrupted sutures, and RALP by antegrade nerve sparing dissection with a continuous suture.

Nerve sparing is an important step in radical prostatectomy and substantially determines functional outcomes, and hence, every attempt should be made to preserve neurovascular bundles (NVB). Two approaches to nerve sparing can be used, that is, from the prostate base to the apex (antegrade) or from the apex to the base (retrograde). During robot or pure laparoscopic surgery, the antegrade approach is mainly adopted because it is believed that it allows for early control of prostatic pedicles, and thus, minimizes bleeding during NS. Furthermore, this approach provides a more natural working angle for instruments during NVB dissection after the bladder neck has been divided.

Vesicourethral anastomosis (VUA) is another important step in radical prostatectomy, and has also been found to affect hospital outcomes. RRP is a modified version of the initial VUA technique described by Walsh et al., which makes use of interrupted sutures and is used in modern

practice. However, interrupted suturing techniques are not used during RALP or laparoscopic radical prostatectomy (LRP) because of technical difficulties. Therefore, VUA using the continuous suturing technique introduced by Van Velthoven et al. (22) and modified by Menon et al. (23) is widely used. Several RALP and LRP studies using VUA and watertight continuous suturing have reported successful urethral catheter removal as early as 7 days after surgery (24, 25). In addition to its use in RALP and LRP, some studies have suggested that VUA with continuous suturing in open RRP could reduce VUA site leakage and alleviate PPI (26-28).

Before the introduction of RALP, we sutured the deep venous complex after opening endopelvic fascia, but this process created broad levator muscle injury, which is related to urinary incontinence. In addition, bleeding of pelvic muscles and adjacent tissues caused during this process can often obstruct the surgical field. These situations can be prevented by preserving the endopelvic fascia, and nerve-sparing procedures tend to be easier when the endopelvic fascia is preserved because it is not detached from muscle and the neurovascular bundle is relatively well-dissected. However, performing this technique was difficult in the narrow surgical field of RRP, which was adopted to prevent excessive bleeding. However, understanding of pelvic anatomy gained through experiences of robot surgery enable us to perform these ways. Furthermore, because the antegrade approach allows early control of prostatic pedicles, bleeding is minimized during NS and suturing of the deep dorsal vein complex is not required. Our VUA technique has several advantages. Because, we placed a Rocco suture and performed VUA simultaneously, posterior reconstruction approximated original anatomy. In particular, we sutured bladder mucosa and serosa separately, as a result, VUAs were watertight and in no patient was an anastomosis site torn intraoperatively. Furthermore, this procedure shortened the duration of indwelling Foley catheterization. In addition, anterior reconstruction was performed by suturing bladder serosa and prostatic fascia to include the prostatic ligament, which aided the recovery of continence and of the original anatomy.

The desirable results after radical prostatectomy include achieving oncologic and functional outcomes. Accordingly, trifecta or pentaecta represent optimal desired outcome and were used to help patients counseling undergoing radical prostatectomy. Antebi et al. reported trifecta following open radical prostatectomy. Trifecta at 2 and 5 years was achieved in 64% and 61% of patients respectively (29). Bianco et al. reported a trifecta rate of 60% at 2 years in 758 men after RRP (30). In our study, pentaecta rate at 12 months was 21.2% and 29.6% for each group respectively. Our results showed low rate relatively for previous other reports. It was considered that there were many high stage cases relatively in our cases for cited reports.

This study has some limitations that should be considered. First, no comparison was made between antegrade RRP and conventional RRP. Second, our results are based on a relatively small sample size because the study was performed using a retrospective design at a single institution. Nevertheless, the study shows that RRP can be improved by adopting what is essentially a RALP procedure. Moreover, our procedure can help to urologist trained with Robot system but affiliated in medical institution not equipped with Robot system.

CONCLUSIONS

The surgical technique used during RALP, that is, antegrade dissection and continuous urethrovesical anastomosis, could be used for RRP. In the present study, antegrade RRP produced perioperative surgical outcomes comparable with that of RALP. We believe that this technique has the potential to be adopted by urologic surgeons as a standard RRP procedure.

ABBREVIATIONS

RRP = Radical retropubic prostatectomy

RALP = Robot assisted laparoscopic radical prostatectomy

PPI = postoperative incontinence

IEEF = International Index of Erectile Function

PSM = positive surgical margin

EBL = estimated blood loss

VUA = Vesicourethral anastomosis

PLND = Pelvic lymph node dissection

NS = Nerve sparing

PSA = Prostate specific antigen

CONFLICT OF INTEREST

None declared.

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Ureteral orifice involvement by urothelial carcinoma: long term oncologic and functional outcomes

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ABSTRACT

Purpose: Bladder cancer (BC) may involve the ureteral orifice, and the resection of the orifice has oncological and functional consequences such as development of upper tract urothelial carcinoma (UTUC), vesicoureteral reflux or ureteral stenosis. The aim of this study was to investigate the oncological and functional outcomes of the ureteral orifice resection in BC patients and determine the predictive factors for UTUC development.

Materials and methods: A total of 1359 patients diagnosed with BC, between 1992 and 2012, were reviewed retrospectively. Patients were grouped with respect to orifice resection and compared for development of UTUC, survival and functional outcomes. Kaplan-Meier method was used to compare survival outcomes. Logistic regression analysis was performed to determine predictors of UTUC development.

Results: Ureteral orifice involvement was detected in 138 (10.2%) patients. The rate of synchronous (10.1% vs. 0.7%, $p=0.0001$) and metachronous (5.3% vs. 0.9%, $p=0.0001$) UTUC development was found to be higher in patients with ureteral orifice involvement. Orifice involvement and tumor stage were found to be associated with development of UTUC in the regression analysis. Overall ($p=0.963$) and cancer specific survival rates ($p=0.629$) were found to be similar. Hydronephrosis was also significantly higher in patients with orifice involved BC, due to the orifice obstruction caused by the tumor (33.3% vs. 13.9%, $p<0.05$).

Conclusions: BC with ureteral orifice involvement has significantly increased the risk of having synchronous or metachronous UTUC. However, orifice involvement was not found to be associated with survival outcomes. Development of stricture due to resection is a very rare complication.

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Keywords:

Urinary Bladder Neoplasms; Hydronephrosis; Therapeutics

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INTRODUCTION

Urothelial carcinoma of the bladder is the most common malignancy of the urinary tract (1, 2). Bladder cancer (BC) may be localized anywhere in the bladder and involvement of ureteral orifi-

ce or its close environment has been reported in 5-35% of the cases (3-7). Involvement of ureteral orifice is a diagnostic and therapeutic dilemma as the disease location itself or the applied treatments may cause oncological and functional derangements in the upper urinary tract (5, 6, 8, 9).

Transurethral resection (TUR) of the ureteral orifice is necessary during treatment of these cases (3, 4, 8, 10) and TUR of the ureteral orifice is suggested to cause vesicoureteral reflux (VUR), due to the destruction of the muscle fibers, which leads to upper tract urothelial carcinoma (UTUC) development (5, 8, 9). Additionally, the electro-resection of the ureteral orifice may cause orifice stenosis, and secondary obstruction of the upper urinary tract as well (3, 6, 11).

In the current literature there are a number of studies that report the treatment outcomes of patients with involvement of the ureteral orifice (3-12). These studies involve either relatively low number of patients (6, 8-11) or insufficient follow-up data (3, 5, 7, 8).

In this study, we investigated the data of 138 patients underwent orifice resection from a cohort of 1359 patients underwent TUR for urothelial carcinoma and aimed to report the oncological and physiological outcomes of the patients underwent TUR of the ureteral orifice in comparison with patients that have no evidence of ureteral involvement.

MATERIALS AND METHODS

This study began after Local Ethics Committee approval, and the medical records were based on the Oncologic Urology Clinics of Tepecik Research and Education Hospital in Izmir, in Turkey. All patients, diagnosed with BC between 1992 and 2012 were reviewed retrospectively, and 1359 patients with available data about tumor localization were evaluated.

The tumors were staged and graded according to the International Union Against Cancer TNM classification and WHO 1973 grading scheme (1, 13). The tumors were classified as <3cm or ≥3cm, and as solitary or multiple. An atrophic kidney was detected in some patients due to obstruction; therefore, the development of hydronephrosis was described as hydronephrosis±atrophic kidney. Tumors involving the ureteral orifice were treated with wide, deep resection, including the entire orifice area, as mentioned in the literature (7, 9, 10). During TUR, pure cutting current was used and selective coagulation was performed

to achieve hemostasis. According to our departmental policy, ureteral stenting was avoided. All patients were routinely evaluated via intravenous urography or ultrasound during the first visit and, if necessary, computed tomography and further imaging were performed. During the follow-up, adjuvant intravesical chemotherapy or immunotherapy, re-TUR, second TUR, imaging, advanced therapy, etc. were performed according to the valid guidelines at the time (13, 14). Survival was calculated from the date of surgery, to either the last follow-up or death.

Statistical analysis was performed using the SPSS 22.0 software program for Windows (SPSS Inc., Chicago, IL, USA). Descriptive statistics for the clinical, pathological and treatment related data were provided. The Student t and Fisher exact tests were used to compare continuous and categorical variables, respectively. Logistic regression analysis was performed to define factors associated with the development of UTUC. Kaplan-Meier analysis was performed to evaluate cancer-specific and overall survival rates of patients with and without ureteral orifice involvement. Cox regression analysis was performed to define the factors associated with survival rates. For statistical significance p-value of 0.05 was accepted.

RESULTS

Among 1359 patients, 138 (10.2%) had BC involving the ureteral orifice. The two groups did not show significant difference in terms of demographic and cancer-related characteristics except, multiple tumors were significantly more frequent in patients without orifice involvement, and hydronephrosis at the initial diagnosis was more prevalent in the group of patients with orifice involvement. The patients and tumor characteristics are summarized in Table-1. One patient had a history of nephrectomy for renal cell cancer (RCC) before the diagnosis of BC.

UTUC development

UTUC was present at the time of diagnosis in 14 of the 138 patients (10.1%) and 8 of 1221 patients (0.7%) in the orifice involved and uninvolved groups respectively (p=0.0001). Rate of

Table 1 - Patients and tumor characteristics.

Characteristics	Orifice involved (n=138)	Non-Orifice involved (n=1221)	Total (n=1359)	P
Age (Mean ± SD)	65.1±10.5	63.4±11.7	63.5±11.6	0.087
Follow-up, months (mean ± IQR)*	45.5 (9-68)	47.1 (9-70)	46.9 (9-69)	0.721
No. Gender (%)				
M	119 (86.2)	1095 (89.7)	1214 (89.3)	0.214
F	19 (13.8)	126 (10.3)	145 (10.7)	
No.TCC tumor grade (%)				
G1	47 (34.1)	493 (40.4)	540 (39.7)	0.067
G2	36 (26.1)	286 (23.4)	322 (23.7)	
G3	40 (29.0)	248 (20.3)	288 (21.2)	
Unspecified	15 (10.8)	194 (15.9)	209 (15.4)	
No.TCC Tumor stage (%)				
Ta	47 (34.1)	349 (28.6)	396 (29.1)	0.565
T1	59 (42.8)	520 (42.6)	579 (42.6)	
≥T2	32 (23.2)	302 (24.7)	334 (24.6)	
Unspecified	-	50 (4.1)	50 (3.7)	
Carcinoma in situ (CIS)(%)				
CIS at initial diagnosis	6 (4.3)	42 (3.4)	48 (3.5)	0.584
CIS progression	3 (2.2)	22 (1.8)	25 (1.8)	0.758
Total CIS	9 (6.5)	64 (5.2)	73 (5.3)	
No. Tumor size (%)				
Tumor < 3 cm	35 (25.4)	348 (28.5)	383 (28.2)	0.305
Tumor ≥ 3 cm	94 (68.1)	755 (61.8)	849 (62.5)	
Unspecified	9 (6.5)	118 (9.7)	127 (9.3)	
No.Tumor number (%)				
Solitary	99 (71.7)	728 (59.6)	827 (60.9)	0.003
Multiple	36 (26.1)	479 (39.2)	515 (37.9)	
Unspecified	3 (2.2)	14 (1.1)	17 (1.2)	
Hydronephrosis (initial diagnosis)(%)				
Hydronephrosis±Atrophic kidney	46 (33.3)	170 (13.9)	216 (15.9)	0.0001
Presence of UTUC (%)				
Synchronous	14 (10.1)	8 (0.7)	22 (1.6)	0.0001
Metachronous*	7 (5.3)	11 (0.9)	18 (1.4)	

* Results of 1299 follow-up patients (132 orifice involved bladder cancer).

metachronous UTUC could be evaluated in 1299 patients (132 orifice involved bladder cancer) and after a mean follow-up of 47 (IQR: 9-69) months, metachronous UTUC developed in 5.3% and 0.9% of the patients in the orifice involved and uninvolved groups of patients respectively (p=0.0001). The results of synchronous and metachronous UTUC are summarized in Figure-1. Logistic regression analysis was performed to determine factors associated with synchronous and metachronous UTUC development. Orifice involvement (OR: 16.044, 95% CI: 6.575-39.151, p=0.0001) and tumor stage (OR: 15.516, 95% CI:1.908-126.182, p=0.01) were identified as the parameters associated with synchronous UTUC development. For metachronous UTUC development, orifice involvement (OR: 9.141, 95% CI: 3.104-26.923, p=0.0001) and T stage (OR: 8.892, 95% CI: 1.163-67.978, p=0.035) were detected as significant. The results of logistic regression analysis are summarized in Table-2.

Survival analysis

Kaplan-Meier analysis was performed to determine the effect of orifice involvement on cancer-specific and overall survival. Both

cancer-specific and overall survival rates of the orifice involved and uninvolved groups were similar. The survival rates are summarized in Table-3 and Kaplan-Meier figures are given in Figure-2.

Functional outcomes

Development of hydronephrosis or renal failure could be evaluated in 132 of the 138 patients with ureteral orifice involvement. One patient underwent nephrectomy due to RCC and hydronephrosis was present in 44 of these patients prior to resection. Seventeen of these 44 patients also had non-functional kidney and 15 of these patients underwent nephrectomy. Hydronephrosis reversed in 10 of the remaining 27 patients (with hydronephrosis and a functioning kidney) after orifice resection. Hydronephrosis at the ipsilateral kidney developed in 17 of the 87 remaining patients without initial hydronephrosis. The underlying cause of hydronephrosis was vesicoureteral reflux in 8 (47%) patients, cancer progression and involvement of orifice in 5 (29%) patients, stone disease in 3 (18%) patients and orifice stenosis in 1 (6%) patient.

Figure 1 - UTUC Status.

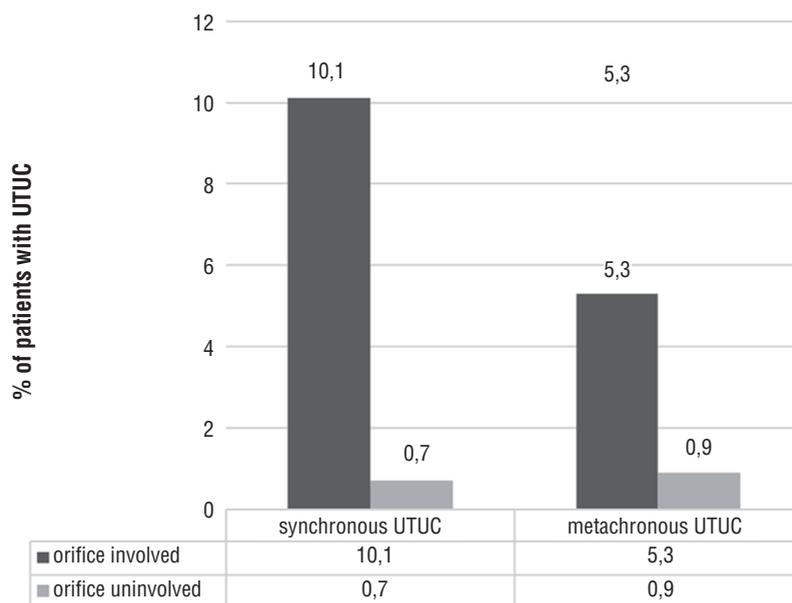


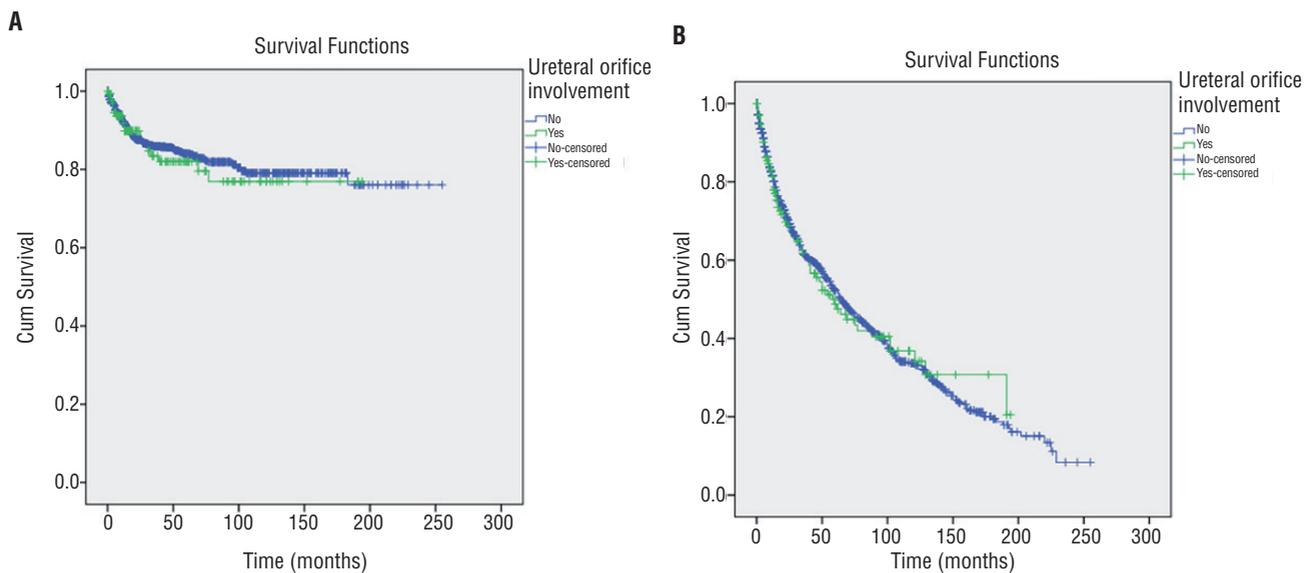
Table 2 - Results of logistic regression analysis for development of synchronous and metachronous UTUC.

Parameter	Synchronous UTUC development			Metachronous UTUC development		
	OR	95% CI	P value	OR	95% CI	P value
Age	1.006	0.964-1.051	0.780	0.964	0.922-1.009	0.113
Sex (male vs female)	0.774	0.200-2.994	0.710	0.572	0.069-4.721	0.604
Tumor grade	2.089	0.896-4.868	0.088	1.650	0.164-16.585	0.670
Tumor stage	15.516	1.908-126.182	0.01	8.892	1.163-67.978	0.035
Tumor multiplicity	0.523	0.166-4.648	0.269	0.443	0.158-1.240	0.121
Tumor size (<3 cm vs. ≥3 cm)	0.579	0.200-1.677	0.314	1.731	0.585-5.127	0.322
Orifice involvement	16.044	6.575-39.151	0.0001	9.141	3.104-26.923	0.0001

Table 3 - Survival rates of the ureter orifice involved and uninvolved patient groups.

Time	Cancer specific survival rates (%)			Overall survival rates (%)		
	Orifice uninvolved	Orifice involved	P value	Orifice uninvolved	Orifice involved	P value
3 years	85.8	82.0		61.2	60.8	
5 years	83.8	79.6	0.629	52.1	47.5	0.963
10 years	76.1	Not reached		33.4	34.2	

Figure 2 - Kaplan-Meier curves for cancer specific (2A) and overall survival (2B).



DISCUSSION

Involvement of ureteral orifice or its close environment by urothelial carcinoma is observed in up to 35% of the cases (4, 6, 7, 9, 12). Resection of the orifice is necessary in these cases and this has potential to result in loss of anti-reflux mechanism and therefore seeding of malignant cells in the upper urinary tract or ureteral orifice stenosis, which may lead to renal function impairment. In this study, we reported the long-term oncological and functional outcomes of 138 patients underwent ureteral orifice resection due to involvement by urothelial carcinoma and ureteral orifice involvement and resection was shown to increase the risk of UTUC development.

Results of resection of the ureteral orifice have been reported as early as 1936 and in a series of 5 patients, no cases of ureteral orifice stenosis were reported (15). Later on, Rees et al. reported their outcomes in 20 patients, which revealed reflux in 12 of the 17 patients with follow-up data and no cases of stenosis was reported (4). In these two early series, no data for development of UTUC was available. The first study with evaluation of UTUC development was published by Gottfries et al. and the authors reported their results of 19 patients with a 12 month mean follow-up. In this, no cases of UTUC or ureteral orifice stenosis were reported, with 9 patients found to have reflux (9). Resection of ureteral orifice seems to provide favorable results based on the results of these very early studies which have either very low number of patients of very short duration of follow-up. However, De Torres Mateos et al. reported 26% rate of reflux following resection and they also found a 22-fold greater risk of UTUC development. Therefore, the authors concluded on close follow-up for UTUC development following resection of the ureteral orifice (5). Palou et al. reported the results of their 19 patients underwent resection of the ureter with a mean follow-up of 57 months and they reported UTUC development in 8 patients (42.1%), and nontumoral stenosis in 3 (16%) of the patients. Therefore, the authors also concluded in closer follow-up of the upper urinary tract (11). In a more recent series, Chou et al. reported the results of 31 patients underwent

ureteral orifice resection and UTUC was observed in 4 (12.9%) of the patients after a mean follow-up of 33.5 months. Orifice stenosis was reported in 3 (10%) patients as well (6). In another recent series, Mano et al. reported results from 79 patients and 89 renal units underwent ureteral orifice resection. The median follow-up duration was 15 months and they reported 11 (13%) patients to develop hydronephrosis. However, orifice stricture was the cause of hydronephrosis in only 3 (4%) of these patients. UTUC development during the follow-up was reported in only one patient (3).

Our study included a high number of patients with ureteral orifice involvement and different from the previous studies we reported synchronous and metachronous UTUC development separately. Ureteral orifice involvement was found to be associated with 14.4 and 5.7 times increased risk of development of synchronous and metachronous UTUC, respectively. This increased rate of development of metachronous UTUC is parallel to the findings of De Torres Mateos et al. (5). But it is much higher compared to the results of Mano et al. (3), which reported UTUC development in only one patient. This difference may be associated with differences in the duration of follow-up.

Also, the logistic regression analysis revealed ureteral orifice involvement as a significant factor for the development of synchronous and metachronous UTUC. The risk factors for UTUC in primary BC are strongly related to the primary tumor risk stratification, where the incidence is as low as 0.7% in the low-risk group, to as high as 24% in high-risk groups (16). Tumor grade, the presence of carcinoma in situ (CIS), tumor stage, and tumor multiplicity were the factors identified to have an association with the development of UTUC (11, 16, 17). Our result revealed an evidence for the significance of ureteral orifice involvement for further development of UTUC.

This increased risk of UTUC in our population also takes into mind the question of the effect of ureteral orifice involvement on survival rates. Therefore, we performed survival analysis and no significant difference in overall and cancer-specific survival rates were detected between patients with and without ureteral orifice involvement.

Our data indicate that resection of the ureteral orifice resulted in resolution of hydronephrosis in 10 of the 27 patients that have hydronephrosis prior to resection. New developed hydronephrosis was observed in 17 of the 87 patients without prior hydronephrosis and orifice stenosis was the cause in only one patient. This result is consistent with the results of the study by Mano et al. (3). In our series, ureteral catheterization following resection was not performed in any of the patients and ureteral stricture developed in only one patient. Therefore, we support the idea of ureteral stenting unnecessary, contrary to the results of the study by Chou et al. which reported 10% obstruction rate (6). Ureteral stenting may be beneficial to prevent the consequences related to ureteral orifice edema, but fibrotic changes were shown to develop after about a month following surgery, which corresponds to the time for extraction of the ureteral stent (18). Therefore, we recommend against routine ureteral stenting following ureteral orifice resection and any symptom related to ureteral orifice edema should be tried to be managed conservatively in the first step.

Our study has some limitations. First of all, retrospective nature and inclusion of patients from a 20 years of time interval limits the homogeneity of follow-up and imaging protocols. Additionally, treatment guidelines showed significant changes during the study period, therefore patients received different adjuvant treatments for urothelial cancer, which has an effect on the survival rates as well.

CONCLUSIONS

The involvement of the ureteral orifice seems to be an important risk factor for both synchronous metachronous UTUC development. However, ureteral orifice involvement was not found to be associated with overall and cancer specific survival outcomes. Resection of ureteral orifice seems to achieve acceptable functional outcome results. Clinicians should suspect UTUC in patients with BC involving the ureteral orifice, especially when associated with hydronephrosis.

ABBREVIATIONS

BC = Bladder Cancer
 CIS = Carcinoma in situ
 TUR = Transurethral resection
 UTUC = Upper tract urothelial carcinoma
 VUR = Vesicoureteral reflux
 RCC = Renal Cell Cancer

CONFLICT OF INTEREST

None declared.

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miR-483-5p promotes prostate cancer cell proliferation and invasion by targeting RBM5

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ABSTRACT

Objective: miR-483-5p has been identified as a miRNA oncogene in certain cancers. However, its role in prostate cancer has not been sufficiently investigated. In this study, we investigated the role of miR-483-5p in prostate cancer and examined RBM5 regulation by miR-483-5p.

Material and methods: Expression levels of miR-483-5p were determined by quantitative real-time PCR. The effect of miR-483-5p on proliferation was evaluated by MTT assay, cell invasion was evaluated by trans-well invasion assays, and target protein expression was determined by western blotting in LNCaP, DU-145, and PC-3 cells. Luciferase reporter plasmids were constructed to confirm the action of miR-483-5p on downstream target gene RBM5 in HEK-293T cells.

Results: we observed that miR-483-5p was upregulated in prostate cancer cell lines and tissues. A miR-483-5p inhibitor inhibited prostate cancer cell growth and invasion in DU-145 and PC-3 cells. miR-483-5p directly bound to the 3' untranslated region (3'UTR) of RBM5 in HEK-293T cells. RBM5 overexpression inhibited prostate cancer cell growth and invasion in LNCaP cells. Enforced RBM5 expression alleviated miR-483-5p promotion of prostate cancer cell growth and invasion in LNCaP cells.

Conclusion: The present study describes a potential mechanism underlying a miR-483-5p/RBM5 link that contributes to prostate cancer development.

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Keywords:

MIRN483 microRNA, human [Supplementary Concept]; RBM5 protein, human [Supplementary Concept]; Prostatic Neoplasms; Growth

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INTRODUCTION

Prostate cancer is the most common type of cancer, and is an universal cause of cancer-related death in men worldwide (1). Therefore, it is necessary to improve prostate cancer detection, diagnosis, treatment and survival (2). However, there are few reliable biomarkers for early prostate cancer diagnosis and prognosis (3). Many microRNAs (miRNAs) have been shown to affect key cellular processes involved in prostate tumorigenesis, and thus, miRNAs may be potential prostate cancer biomarkers (4).

miRNAs are a group of small non-coding RNAs of 17-25 nucleotides in length that are conserved across species (5-7). miRNAs are involved in several developmental and physiological processes, and their dysregulation has been associated with disease development, including cancer (8, 9). They have been implicated in tumor formation, progression, invasion and metastasis. Depending on its target gene, a miRNA can act as an oncogene or tumor suppressor gene (10). Previous studies have suggested miR-483-5p as a potential hepatocellular carcinoma biomarker (11) and a marker of

poor adrenocortical carcinoma prognosis (12, 13). Furthermore, miR-483-5p is a potential predictor of myeloma survival (14). It also promotes lung adenocarcinoma invasion and metastasis (15). miR-483-5p can be detected in the cell-free, non-exosome-enriched fraction of urine collected from patients with prostate cancer (16), however, its role in prostate cancer is unclear.

RBM5 is a well-known tumor suppressor gene, and it inhibits cell growth by modulating apoptosis (17). RBM5 inhibits lung adenocarcinoma formation through diverse apoptotic signaling pathways (18). RBM5 has been implicated as a tumor suppressor gene in lung cancer (19) and prostate cancer (20), but it is unclear whether RBM5 is a miR-483-5p target.

In this study, we explored the role of miR-483-5p in prostate cancer development. Our results suggested that miR-483-5p plays a critical role in cell proliferation and invasion by regulating its target gene RBM5 in human prostate cancer. The present study describes a potential mechanism underlying a miR-483-5p/RBM5 link that contributes to prostate cancer development. Our results demonstrated that miR-483-5p is a potential target in prostate cancer therapy.

MATERIALS AND METHODS

Cell lines

The human prostate cancer cell lines VCaP, LNCaP, DU-145, and PC-3, human prostate epithelial cell line RWPE-1, and HEK 293T cells were purchased from the American Type Culture Collection (ATCC). Prostate cancer cells were cultured in RPMI-1640 medium (Invitrogen) supplemented with 10% fetal bovine serum (Gibco) and in a 37°C humidified atmosphere of 5% CO₂. RWPE-1 cells were cultured following the ATCC instructions. HEK 293T cells were grown in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum (Gibco).

Transfection

MiR-483-5p mimics and the miR-483-5p inhibitor were purchased from Sigma-Aldrich. We used mirVana miRNA mimic or mirVana

miRNA inhibitor (Ambion, Austin, TX, USA) for the negative control. Furthermore, a RBM5 expression vector was generated into a pCMV-N-FLAG vector (Beyotime, Jiangsu, China) and pCMV-N-FLAG vector for the negative control. Cells were allowed to reach 70% to 80% confluence in 6-well plates before transfection. Cells were transfected using Lipofectamine²⁰⁰⁰ according to the manufacturer's instructions. After 48 hours of transfection, the cells were harvested for further study.

Prostate tissues

Fresh tumor tissues were obtained from 26 prostate cancer patients during surgery at Baotou Central Hospital. The selected prostate cancer specimens were immediately frozen in liquid nitrogen and stored at -80°C for RNA extraction. The present study was approved by the Ethics Committee of Baotou Central Hospital.

RNA preparation and quantitative real-time PCR

Total RNA was extracted from cells using TRIzol® Reagent (Invitrogen, Carlsbad, CA, USA) and treated with DNase I (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's protocol. RNA (1µg) from each sample was reverse transcribed into complementary DNA (cDNA) using random primers, and the cDNA was subjected to quantitative reverse-transcription polymerase chain reaction (qRT-PCR). Subsequently, 1µg RNA was transcribed into cDNA using a miR-483-5p-specific stem-loop primer, and qRT-PCR was performed with miR-483-5p-specific primers using a 7500 Real-Time PCR System (Applied Biosystems, Mannheim, Germany). All miR-483-5p and U6 primers were synthesized by GenePharma, Shanghai.

Protein extraction and Western blot analysis

Proteins were extracted with RIPA buffer (Beyotime, Shanghai, China) containing protease inhibitors. Equal amounts of protein samples were separated by 10% SDS-PAGE and electrotransferred to PVDF membranes (Millipore, Billerica, MA, USA). After blocking, the membranes were immunoblotted overnight at 4°C with primary an-

tibodies, followed by HRP-conjugated secondary antibodies at 37°C for 1h. Signals were detected using an ECL system. Primary antibodies against RBM5 (Abcam) and GAPDH (KangChen Bio-tech) were used.

Luciferase reporter assays

The RBM5 3'untranslated region (UTR) luciferase reporter plasmid and plasmid containing the RBM5 3'UTR (base pairs 416-438) with the miR-483-5p target site deleted were constructed using the pMIR-REPORT vector (Ambion, Austin, TX, USA). The two constructs were confirmed by DNA sequencing, and luciferase activity assays were performed. Briefly, luciferase activities were measured 48h post-transfection using a Dual-Luciferase Reporter Assay System (Promega, Beijing, China) following the manufacturer's instructions. The data were normalized by dividing firefly luciferase activity by that of Renilla luciferase.

Cell proliferation assay

Cell proliferation was analyzed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells were seeded in 24-well plates and cultured for 1 to 4 days, following by the addition of MTT solution for 2 hours. After removing the medium, the remaining MTT formazan crystals were solubilized in DMSO and measured with a microplate reader at 570nm.

Trans-well invasion assay

For invasion assays, 1.0×10^5 cells were seeded in a Matrigel-coated chamber (BD Biosciences). Cells were seeded in serum-free media and then cultured in complete growth media. After 24 hours of incubation at 37°C, cells that had invaded were fixed and stained in dye solution containing 20% methanol and 0.1% crystal violet. Invasive cells were imaged using a BH-2 inverted microscope (Olympus). The mean values of three duplicate assays for each experimental condition were used for statistical analysis.

Statistical analysis

Statistical significance was determined using two-tailed Student's t-tests between the

means of the control and experimental groups. All statistical calculations were performed and graphs were generated using Graphpad Prism 5.0 software.

RESULTS

miR-483-5p is significantly upregulated in prostate cancer cell lines and tissues

We first performed quantitative PCR to detect miR-483-5p levels in the following prostate cancer cell lines: VCaP, LNCaP, DU-145, and PC-3. We compared them to the miR-483-5p levels in the non-tumorigenic RWPE-1 human prostate epithelial cell line. miR-483-5p was upregulated in all 4 prostate cancer cell lines compared to RWPE-1 cells (Figure-1A). To ascertain the clinical significance of miR-483-5p, we analyzed miR-483-5p expression levels by qRT-PCR in human prostate cancer tissues compared with its expression in normal human prostate tissues. We found that miR-483-5p expression dramatically increased in prostate cancer tissues ($P < 0.01$, Figure-1B).

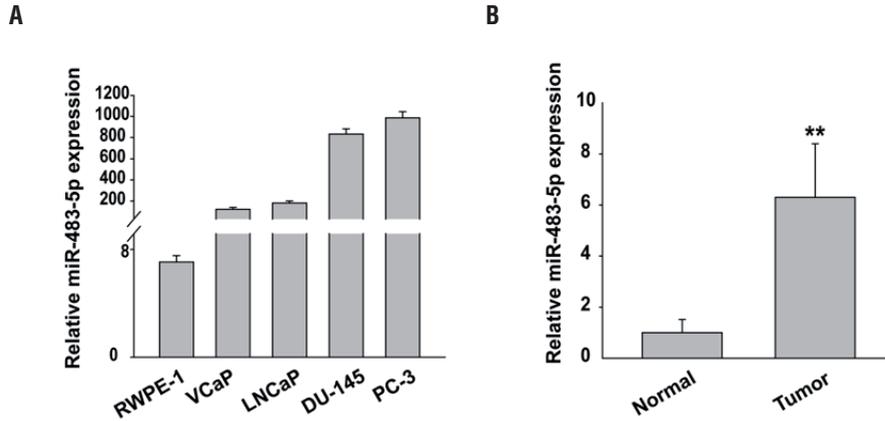
MiR-483-5p inhibition decreases prostate cancer cell growth and invasion

To determine whether miR-483-5p promotes oncogenic phenotypes of prostate cancer, we performed inhibition function assays in prostate cancer cells by using a miR-483-5p inhibitor. As shown in Figures 2A and 2B, miR-483-5p inhibition significantly reduced prostate cancer cell growth compared to the scramble control cells as measured by MTT assay. Furthermore, we performed Trans-well invasion assays to determine whether miR-483-5p regulates prostate cancer cell invasiveness (Figures 2C and 2D). We found that miR-483-5p inhibition reduced prostate cancer cell invasion through Matrigel.

RBM5 is a direct miR-483-5p target gene in prostate cancer

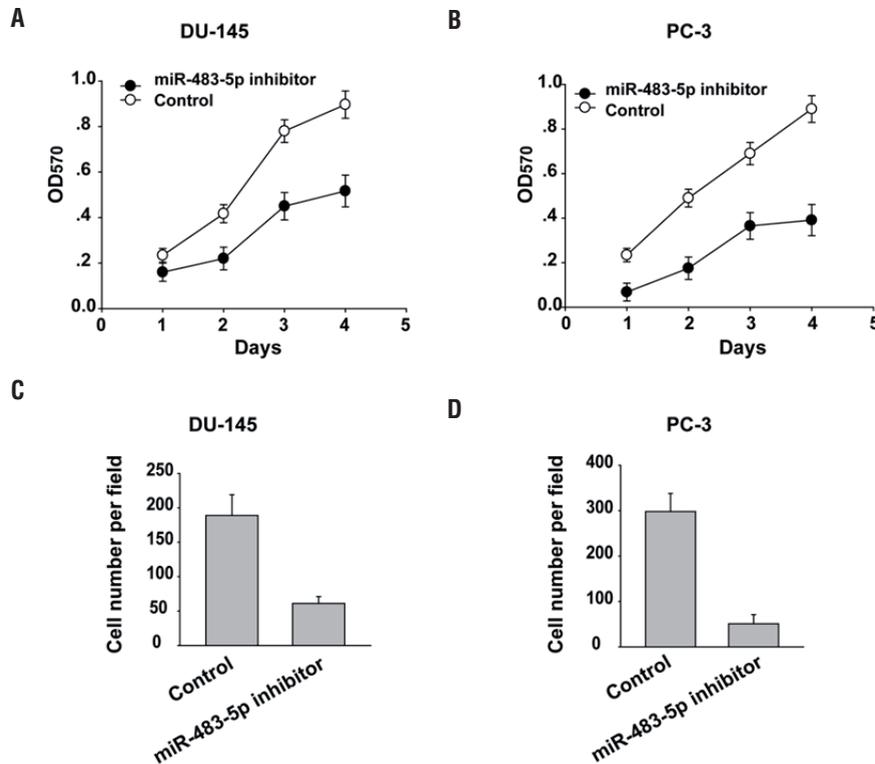
By utilizing a bioinformatic algorithm (miRNAdb), we identified RBM5 as a miR-483-5p target gene. The predicted miR-483-5p binding site within the RBM5 mRNA 3' UTR is shown in Figure-3A. Western blot analysis further

Figure 1 - miR-483-5p is significantly upregulated in prostate cancer cell lines and tissues. A, histograms of the average relative expression of miR-483-5p in a normal prostate epithelial cell line and prostate cancer cell lines as shown. B, relative ratios of miR-483-5p expression in 26 prostate cancer tissues compared with 10 normal prostate tissues.



** , P<0.01. Data are presented as mean±SD from three independent experiments.

Figure 2 - MiR-483-5p inhibition decreases prostate cancer cell growth and invasion. (A) (B) MTT assay was performed after transfection of DU-145 cells (A) or PC-3 cells (B) with the miR-483-5p inhibitor for the indicated time. (C) (D) Invasion assays were used to determine DU-145 cells (C) or PC-3 cells (D) motility. Experiments were performed in triplicate.



Data are presented as mean±SD from three independent experiments.

demonstrated that miR-483-5p overexpression dramatically suppressed endogenous RBM5 protein levels (Figure-3B).

We demonstrated that miR-483-5p directly regulates RBM5 using a Dual-Luciferase Reporter Assay System. miR-483-5p strongly reduced luciferase activity only in the presence of the RBM5 3' UTR. Mutation of the miR-483-5p seed recognition motif abrogated these effects, confirming that RBM5 is a miR-483-5p target (Figure-3C).

Enforced RBM5 expression mitigates miR-483-5p promotion of prostate cancer cell growth and invasion

We next investigated the role of RBM5 in prostate cancer progression. RBM5 overexpression reduced cell growth (Figure-4A) by MTT assay and invasion by trans-well invasion as-

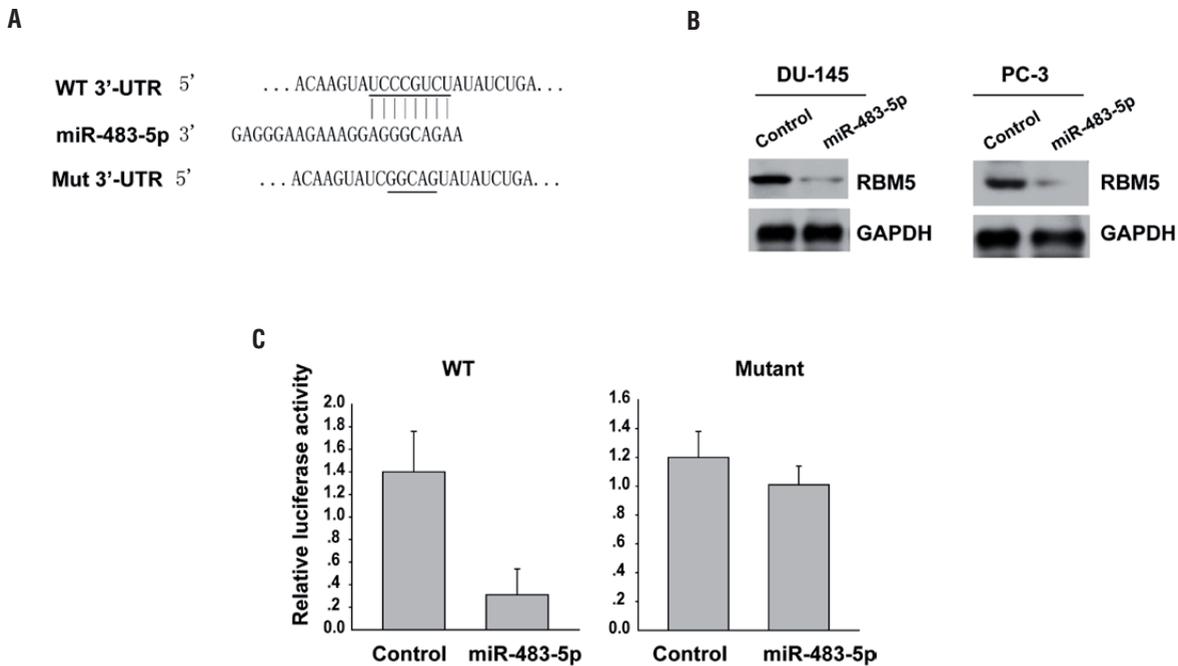
say (Figure-4B), respectively. RBM5 overexpression was confirmed by Western blot analysis (Figure-4C). These results suggested that RBM5 inhibits prostate cancer cell growth and invasion.

To determine whether miR-483-5p targeting of RBM5 was required for inhibiting prostate cancer cell proliferation and invasion, we employed an expression construct that encodes the entire RBM5 coding sequence but lacks the 3'-UTR. Enforced RBM5 expression partially rescued the miR-483-5p-mediated decrease in cell growth by MTT assay and invasion by trans-well invasion assay (Figures 4D and 4F), respectively. RBM5 overexpression was confirmed by Western blot analysis (Figure-4E).

DISCUSSION

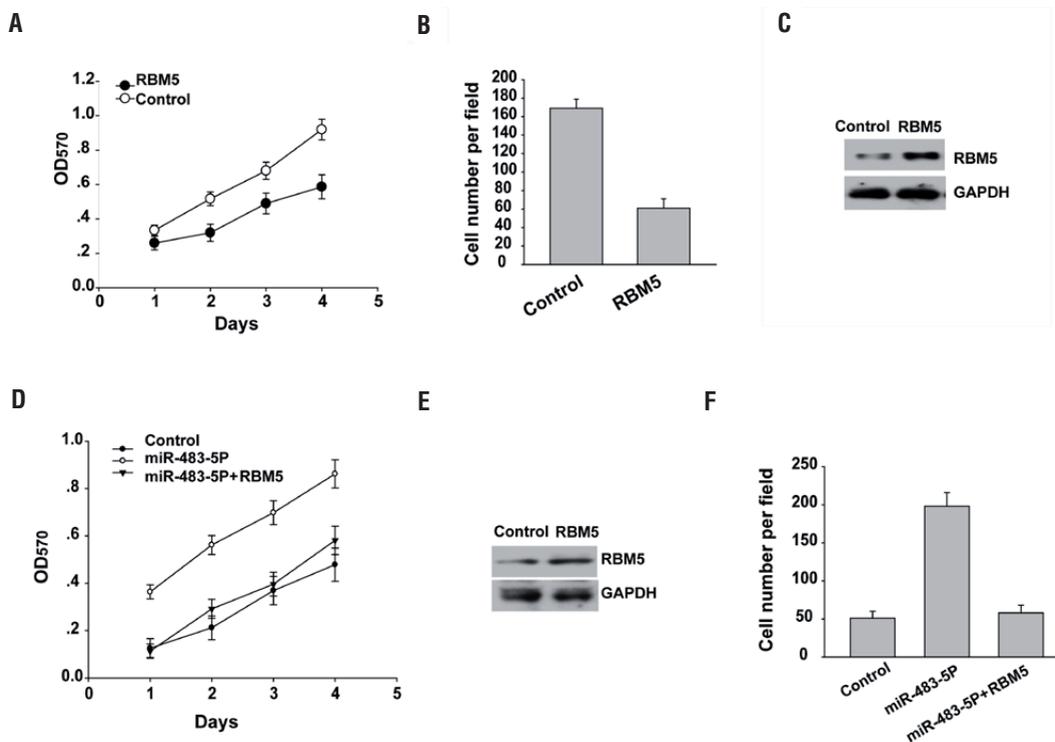
Prostate cancer is one of the leading causes of cancer-related mortality. Although

Figure 3 - RBM5 is a direct miR-483-5p target gene in prostate cancer. (A) Schematic representation of human RBM5 mRNA; highly conserved miR-483-5p binding sites were located in the 3' UTR. (B) RBM5 levels were examined by western blot analysis. GAPDH was also determined as a loading control. (C) Luciferase assay results from HEK-293T cells co-transfected with the RBM5 3' UTR-wt or RBM5 3' UTR-mut reporter plasmids and either miR-483-5p or miR-NC.



Data are presented as mean±SD from three independent experiments.

Figure 4 - Enforced RBM5 expression mitigates miR-483-5p promotion of prostate cancer cell growth and invasion. (A) MTT assay was performed after transfection of LNCaP cells for 1 to 4 days. **(B)** Invasion assays were used to determine LNCaP cell motility. The invaded cell numbers were quantified 24h after cells were seeded. Data are presented as mean±SD from three independent experiments. **(C)** Western blot analysis of RBM5 levels in the fourth day was examined. GAPDH was also determined as a loading control. **(D)** MTT assay was performed after transfection of LNCaP cells for 1 to 4 days. **(E)** Western blot analysis of RBM5 levels in the fourth day was examined. GAPDH was also determined as a loading control. **(F)** Invasion assays were used to determine LNCaP cell motility. The invaded cell numbers were quantified 24h after cells were seeded.



Data are presented as mean±SD from three independent experiments.

major breakthroughs in prostate cancer treatment have been achieved in recent years, it is important to identify novel and versatile molecular biomarkers for the disease (21).

Emerging evidence suggest that the dysregulation of miRNAs contributes to carcinogenesis. Moreover, accumulating evidence has demonstrated miR-483-5p expression in multiple types of tumors, including tongue squamous cell carcinoma, multiple myeloma, lung adenocarcinoma, hepatocellular carcinoma, and glioma (11-15, 22, 23). However, it remains unclear whether miR-483-5p is expressed in prostate cancer and whether it plays a role in prostate cancer.

In the present study, we observed increased miR-483-5p expression in prostate cancer

cell lines or tissues compared to a normal prostate epithelial cell line tissues. Moreover, miR-483-5p inhibition suppressed prostate cancer cell growth and invasion in vitro. In the future, we will further perform analysis of miRNA arrays to reveal many genes difference in expression between benign and malignant tissue samples.

Despite previous studies indicating the oncogenic role of miR-483-5p, its role in tumor cell growth and invasion and its molecular mechanisms regulating growth and invasion are unknown. Here, we found that miR-483-5p expression was upregulated in prostate cancer cell lines. Moreover, we identified miR-483-5p as a pro-metastatic miRNA and a negative regulator of the key metastasis suppressor RBM5.

Our study describes the regulatory link between miR-483-5p and RBM5 and identifies a potential mechanism of RBM5 dysregulation and its contribution to prostate cancer progression. We found that miR-483-5p expression is significantly upregulated in prostate cancer cell lines and is negatively associated with RBM5 protein levels. miR-483-5p suppression inhibits prostate cancer cell growth and invasion by directly targeting RBM5. However, further investigation in other types of cancer is necessary to explore the function and mechanisms of miR-483-5p.

In summary, the present study is the first to suggest the potential prognostic significance of miR-483-5p mediated the downregulation of RBM5 expression that promotes cancer cell proliferation and invasion in prostate cancer.

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CONFLICT OF INTEREST

None declared.

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Fosfomycin: a good alternative drug for prostate biopsy prophylaxis the results of a prospective, randomized trial with respect to risk factors

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ABSTRACT

Purpose: To determine the risk factors and the efficiency of rectal swab samples to prevent infectious complications in prostate biopsy, and compare fosfomycin with ciprofloxacin use in prophylaxis.

Materials and methods: Between May and October 2014, pre-biopsy risk factors and their effect in ciprofloxacin and fosfomycin prophylaxis were determined. Pre-biopsy urinalysis, urine culture and rectal swab samples were obtained from all of the patients. Rectal swabs were obtained upon admission, and biopsy was performed in the following 3-7 days. The place of rectal swab samples and efficiency of fosfomycin use was evaluated.

Results: Pre-biopsy rectal swabs were obtained from 110 patients who revealed 60.9% fluoroquinolone resistance (FQR), and 32.7% fluoroquinolone sensitivity (FQS). Fosfomycin resistance was present in 3 patients. Ciprofloxacin use in last 6 months was the only risk factor for FQR. Antibiotic prophylaxis was given to both groups with and without risk factors, according to swab results, and no infective complications were observed. Among the group where fosfomycin was used empirically, one patient had an infection needing hospitalization, however this constitutes no statistical difference between the Group that fosfomycin used empirically or according to swab results ($p=0.164$).

Conclusions: In prostate biopsy prophylaxis, ciprofloxacin may be used liberally in patients without risk factors, but it should be given according to the rectal swab results in the patients with risk, and fosfomycin may be used independently of risk factors and rectal swab results.

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Fosfomycin; Prostate; Biopsy

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INTRODUCTION

Prostate cancer (PCa) is an important disease because of its high incidence and being the second cancer mortality cause (1). Transrectal ultrasound guided biopsy (TRUSG-Bx) is a generally accepted standard method for diagnosis of PCa. In-

fection is the most serious complication of biopsy. It is mostly afebrile, non-complicated (1.2-11.3%), but rarely it can become pyretic (1.4-4.5%), may cause severe sepsis (0.3-3%), needing hospitalization and lead to a life threatening condition (2). Ciprofloxacin is widely used in TRUSG-Bx prophylaxis. Up to 20% increase in the fluoroquino-

lone resistance (FQR) in rectal swab samples and the observation of the FQR bacteria in about 50% of the infections have created a need for alternative prophylaxis (3). For TRUSG-Bx prophylaxis, fosfomycin may be preferred, because it's more reliable than the fluoroquinolones. It has lower resistance rate and oral single-dose usage (4).

The aim of this study is to consider FQR in order to determine the risk factors prior to TRUSG-Bx as well as to determine the reliability of taking rectal swab samples, and to compare the efficiency of fosfomycin and ciprofloxacin prophylaxis.

MATERIALS AND METHODS

Between May and October 2014, 110 patients were included in this study, for which TRUSG-Bx was planned because of PCa suspicion. The patients were informed about the study, and written consents as well as local ethics committee decision were obtained. Pre-biopsy urinalysis, culture and rectal swab samples were obtained from all patients. For the antibiotic prophylaxis, the patients were divided into 2 main groups according to risk factors (ciprofloxacin or other antibiotic use in the last 6 months, diabetes mellitus (DM), urethral catheterization, genitourinary system (GUS) operation history).

Group A included patients with no risk factors. It was divided into 2 sub-groups: patients using single dose fosfomycin the night before the biopsy (A1), and those using ciprofloxacin twice daily for 5 days, beginning the day before (A2). In both groups the prophylaxis was started before getting the swab results.

Group B included those with risk factors. It was divided into 3 sub-groups: patients who took fosfomycin (B1) or oral ciprofloxacin (B2) according to the swab results, and those who took fosfomycin (B3) empirically (Figure-1).

Rectal swabs were obtained upon admission, and biopsy (standard 12 quadrant) was performed in the following 3-7 days. Using Kirby-Bauer disc diffusion method, in line with the suggestions of the "Clinical and Laboratory Standards Institute (CLSI)" fosfomycin and cip-

rofloxacins sensitivities of *Escherichia coli* (*E. coli*) were examined.

The patients were contacted by telephone 24 hours after biopsy, and were asked if they had fever, and their conditions were evaluated by urinalysis and cultures in the 1st and 4th weeks.

Statistical Analysis

Data was analyzed using SPSS for Windows. Risk factors predicting the FQR were determined using multivariate logistic regression analysis. Odds ratio and 95% confidence intervals for each risk factor were calculated. The results with $p < 0.05$ were considered significant.

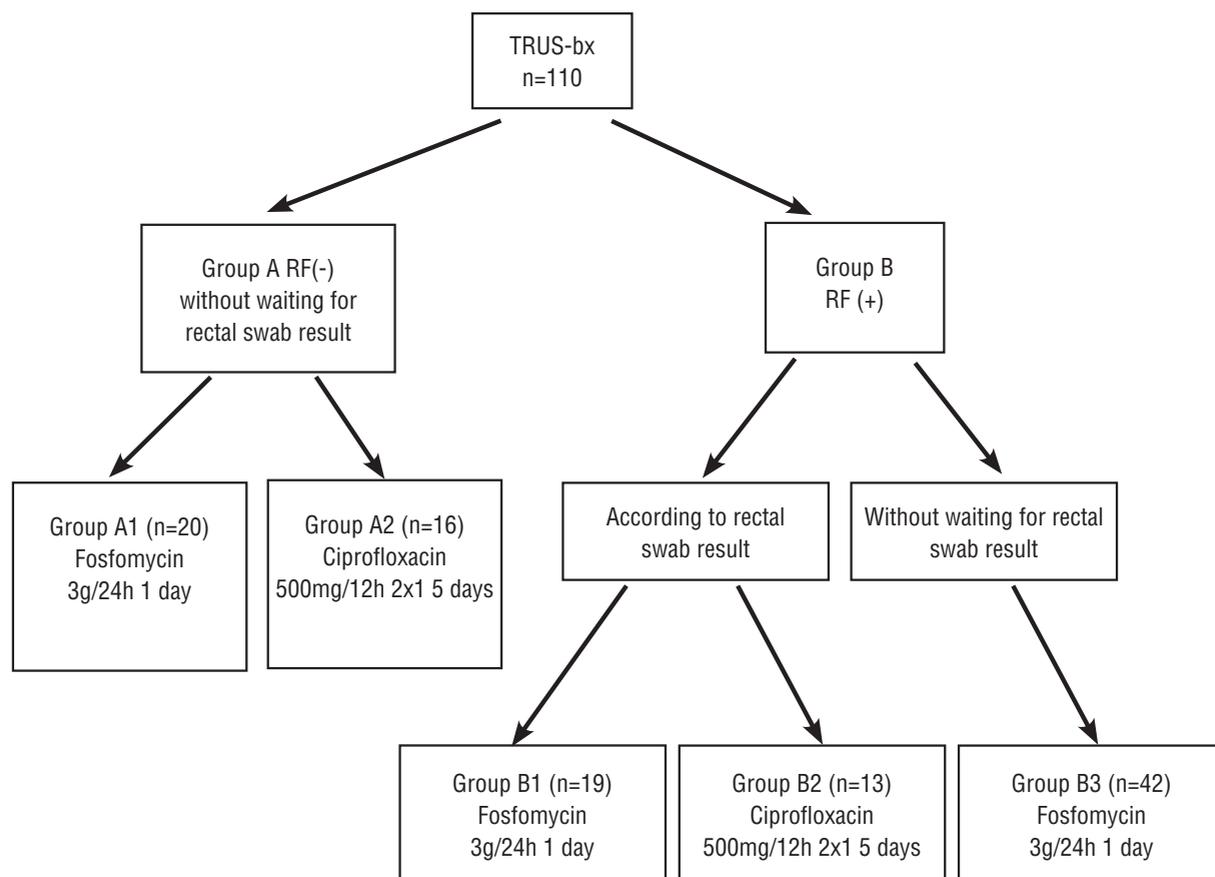
RESULTS

Between May and October 2014, pre-biopsy swabs were obtained from 155 patients. Following the evaluations of the patients, 110 were included in the study and 45 excluded because of contamination.

Mean age of the patients was 63.8. Mean PSA was 13.3ng/mL 18 patients had repeated biopsy, and 7 had indwelling catheter. Among the comorbidities 16 had diabetes mellitus. When the swabs were evaluated *E.coli* had grown in 93.6% (103/110). FQS was present in 67 (60.9%), and FQR in 36 (32.7%). Fosfomycin resistance was seen in 3 (2.7%), sensitivity in 100 (90.9%). When the risk factors were evaluated, they were negative in 36 (32.72%), and positive in 74 (67.27%) patients.

FQR was present in 16.7% in Group A (6/36), and in 40.5% in Group B (30/74). The increase in FQR of Group with positive risk factors was found to be statistically significant ($p=0.012$). 30 of 36 patients with FQR and 39 of the 67 patients with FQS had risk factors. The risk factor positivity was significantly associated with the FQR ($p=0.010$). Ciprofloxacin use in the last 6 months was the only risk factor for FQR ($p=0.002$); 17 of the 36 patients (47.2%) who had FQR, and 12 of the 67 patients (17.9%) who had FQS used ciprofloxacin in the last 6 months, which increased FQR 4.10 times (95% CI: 1.66-10.13). When the relation of diabetes mellitus and FQR was evaluated, DM was present in 25% of the patients with FQR,

Figure 1 - Study Design (Algorithm of TRUSG-bx patients).



RF = risk factors; TRUSG-Bx = transrectal ultrasound guided biopsy; UTI = urinary tract infection

and in 10.4% with FQS. Although the presence of DM was not statistically significant it increased the FQR risk by 2.86 folds (95% CI: 0.96-8.47) ($p=0.052$). No association was observed between the antibiotic use (except ciprofloxacin) and catheter history ($p=0.394$ and $p=0.142$). In the analysis of the 6 patients who had GUS operation history FQR was not detected in the rectal swab.

In the multivariate analysis of the risk factors the most determining factor for the FQR in swab was the use of ciprofloxacin in the last 6 months and was an independent risk factor.

There was no statistically significant difference, in terms of UTI, between A1 and A2 Groups without risk factors ($p=1.000$) and fosfomycin or ciprofloxacin can be used safely in these patients.

There was no difference, in terms of UTI, between the fosfomycin (A1) Group which had no

risk factor and received prophylaxis without the swab result and the fosfomycin (B1) Group with risk factors and received prophylactically according to the swab result ($p=0.487$). Also, there was no statistically significant difference between the groups empirically using fosfomycin (Group B3 and A1) with and without risk factors ($p=1.000$). Whether the presence of risk factors in the patient has an effect on the fosfomycin use could not be shown. However, the observed 4 infections without fever in Group B3 were explained as increased asymptomatic bacteriuria risk due to indwelling catheter ($p=0.002$).

There was no difference, in terms of UTI, between the fosfomycin Group B1 with risk factors and received prophylaxis according to the swab, and Group B3 with risk factors using fosfomycin empirically ($p=0.164$). It was concluded that che-

cking the rectal swab before fosfomycin prophylaxis was not necessary in terms of decreasing UTI.

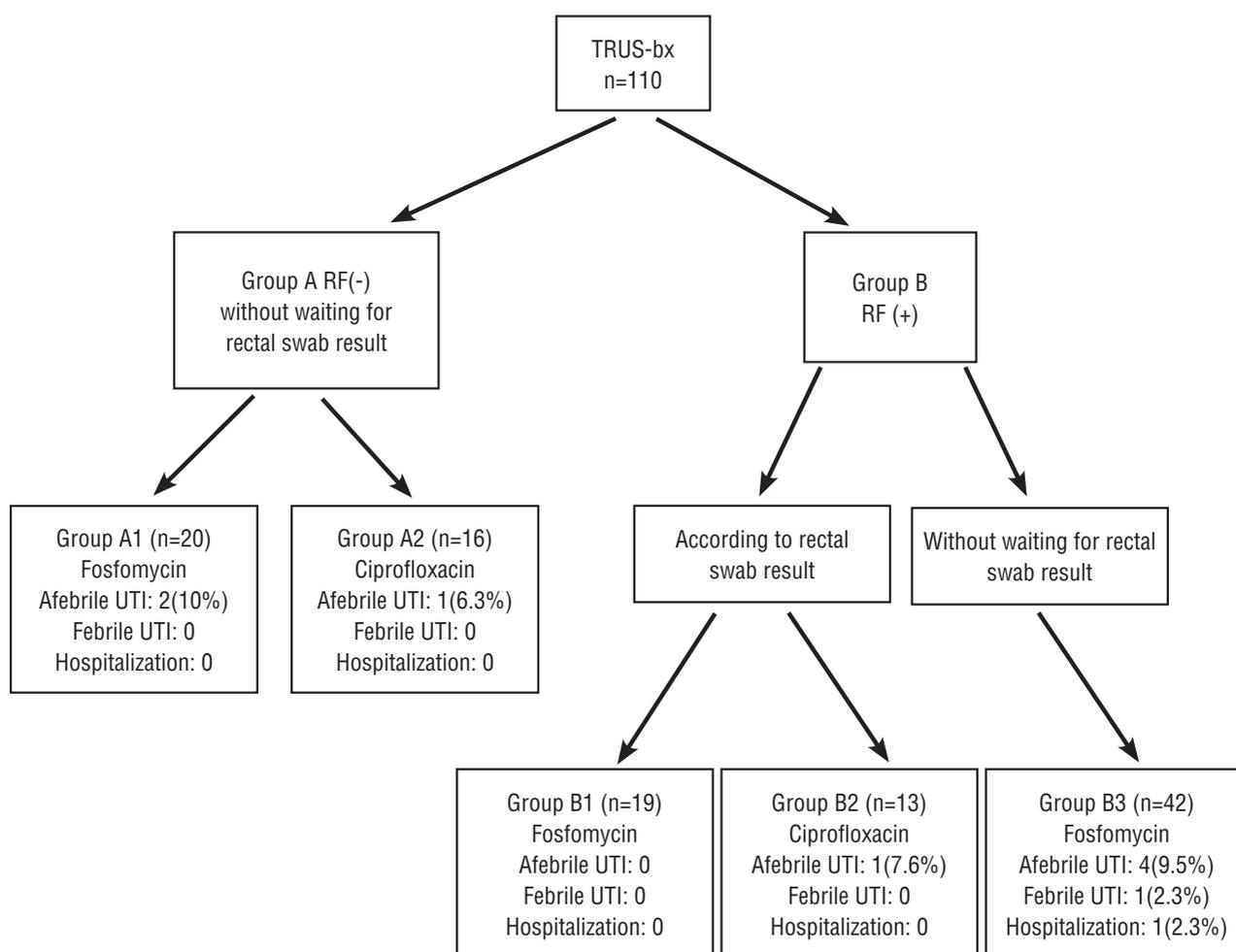
There was no difference, in terms of UTI, between the ciprofloxacin Group A2 without risk factors and received prophylaxis without checking the swab, and the B2 group with risk factor and received prophylaxis according to ciprofloxacin sensitivity in the swab ($p=1.000$). It was understood that the patients who developed UTI in both Groups had asymptomatic bacteriuria due to indwelling catheter.

When we examined the 30 patients in the Groups B1, B2, and B3, all with risk factors, we found that 56.7% ($n=17$) with FQR and 34.3%

($n=23$) with FQS used ciprofloxacin in the last 6 months and the effect of this on the FQR was not different between the Groups ($p=0.235$). However, it was understood in the multivariate analyses that the ciprofloxacin exposure affected FQR [OR=2.839, 95% CI: 1.055-7.640, $p=0.039$]. On the other hand, DM, catheter history, antibiotic use, GUS operation history did not affect FQR in these Groups.

Infectious complications occurred in 10 (9%) patients. There was asymptomatic bacteriuria in 5 (4.5%), UTI without fever in 3 (2.7%), and fever in 2 patients (1.8%) (Figure-2). The urine cultures of the patients with asymptomatic bacteriuria was repeated after 2 weeks and treatment was initiated in

Figure 2 - Infectious complication rates of the patients in the study Group.



RF = risk factors; TRUSG-Bx = transrectal ultrasound guided biopsy; UTI = urinary tract infection

the ones with bacterial growth. UTIs without fever were treated according to urine cultures.

DISCUSSION

PCa is the most prevalent solid tumor in Europe and diagnosed mainly by TRUSG-Bx. There is not yet a standard antibiotic prophylaxis protocol, but mostly fluoroquinolones are used (1). In a study, it was revealed that fluoroquinolones were used in 2 million prostate biopsies every year (5). In another study, antibiotic sensitivity of *E. coli* was evaluated. It was shown that ciprofloxacin resistance rate of 0.8% in 810 *E. Coli* strains between 1994 and 1996, has climbed to 12% in 1163 *E. Coli* strains between 2000 and 2002 (6).

The increase in the infectious complications despite prophylaxis has been associated with the presence of ESBL positive bacteria and especially to the FQR in the rectal swab (7). While in the initial studies of FQR in rectal swab in 2010 it was found that FQR was only 10.6%, in the more recent studies it was shown to increased up to 22% (8-9). In our study, FQR rate in swab was found in 32.7% (36/110), which is higher than other studies (10-12). Because the most determining risk factor for the post TRUSG-Bx infectious complications is FQR bacteria and because the FQR rate in our region is high, it seems necessary to review the ciprofloxacin use in prophylaxis (11).

In the largest prospective study on FQR, swab of 849 patients were examined and the resistance rate was found 19% (n=161), with the most determining factor for FQR being fluoroquinolone use in the last 3 months and the patients with heart valve prosthesis. FQR patients comprised 48% (15/31) of all infectious patients. With this result, it was emphasized that it may be beneficial to determine FQR in rectal swab and to start targeted antibiotic prophylaxis (TAP) or TAP could be used by considering the antibiotic profiles in the swabs (12).

In our study, having a GUS operation or repeat biopsy didn't affect FQR, although they used ciprofloxacin during these procedures. The reason for this might be the time that passed after the ciprofloxacin exposure.

It was shown in many studies that FQR

bacteria present in swab increases the infection and sepsis rates. Liss et al. in their meta-analysis, have determined the FQR rate was 20.5% (n=549) in 2673 patients. The difference of infection rates of FQR and FQS were found to be 6.6% and 1.1%, and hospitalization rates were 4.4% and 0.9% respectively. Both results were statistically significant. Among the patients who received fluoroquinolone prophylaxis the infection rate was 8.2% in those with FQR and 1.8% in those with FQS. In the same study, it was shown that the presence of FQR organisms in the swab increased the infection and hospital admission rate 3 times, and the presence of FQR organisms in both swab sample and fluoroquinolone prophylaxis increased the hospital admission 6 times. The presence of FQR bacteria in swab was the risk factor contributed the most to the infection rate increase. In FQR (-) patients fluoroquinolone prophylaxis would be sufficient and the infection rate remains in 1% (3).

When the FQR and FQS patients in our study were evaluated, there were 5 UTIs with 2 asymptomatic bacteriuria among the 67 patients in FQS, and 4 UTIs with 2 asymptomatic bacteriuria among the 36 patients in FQR Group. There was no statistically significant difference between the Groups (p=1). It was shown in many studies that FQR being an independent risk factor in terms of UTI in the patients receiving ciprofloxacin prophylaxis (3-10). However, in our study, out of 6 patients with FQR in Group A, 5 of them received fosfomycin and only 1 patient received ciprofloxacin prophylaxis. Furthermore, the patients with FQR in Group B1 and those with FQR in B3 who took fosfomycin prophylaxis, lead to low infection rates like that in FQS patients. Thus, we conclude that infectious complication rates could be decreased by not giving ciprofloxacin to the patients who have or with risk of having FQR in swab.

To decrease infectious complications, the use of antibiotics according to the rectal swab is a promising method. In the study by Taylor et al. Fluoroquinolone was started according to fluoroquinolone sensitivity in the swab in 112 patients, and empirically in 345 patients without taking swab. Among the 112 patients 19.6% had FQR in swab. Ciprofloxacin was used in the patients with

FQS and various prophylaxis (TMP/SMX, cephalosporin) in those with FQR. While no infectious complications were observed in any of these 112 patients, 9 (2.6%) infectious complication and 1 sepsis were observed in the other group. This study was exciting and promising with the TAP in TRUSG-Bx prophylaxis. In the same study, it was suggested that using fluoroquinolone in prophylaxis of patients with FQR risk was no longer logical. Another point mentioned was that the risk of complication due to FQR increased in 68 of the 345 patients (19.6%), and infectious complication was not observed only in 9 patients (13%). It was emphasized that the infections following TRUSG-Bx were not only due to FQR in the swab or the preference of the antibiotic, but also to some other factors (humoral immunity, procedure technique, bacterial inoculums). Taylor et al. stated in their study that it was necessary to obtain 38 rectal swabs in order to prevent 1 infectious complication, but despite this, TAP was beneficial in terms of cost (10).

Liss et al. in their meta-analysis, stated that infectious complication risk would increase when the patients with risk of FQR would take fluoroquinolone prophylaxis, and starting TAP according to swab would be beneficial for those patient. Furthermore, in the future it wouldn't be possible for everybody to take the same antibiotic for prophylaxis (3). In our study, we used TAP to the 12 patients in Group B2 with risk factor and FQS according to swab, and did not observe any infection except one patient with indwelling catheter, who showed asymptomatic bacteriuria.

Fosfomycin, was first used by Ongün et al. they compared single dose fosfomycin with ciprofloxacin 2x500mg and levofloxacin 500mg. They found that it is an alternative prophylaxis and is effective against FQR bacteria, moreover it is easily used and well tolerated (4). In another study, Lista et al. compared fosfomycin and ciprofloxacin for prophylaxis. No significant difference was observed in terms of infection and sepsis, and it was emphasized that fosfomycin may decrease FQR related infections (13).

In our study, there was no significant difference, in terms of infection, between the A1 and the B1 fosfomycin groups, which reveals that fos-

fomycin prophylaxis could be used independently of risk factors. Moreover, this may also prove that fosfomycin could be preferred to ciprofloxacin in patients who had FQR. The observation of asymptomatic bacteriuria in the 4 patients with indwelling catheter in the B3 Group can be explained with fosfomycin not staying enough in the bladder to show its effect (it reaches maximum concentration in 4 hours).

CONCLUSIONS

In this study, it was shown that using fosfomycin empirically or according to the rectal swab, regardless of patients having or not having risk factors, does not affect infection rates. There is no need for rectal swab sampling if fosfomycin prophylaxis is planned. The result was the same for ciprofloxacin Group who had no risk factor; we found similar infection rates which shows that both prophylaxis could be used in the patients without risk factors. But if ciprofloxacin prophylaxis is planned for the patients with risk factors, it must be used according to rectal swab results and infection rates may decrease with targeted antibiotic prophylaxis.

When fosfomycin and ciprofloxacin were compared in terms of cost effectiveness, considering increase in the infection rates due to ciprofloxacin, it could be suggested that fosfomycin would become more economical in the long run.

ABBREVIATIONS

FQR = Fluoroquinolone-resistance

FQS = Fluoroquinolone-sensitivity

DM = Diabetes mellitus

UTI = Urinary tract infection

GUS = Genitourinary system

E.coli = Escherichia coli

PCa = Prostate cancer

TRUSG-Bx = Transrectal ultrasound guided biopsy

TAP = Targeted antibiotic therapy

CONFLICT OF INTEREST

None declared.

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Nephrometry scores and perioperative outcomes following robotic partial nephrectomy

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ABSTRACT

Objectives: Based on imaging features, nephrometry scoring systems have been conceived to create a standardized and reproducible way to characterize renal tumor anatomy. However, less is known about which of these individual measures are important with regard to clinically relevant perioperative outcomes such as ischemia time (IT), estimated blood loss (EBL), length of hospital stay (LOS), and change in estimated glomerular filtration rate (eGFR) after robotic partial nephrectomy (PN). We aimed to assess the utility of the RENAL and PADUA scores, their subscales, and C-index for predicting these outcomes.

Materials and Methods: We analyzed imaging studies from 283 patients who underwent robotic PN between 2008 and 2014 to assign nephrometry scores (NS): PADUA, RENAL and C-index. Univariate linear regression was used to assess whether the NS or any of their subscales were associated with EBL or IT. Multivariable linear regression and linear regression models were created to assess LOS and eGFR.

Results: The three NS were significantly associated with EBL, IT, LOS, and eGFR at 12 months after surgery. All subscales with the exception of anterior/posterior were significantly associated with EBL and IT. Collecting system, renal rim location, renal sinus, exophytic/endophytic, and nearness to collecting system were significant predictors for LOS. Only renal rim location, renal sinus invasion and polar location were significantly associated with eGFR at 12 months.

Conclusions: Tumor size and depth are important characteristics for predicting robotic PN outcomes and thus could be used individually as a simplified way to report tumor features for research and patient counseling purposes.

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INTRODUCTION

Partial nephrectomy (PN) is the preferred technique in the treatment of small (<4cm) and mid-size (<7cm) kidney masses and can entail varying degrees of technical challenges based on anatomic features of the tumor (1, 2). The growing

interest in PN has highlighted the need for a standardized method for characterizing renal masses that provides clinically meaningful information when different approaches and techniques for PN are compared (3, 4).

In this context, different nephrometry scores (NS), principally based on renal imaging,

have been proposed (5-7). Their main objective is to provide a reproducible way of characterizing anatomy and classifying renal masses with emphasis on the most surgically-relevant features. However, subscale metrics have not been validated individually and little is known about which of them are truly related to complications and outcomes after PN. Although some studies have investigated the impact of the individual components of NS on complications or other outcomes, their results should be taken cautiously because of small series (8, 9) and because they only compared subscales within the same NS (10-12). Furthermore, very few of them focused on robotic procedures (13, 14). This is important because trend analyses show that robotic PN is likely to become the most frequently performed operation for renal masses (15).

Here we assessed the utility of the RENAL and PADUA scores, their subscales, and C-index for predicting perioperative parameters and postoperative outcomes from a single institution series of robotic PN. We hypothesized that some subscales hold more value than others for predicting some of these outcomes, and that in daily practice the use of the most relevant subscales is as effective as the use of one of the complexity scores.

MATERIALS AND METHODS

Patients

After obtaining institutional review board approval, we identified 317 patients who underwent robotic PN between May 2008 and August 2014 at Memorial Sloan Kettering Cancer Center (MSKCC). Patients with benign histology (n=16) and whose imaging exams were not available (n=18) were excluded, leaving 283 patients with malignant tumors for final analysis. Baseline characteristics were extracted from a prospectively maintained database and included patient age, gender, American Society of Anesthesiologists (ASA) score, and race.

Surgical Procedures

All procedures were performed by surgeons with over five years of experience in mi-

nimally invasive and robotic surgery including PN. Normothermic ischemia was utilized during sharp excisional resection and surgical repair was conducted by renorrhaphy with absorbable sutures over nitrocellulose and thrombin-based procoagulant materials. Cases were performed trans or retroperitoneally according to surgeon preference and tumor location. Early unclamping and enucleo-resection techniques were not utilized. Postoperative care was managed under a standardized clinical care pathway overseen by care providers independent from the surgical team (16). Surgical complications reported within 30 days were obtained prospectively using a standardized grading system (17).

Outcome measures

Pathologic data

Kidney tumor specimens were evaluated according to standard pathology protocol. Pathologic data included tumor size and tumor stage according to the 2009 AJCC TNM classification (18).

Postsurgical complications

Postsurgical complications within 30 days were collected prospectively and graded using the modified Clavien classification system (19). Regular correspondence with patients and their physicians ensured that treatment received outside of our institution was accounted for in the database.

Perioperative outcomes

Estimated blood loss (EBL), ischemia time (IT) and length of stay (LOS) were collected from our prospectively maintained database. Data regarding IT and LOS were not available for 4 and 56 patients, respectively.

Renal function outcomes

Baseline eGFR was calculated from creatinine readings using the Chronic Kidney Disease Epidemiology Collaboration (CKD-Epi) equation taken one month prior to surgery (20). Postoperative eGFR was calculated at 12 months or from the closest measurement between 6 and 12 months following surgery.

Nephrometry scoring

RENAL scores were assigned as described by Kutikov and Uzzo (6). The components of this score are radius (R), exophytic/endophytic (E), proximity to collecting system or sinus (N), anterior/posterior (A), and location relative to polar lines (L). R, E, N and L are scored from 1 to 3. Subscale A is a categorical variable defined as anterior, posterior or X when a designation relative to anterior/posterior is not possible.

The PADUA nephrometry score, described by Ficarra et al. (5), is similar to the RENAL nephrometry score. Longitudinal location, renal rim, renal sinus, and urinary collecting system are scored with either 1 or 2 points; exophytic rate and tumor size are scored from 1 to 3 points. Tumor size was defined as the maximum tumor diameter as described in the original articles from Ficarra et al. and Kutikov et al. (5, 6),

C-index measurements are described by Simmons et al. (7) and were made according to the authors instructions contained in the original article where this NS is described (7).

All NS measurements were made by the same urologic surgeon and were based on the original studies described.

Statistical analyses

Univariate linear regression was used to assess the association between C-index, RENAL, PADUA and any of the RENAL or PADUA NS subscales with EBL and IT. Multivariable linear regression models adjusted for age and ASA score were used to determine whether NS is associated with LOS. Linear regression models adjusted for preoperative eGFR were used to assess the association of the NS with eGFR at 12 months after surgery. The effects of the renal NS on oncologic outcomes were not studied because of the limited number of events. All analyses were performed using Stata 12 (StataCorp, College Station, TX).

RESULTS

Patient and tumor characteristics are described in Table-1. Renal nephrometry subscale scores are reported in Table-2.

Table 1 - Patient and tumor characteristics, N = 283. Data are reported as frequency (%) or median (IQR).

Female	93 (33%)
Age	60 (51, 67)
ASA score	
1	17 (6%)
2	101 (36%)
3	160 (57%)
4	5 (2%)
Pathologic T stage	
T0	4 (1%)
T1	240 (85%)
T2	8 (3%)
T3	31 (11%)
Pathologic N stage (N=277)	
N0	138 (50%)
N1	1 (<1%)
N2	4 (1%)
NX	134 (48%)
Pathologic M stage (N=231)	
M0	212 (92%)
M1	6 (3%)
MX	13 (5%)
Positive margins (N=279)	22 (8%)
Tumor size (cm) (N=280)	2.9 (2.0, 4.3)
Grade 3+ complications within 30 days	8 (3%)

C-index, RENAL and PADUA scores were all significantly associated with EBL, IT, LOS, and eGFR at 12 months after surgery. The effect of a one-unit increase in RENAL score and a one-unit increase in PADUA score were similar for all outcomes.

With regard to subscales, tumor size, a component of both the PADUA and RENAL scores, was associated with perioperative outcomes: larger tumors resulted in significantly increased EBL and IT (Table-3). With the exception of the anterior/posterior scale, all other scales describing tumor location were significantly associated with EBL and IT, with tumors in complex locations or centrally located having increased EBL and longer IT (Table-3).

Table 2 - RENAL and PADUA subscale scores, N=283. Data are reported as frequency (%).

Collecting sinus (PADUA)	
Absent	64 (23%)
Dislocated/infiltrated	219 (77%)
Renal rim (PADUA)	
Lateral	194 (69%)
Medial	89 (31%)
Renal sinus (PADUA)	
Absent	184 (65%)
Renal sinus location	99 (35%)
Sinus line (PADUA)	
Entirely above/below or <50% crossing sinus lines	162 (57%)
Entirely between / ≥50% crossing	121 (43%)
Nearness to collecting sinus (RENAL)	
≥7mm	45 (16%)
4 mm-7 mm	35 (12%)
≤4 mm	203 (72%)
Anterior/Posterior (RENAL)	
Anterior	114 (40%)
Posterior	114 (40%)
Neither	55 (19%)
Location relative to polar line (RENAL)	
Entirely above/below	143 (51%)
Crosses polar lines <50%	46 (16%)
Entirely between / ≥50% crossing	94 (33%)
Exophytic / endophytic (PADUA/RENAL)	
≥50% exophytic	108 (38%)
<50% endophytic	96 (34%)
Entirely endophytic	79 (28%)
Size (PADUA/RENAL)	
≤4cm	183 (65%)
4 cm - 7cm	81 (29%)
≥7cm	19 (7%)

Regarding LOS, collecting system, renal rim location, renal sinus, exophytic/endophytic, and nearness to collecting system were significant predictors (Table-4). Conversely, tumor size was not associated with LOS. Furthermore, only renal rim location, renal sinus invasion and polar loca-

tion were significantly associated with eGFR at 12 months. It has to be noted that anterior/posterior location was not significantly associated with any peri-operative or postoperative outcomes, while renal rim location and renal sinus invasion, both from PADUA, were the only subscales associated with all four outcomes.

Tumor location relative to polar lines was found to significantly increase both EBL and IT, with patients with centrally located tumors having the worst outcomes. As a sensitivity analysis, we compared patients with centrally located tumors (entirely between or crossing polar lines more than 50%) with patients whose tumors did not cross polar lines or crossed less than 50%. Patients with centrally located tumors had an increase in EBL of 60mL (95% confidence interval [CI] 12, 109, $p=0.02$) and increase in IT of 6.5 minutes (95% CI 3.8, 9.2, $p < 0.0001$) compared to patients without centrally located tumors. When comparing patients with centrally located tumors to all others, we found some evidence that these patients had longer LOS (0.30 days, 95% CI-0.02, 0.62, $p=0.07$), although this association did not reach conventional levels of statistical significance. These patients also had eGFR at 12 months that was 3.56 points lower (95% CI-7.04, -0.08, $p=0.045$) compared to patients with tumors that did not cross the polar line or crossed <50%.

DISCUSSION

Prior to the introduction of NS only limited and mainly quantitative information regarding surgically-relevant anatomical features of solid enhancing renal masses was available. By defining the characteristics of tumors treated with PN, integrated anatomical systems can be used to predict the risk of surgical complications and allows comparisons among different surgeons and techniques. Given the variability in techniques and possibly outcomes inherent to surgical approaches, here we offer a comprehensive analysis on the value of all previously described NS and their subscales for robotic PN.

The RENAL and PADUA NS contain subscales related to tumor size and location within the renal parenchyma, as well as relative to portions

Table 3 - Univariate linear regression models for the association between C-index, RENAL and PADUA total scores and subscales and peri-operative outcomes: estimated blood loss (EBL) in mL (N = 283) and ischemia time (IT) in minutes, (N=279).

	EBL (mL)			Ischemia time (min.)		
	β	95% CI	p value	β	95% CI	p value
C-Index (per 1 unit increase) (N=282)	-13	-24, -2	0.020	-2.4	-3.0, -1.9	<0.0001
RENAL (per 1 unit increase)	28	16, 39	<0.0001	2.8	2.2, 3.4	<0.0001
PADUA (per 1 unit increase)	29	18, 39	<0.0001	2.7	2.1, 3.2	<0.0001
Collecting system						
Not involved	Ref.	-	0.013	Ref.	-	<0.0001
Dislocated/infiltrated	70	15, 125		8.8	5.8, 11.8	
Renal rim						
Lateral	Ref.	-	0.0002	Ref.	-	<0.0001
Medial	93	44, 141		6.6	3.9, 9.4	
Renal sinus						
Not involved	Ref.	-	<0.0001	Ref.	-	<0.0001
Involved	127	81, 173		10.4	7.9, 12.9	
Longitudinal (polar) location						
Superior/inferior	Ref.	-	0.006	Ref.	-	<0.0001
Middle	64.5	19, 111		7.0	4.4, 9.5	
Exophytic/endophytic						
≥50% exophytic	Ref.	-	0.030	Ref.	-	0.001
1% – 50% exophytic	68	14, 122		5.0	2.0, 8.1	
Entirely endophytic	58	1, 115		5.5	2.3, 8.7	
Tumor radius						
≤4cm	Ref.	-	<0.0001	Ref.	-	<0.0001
>4 cm and <7cm	80	29, 130		8.2	5.6, 10.9	
≥7cm	168	77, 259		16.4	11.6, 21.2	
Nearness to collecting system						
≥7mm	Ref.	-	0.008	Ref.	-	<0.0001
<7mm and >4mm	-1.0	-88, 85		2.8	-2.0, 7.5	
≤4mm	81	17, 144		9.7	6.3, 13.2	
Anterior/posterior						
Anterior	Ref.	-	0.4	Ref.	-	0.5
Posterior	-26	-78, 26		1.7	-1.2, 4.7	
Neither	15	-49, 79		1.2	-2.4, 4.9	
Polar lines						
Entirely above/below	Ref.	-	0.003	Ref.	-	<0.0001
<50% crossing	78	13, 143		6.3	2.8, 9.9	
≥50% crossing or entirely between	82	31, 133		8.2	5.4, 11.0	

β = difference between patients who have that subscale score and those in the reference group

Ref. = reference group

Table 4 - Multivariable linear regression models for the association between C-index, RENAL and PADUA total scores subscales and postoperative outcomes. The model for length of stay (LOS) in days was adjusted for ASA score and age (N = 227).

	Length of stay			eGFR at 12 months		
	β	95% CI	p value	β	95% CI	p value
C-Index (per 1 unit increase) (N=226)	-0.1	-0.2, -0.02	0.014	0.95	0.17, 1.72	0.017
RENAL (per 1 unit increase) (N=227)	0.14	0.07, 0.22	0.0002	-1.05	-1.86, -0.23	0.012
PADUA (per 1 unit increase) (N=227)	0.14	0.07, 0.21	0.0002	-1.06	-1.83, -0.29	0.007
Collecting system						
Not involved	Ref.	-	0.001	Ref.	-	0.6
Dislocated/infiltrated	0.6	0.2, 1.0		-1.12	-5.04, 2.80	
Renal rim						
Lateral	Ref.	-	0.001	Ref.	-	0.019
Medial	0.6	0.3, 0.9		-4.24	-7.76, -0.72	
Renal sinus						
Not involved	Ref.	-	0.015	Ref.	-	0.003
Involved	0.4	0.1, 0.7		-5.17	-8.58, -1.77	
Longitudinal (polar) location						
Superior/inferior	Ref.	-	0.2	Ref.	-	0.014
Middle	0.2	-0.1, 0.5		-4.15	-7.47, -0.84	
Exophytic/endophytic						
≥50% exophytic	Ref.	-	0.049	Ref.	-	0.6
1% – 50% exophytic	0.3	-0.1, 0.7		-1.97	-5.85, 1.92	
Entirely endophytic	0.5	0.1, 0.8		-0.23	-4.35, 3.89	
Tumor radius						
≤4cm	Ref.	-	0.082	Ref.	-	0.057
>4cm and <7cm	0.4	0.04, 0.7		-2.60	-6.27, 1.06	
≥7cm	0.3	-0.3, 0.9		-7.26	-13.85, -0.68	
Nearness to collecting system						
≥7mm	Ref.	-	0.002	Ref.	-	0.11
<7mm and >4mm	0.1	-0.5, 0.7		1.83	-4.40, 8.07	
≤4mm	0.7	0.2, 1.1		-2.96	-7.50, 1.57	
Anterior/posterior						
Anterior	Ref.	-	0.6	Ref.	-	0.8
Posterior	-0.2	-0.5, 0.2		-0.78	-4.45, 2.90	
Neither	0.01	-0.4, 0.4		-1.68	-6.25, 2.89	
Polar lines						
Entirely above/below	Ref.	-	0.2	Ref.	-	0.073
<50% crossing	0.3	-0.2, 0.7		-3.05	-7.69, 1.59	
≥50% crossing or entirely between	0.3	-0.1, 0.6		-4.14	-7.81, -0.46	
Polar lines						
Did not cross or <50% crossing	Ref.	-	0.069	Ref.	-	0.045
≥50% crossing or entirely between (centrally located tumors)	0.3	-0.02, 0.62		-3.56	-7.04, -0.08	

The model for post-operative eGFR at 12 months was adjusted for pre-operative eGFR (N=279).

β = difference in length of stay between patients with that subscale score and patients in the reference group with similar ages and ASA scores

Ref. = reference group

of the collecting system or the renal sinus. The C-index is calculated based on the position of the tumor relative to the center of the kidney and is adjusted by tumor size. Any renal mass with a C-index <1 has some part superimposed on the renal center and a C-index of 1 equates to a tumor with its edge touching the middle of the kidney. Simmons and coworkers from the Cleveland Clinic (7) showed that a C-index <2 was associated with longer IT, higher EBL and rates of intra- and postoperative complications after laparoscopic PN. Here, concordant findings were demonstrated for robotic PN, as a one-unit increase in C-index was associated with lower EBL and shorter IT.

In the current analysis, PADUA and RENAL were associated with all outcomes studied, which is in line with previous non-robotic analyses. In a large retrospective multicenter study, Ficarra et al. showed that PADUA stratification was an independent predictor of IT longer than 20 minutes and overall complications within 3 months (12).

Smaller series also demonstrated associations between RENAL, PADUA and C-index with warm IT (4) and change in renal function (8, 21).

However, a main issue remains as to whether, for robotic PN, there is significant added value of using a composite index scoring system over the simple use of individual measures used to calculate the index. In our analysis that included a large series of robotic PN, all subscales from the PADUA and RENAL scores, except for anterior/posterior, were independently associated with two important surrogates of challenging tumor resections (1, 22), EBL and IT. Tumor size ≥ 7 cm had the biggest impact on IT and EBL, being associated with IT on average 16 minutes and a mean EBL 168mL higher than for tumor size ≤ 4 cm. Concordant findings were seen in a preliminary study from our institution including 90 open, laparoscopic and robotic PN procedures in which location relative to polar line, sinus involvement, tumor size, collecting system involvement, and C-index score were all associated with increased IT (9). Along these lines, Tsivian et al. reported that tumor size, endophytic growth and both central and hilar mass location were associated with increased IT (23). It has to be stressed that other parameters such as the amount of perinephric fat can bring

additional difficulty to the surgical procedure (24) and affect perioperative outcomes. Along the same line, the volume of preserved parenchyma has shown prognostic value for postoperative renal function (25). Our study aimed at evaluating subscales of NS and therefore we did not evaluate these other factors.

LOS is an important outcome when evaluating surgical procedures and an accepted surrogate for immediate post-operative convalescence features including complications (1). Moreover, shortening of LOS is one of the assumed benefits of the robotic approach. Here LOS was associated with features that evaluate tumor depth of invasion, demonstrating roughly changes per each of the subscales unit increase. All cases analyzed were performed by the same operative approach (robotic PN) using a common care pathway: the LOS outcome is likely a surrogate for immediate postoperative convalescence features including complications (1). These data are consistent with those from Simhan et al. (26). Performing a multivariable analysis on 390 patients who underwent PN, the authors found that high tumor complexity using the RENAL US was associated with the occurrence of major complications. The low rate of major postoperative complications (Table-1) in our cohort precluded comparative analyses between NS and subscales.

Renal function after PN depends on several factors, mainly pre-operative eGFR, IT and the volume of preserved kidney parenchyma (1, 25, 27, 28) It seems intuitive that the more complex the renal tumors are, the longer the vessels need to be clamped to achieve safe excision and reconstruction (29). Using multivariable analysis we showed that only three PADUA subscales and nephrometry composite scores were associated with functional loss. Moreover, although the composite indexes were associated with all outcomes studied, the magnitude of difference per unit change in index score was less than for renal rim, sinus involvement and polar location. Thus, our data question the utility of composite index scoring compared to individual subscale for robotic PN.

The ideal method to describe renal masses should not only accurately reflect surgical complexity, but also be reproducible and objective in or-

der to be applicable in daily practice. The reduction of subscales reported in each score might augment each method's reproducibility and applicability.

Although our database is prospectively maintained, LOS missing for 56 patients due to loss of electronic data. Another limitation of our study is the retrospective nature of the analysis, which makes our results vulnerable to caveats in patient selection and other unrecognized confounding factors. However, NS scoring was done prospectively as we evaluated all previously described NS. Another strength of the study was the use of a large series of robotic PN, thus limiting variabilities related to technique compared to previous studies.

In our analysis, we show that the relevant NS features are those that describe tumor depth of invasion and size. It is interesting that tumor size was a significant predictor despite the fact that median tumor size in this study was 2.9cm. Intuitively, this would be expected for larger tumors only. This suggests potential for solely using subscales as a simplified way to stratify kidney tumors for case mix adjustments in programs of quality assurance, for example, when comparing the outcomes of different surgeons and techniques. Furthermore, individual subscale metrics can be a simplified way for the practicing urologist in his/her clinical practice to counsel patients about surgical risks.

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CONFLICT OF INTEREST

None declared.

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Which is best method for instillation of topical therapy to the upper urinary tract? An *in vivo* porcine study to evaluate three delivery methods

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ABSTRACT

Purpose: To compare the staining intensity of the upper urinary tract (UUT) urothelium among three UUT delivery methods in an *in vivo* porcine model.

Materials and methods: A fluorescent dye solution (indigo carmine) was delivered to the UUT via three different methods: antegrade perfusion, vesico-ureteral reflux via indwelling ureteric stent and retrograde perfusion via a 5F open-ended ureteral catheter. Twelve renal units were tested with 4 in each method. After a 2-hour delivery time, the renal-ureter units were harvested en bloc. Time from harvesting to analysis was also standardised to be 2 hours in each arm. Three urothelium samples of the same weight and size were taken from each of the 6 pre-defined points (upper pole, mid pole, lower pole, renal pelvis, mid ureter and distal ureter) and the amount of fluorescence was measured with a spectrometer.

Results: The mean fluorescence detected at all 6 predefined points of the UUT urothelium was the highest for the retrograde method. This was statistically significant with p-value less than <0.05 at all 6 points.

Conclusions: Retrograde infusion of UUT by an open ended ureteral catheter resulted in highest mean fluorescence detected at all 6 pre-defined points of the UUT urothelium compared to antegrade infusion and vesico-ureteral reflux via indwelling ureteric stents indicating retrograde method ideal for topical therapy throughout the UUT urothelium. More clinical studies are needed to demonstrate if retrograde method could lead to better clinical outcomes compared to the other two methods.

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INTRODUCTION

The gold standard for treatment of upper urinary tract urothelial carcinoma (UUT-UC) is nephroureterectomy with bladder cuff excision (1). However, with advancement in endourologic techniques, nephron-sparing treatments have been used by open, percutaneous or ureteroscopic approaches with reasonable oncologic outcomes. This is offered in low risk UUT-UC with normal

contralateral kidney and also in imperative situations such as bilateral tumours, renal insufficiency or solitary kidney (2-4). Similar to adjuvant intravesical therapy for urothelial carcinoma of the bladder after trans-urethral resection, nephron sparing treatments have been paired with the instillation of adjuvant agents to the upper urinary tract (UUT) to reduce recurrence rates (5).

Three main methods have been described in the literature:

1) Antegrade perfusion via a percutaneous nephrostomy tube (6-8);

2) Intravesical administration with vesico-ureteral reflux via an indwelling ureteric stent (9-10);

3) Retrograde perfusion via an open-ended ureteric catheter (11, 12).

There is little data comparing on which of the three methods is the best. It has been shown in an ex-vivo porcine model that retrograde perfusion via an open-ended ureteral catheter results in better mean percentage of surface area stained and with higher mean staining intensities of the UUT, hence suggesting retrograde method the most efficient of the three (13). However, there is a lack of natural ureteral peristalsis, continuous urine production and intra-abdominal pressure in an ex vivo study which could have influenced these results. Hence, this study aims to compare the staining intensity of the UUT urothelium among the three UUT delivery methods in an in vivo porcine model.

MATERIALS AND METHODS

Experimental setup

Healthy live female pigs of the same species and weighing between 40-45kg were obtained from a local livestock company. Pigs were chosen because of their anatomic similarities to the collecting systems of humans (14). Permission was obtained from our institution for the use of the pigs and we strictly followed their ethical guidelines in this study. We carried out each of the three methods of delivering topical therapy to the UUT with 2 pigs consisting of 4 kidney-ureter units in each arm. We used indigo carmine dye as a substitute for topical therapy agents. It is known to stain the superficial layer of the urothelium and not penetrate tissue and it is a fluorescent dye. Hence the amount of fluorescence can be measured as a marker for staining intensity of the UUT urothelium. The dwell time was 2 hours in all 3 arms. After completion of the delivery method, the kidneys and ureters were harvested using open technique and sent for analysis. The time from completion of delivery method to the analysis was the same in all arms at 2 hours.

Nephrostomy Tube technique

A flank incision was made on each side and the kidney and renal pelvis were exposed. We used a 20G intravenous plug as a substitute for nephrostomy tube. Due to technical difficulties in puncturing the calyces in live pigs, we punctured the renal pelvis and angled the plug away from the ureter to prevent the dye solution from flowing straight down into the ureter and avoiding the calyces. An intravenous tubing was attached to the plug and 150mLs of normal saline/indigo carmine solution was infused in by gravity using a burette to control the rate to be 1mL/min and have continuous perfusion of the UUT for 2 hours. The burette was placed 20cm above the renal pelvis. There was no leakage of dye around the plug. The rate of infusion and height was as described in earlier published data so as to maintain intra-renal pressure below 20-25cm H₂O and mimic physiological conditions (7, 8).

Reflux via indwelling ureteric stent technique

For the indwelling ureteric stent delivery method, a cystostomy was first made in the pig's bladder. The ureteric orifices were identified and a hydrophilic tip guidewire was inserted to the renal pelvis and then a 6F double pigtail stent was inserted in a retrograde fashion on each side. The cystostomy was closed with vicryl 3/0 sutures and a suprapubic catheter was placed. The skin and fascia were closed with sutures. The bladder was filled with 150mL of normal saline/indigo carmine solution via the suprapubic catheter and drained after 2 hours of dwell time (9, 10).

Unfortunately, there were no fluoroscopy facilities available in our centre's animal holding unit where the study was carried out to demonstrate vesico-ureteral reflux with the indwelling ureteric stents. To determine the volume required to have vesico-ureteral reflux, we first tested this in a separate pre-study using cadaveric pig kidney-ureter-bladder units. A cystostomy was made and the ureteric orifices were identified. A guidewire was inserted into the ureteric orifice and a 5F ureteric catheter was inserted to the renal pelvis. The renal collecting system was distended with water through the ureteric catheter. A 20G intravenous plug was inserted into the

lower pole calyx by blind technique. Position in the calyx/collecting system was confirmed with good efflux of water seen. The renal pelvis was then emptied of water and a guidewire introduced to the renal pelvis via the ureteric catheter. Indwelling 6F double pigtail ureteric stent was then inserted in retrograde method and position confirmed with palpation. The cystostomy was then closed with vicryl 3/0 sutures and the bladder filled with 100mL of water. Water was seen effluxing from the intravenous plug, demonstrating vesico-ureteral reflux with indwelling ureteric stent. A total of 4 cadaveric renal units were tested. We could not test for reflux in the *in vivo* study but considering the larger volume placed into the bladder (150mLs) compared to the volume demonstrated to have reflux in the cadaveric units (100mLs) and also the presence of intra-abdominal pressure in live pigs, we assumed there would be vesico-ureteral reflux in the indwelling ureteric stent arm.

Retrograde ureteric catheter technique

For the retrograde ureteric catheter technique, a cystostomy was first made in the pig's bladder. The ureteric orifices were identified and a hydrophilic tip guidewire was inserted to the renal pelvis and then a 5F open ended ureteric catheter was inserted in a retrograde fashion on each side. The cystostomy was closed with vicryl 3/0 sutures and a suprapubic catheter was placed. The ureteric catheters were brought out to the skin and the suprapubic catheter was left open to prevent the pressure from building up as the bladder fills which could lead to iatrogenic vesico-ureteral reflux. A tubing connected the ureteric catheters to a burette and 150mL of normal saline/indigo carmine solution was infused via gravity into the nephrostomy tube technique described previously (11, 12).

Analysis

After completion of the delivery method to the UUT, the kidneys and ureters were harvested surgically by open technique. The kidneys-ureters were removed en-bloc with a cuff of bladder taken. The time from completion of delivery method to the time of analysis was standardised

to be 2 hours in all 3 arms. The 6 predefined points were upper pole, mid pole, lower pole, renal pelvis (centre portion), mid ureter (midway of the ureter length) and distal ureter (2cm from the cuff of bladder excised). Three samples of the urothelium were harvested from each of these 6 points to represent that area of the UUT and sent for analysis. The harvested urothelium were sectioned with the same dimensions and they were also weighed to confirm their uniformity. The excised tissues were then homogenized in phosphate buffered saline (PBS) with a tissue homogenizer (Omni International, GA, USA) before the fluorescence was measured using the SpectraMax M2 Microplate reader (Molecular Devices, CA, USA) with excitation and emission at 436 and 528 nm respectively.

Statistical analysis was performed using IBM SPSS Statistics and analyses of variance (ANOVA) was used to calculate the differences among the three arms means. Significance was defined as a P value <0.05.

RESULTS

We followed standard protocol to test 4 renal units (2 pigs) in each of the three arms. There were no complications of setup or experimentation. A sample image for stained kidney and ureter urothelium for each of the three methods are shown in Figures 1-3. Three samples of the same size and weight from each of the 6 pre-defined points of the UUT (upper pole, mid pole, lower pole, renal pelvis, mid ureter and distal ureter) were taken from each porcine unit. Results show that for the retrograde infusion of a fluorescent dye solution (indigo carmine) via an open ended ureteral catheter, the mean amount of fluorescence detected at the 6 pre-defined points was higher than the other two methods and this is significant at all the 6 points with P-value <0.05 on ANOVA analysis comparing the three methods. Comparing the antegrade perfusion method to the vesico-ureteral reflux via indwelling ureteric stent method, the mean amount of fluorescence detected was lower at all points except for the mid ureter. Results are shown in Table-1.

Figure 1 - Representative image after retrograde dye delivery with ureteral catheter.

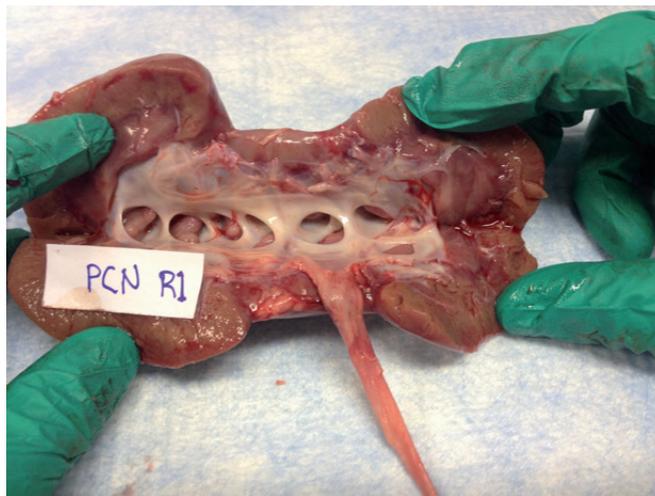


Figure 2 - Representative image after double-pigtail stent dye delivery.

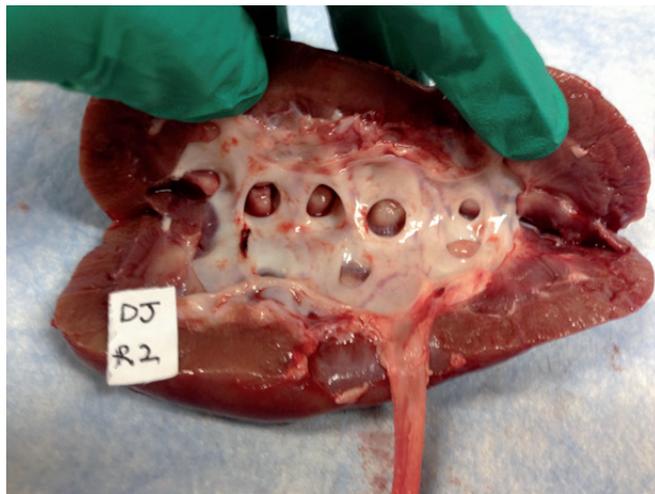


Figure 3 - Representative image after percutaneous dye delivery.

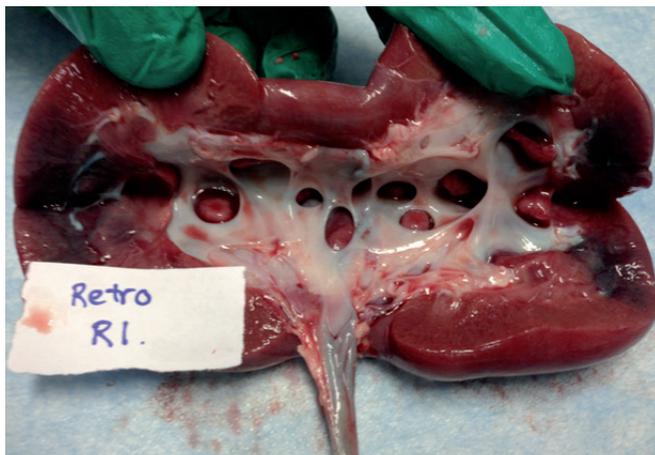


Table 1 - Mean fluorescence, standard deviation and ANOVA analysis of the 3 methods at 6 pre-defined points.

Mean fluorescence (SD)	UP	MP	LP	RP	MU	DU
Antegrade	12.132 (3.646)	12.452 (3.206)	12.353 (3.106)	13.357 (2.964)	17.807 (4.513)	17.406 (2.282)
Retrograde	22.515 (3.508)	25.778 (8.089)	25.267 (3.535)	23.508 (7.482)	24.399 (8.167)	25.396 (9.394)
Reflux via DJS	15.005 (2.863)	18.791 (3.069)	18.520 (5.010)	15.604 (4.506)	14.561 (4.310)	18.005 (3.552)
ANOVA/p-value	<0.0001	<0.0001	<0.0001	<0.0001	0.001	0.004

SD = standard deviation; **UP** = upper pole; **MP** = mid pole; **LP** = lower pole; **RP** = renal pelvis; **MU** = mid ureter; **DU** = distal ureter; **DJS** = double-pigtail stent/ureteric stent
ANOVA = analysis of variance

DISCUSSION

Nephron sparing treatment options have shown good disease control similar to that of nephroureterectomy (NU) with 5 year cancer-specific survival ranging from 87-100% and 89-93% (4, 15) in low grade, non-muscle invasive UUT-UC. This is often paired with adjuvant topical therapy delivered to the UUT to improve oncological outcomes. Instillation of topical bacillus Calmette-Guerin (BCG) to the UUT has also been used as primary treatment for UUT carcinoma-in-situ (CIS). Reported case series on BCG infusion for UUT CIS is associated with uniformly high (63-100%) positive response in terms of short term normalisation of urinary cytology but with a recurrence or progression rate ranging from 0% to 50% (10, 11, 16-21).

However, results for instillation of adjuvant topical therapy to the UUT have been mixed. A non-systemic review by Rastinehad et al. (22) demonstrated efficacy of BCG in the management of upper tract CIS but no definitive efficacy of adjuvant topical therapy after endoscopic resection of Ta/T1 UUT-UC. Other centres have more encouraging results. Giannarini et al. reported their 25 year experience with antegrade infusion of BCG in curative intent for CIS and adjuvant therapy after ablative therapy in 55 patients (23). Recurrence occurred in 40% of CIS and 59% of Ta/T1 UUT-UC and progression occurred in 5% of CIS and 41% with Ta/T1 UUT-UC after a median follow-up of 42 months 11% eventually needed NU. Adverse events occurred in 20% of patients, mostly minor

with one case of fatal E.coli septicaemia. Most of these patients were not medically fit for radical surgery to begin with hence the authors concluded that antegrade instillation of BCG results in high kidney preservation rate and treatment tolerability was good. Katz et al. instilled BCG and interferon- α 2B in 10 patients with median age of 72 years in 11 renal units for adjuvant therapy post endoscopic ablation of UUTUC via a retrograde ureteral catheter (12). Follow-up ureteroscopy with or without biopsy was performed after a 6 week induction to evaluate response. Complete responders were placed on a maintenance regimen. With a median follow-up of 24 months, 8 patients (80%) showed a complete response to therapy and 2 had a partial response. There were no reported side effects or complications.

Several methods have been described for the instillation of topical therapy to the UUT including percutaneous nephrostomy for antegrade instillation, retrograde catheterisation and those using vesico-ureteral reflux with indwelling ureteric stents and each method has its own advantages and limitations (24). Currently, there is no consensus on which is the best method. In the first head to head comparison study between these three methods in an ex-vivo porcine model, Pollard et al. showed retrograde infusion via open ended ureteral catheter is the most efficient method of UUT therapy delivery (13). However, there are several inherent deficiencies in the ex vivo porcine model including lack of natural ureteral peristalsis, continuous urine production and the influence of intra-abdominal pressure on vesico-ureteral

reflux (although the authors used a blood pressure cuff around the bladder to inflate to 8mmHG to mimic intra-abdominal pressure) which could influence the intensity of dye found in the UUT after delivery to the UUT. Hence, there was a need for an in vivo study to compare these three methods.

In this study, we aimed to compare the staining intensity of the UUT urothelium between the three UUT delivery methods in an in vivo porcine model. We measured the amount of fluorescence at 6 predefined points of the UUT with 3 samples measured at each point after instillation of the UUT with a fluorescent dye solution (indigo carmine) via the three methods of UUT delivery. For the antegrade infusion via nephrostomy arm in our study, we used an intravenous plug to access the renal pelvis instead of the calyces for infusion of indigo carmine dye due to technical difficulties in puncturing the calyces in the in vivo pig model. We also did not use a nephrostomy tube because antegrade insertion of a nephrostomy tube in a live pig with a non-dilated collecting system was anticipated to be very challenging and time consuming. In the ex vivo porcine study by Pollard et al., (13) retrograde insertion of nephrostomy tube via puncturing the renal pelvis with an angio-catheter and then inserting a stiff guidewire out through the renal parenchyma was found to have leakage of the dye solution around the nephrostomy tube and the risk of leakage around the defect in the collecting system created. These differences from the actual antegrade delivery method by nephrostomy tube in our study may have contributed to the lower mean amount of fluorescence detected in all the points measured compared to that of retrograde catheterisation and reflux via ureteric stent (except for the mid ureter). Other potential problems with percutaneous nephrostomy include risk of tumour seeding because of the breach in the collecting system, invasive nature and the potential to miss calyces if the therapy solution flows straight down into the ureter. We found that with antegrade perfusion via the intravenous plug in the renal pelvis and angled away from the ureter, the renal collecting system and ureter were uniformly stained and there were no missed calyces in our study. However, the mean fluorescence detected at the 4 points within the

kidney were all lower than the 2 points in the ureter. This could be explained by the position of the plug in the renal pelvis resulting in preferential flow down the ureter despite angling it away from the ureter highlighting the possible effect of the position of the nephrostomy tube in the staining of the UUT urothelium.

The main problem with vesico-ureteral reflux via indwelling ureteric stent is that reflux is not guaranteed with the indwelling ureteric stent. Yossepowitch showed that only 59% of patients had reflux with ureteric stents (25), making this potentially an unreliable method for delivery of topical therapy to the UUT. In our study, we previously tested in cadaveric pig units, the bladder volume required to have vesico-ureteral reflux with indwelling ureteric stents as described previously. We could not test for reflux in the in vivo study but considering the larger volume placed into the bladder (150mLs) compared to the volume demonstrated to have reflux in the cadaveric units (100mLs) and also the presence of intra-abdominal pressure in live pigs, we assumed there would be reflux in the indwelling ureteric stent arm in our study. Our results show that this method stains the pre-defined points more than the antegrade perfusion arm except for the mid ureter.

Retrograde instillation of topical therapy to the UUT via open ended ureteral catheter has been published in the literature (11, 12). Pollard et al. have demonstrated in the ex vivo model that this method has advantage both in terms of area coverage and staining intensity (13). Similarly in our study, this method also resulted in the highest mean fluorescence detected at all the 6 pre-defined points and this was significant for all points on ANOVA analysis compared to the other two delivery methods stents indicating retrograde method results in the greatest staining of topical therapy throughout the UUT urothelium. More clinical studies are needed to investigate if this could correlate to better clinical outcomes by using this method compared to the other two.

One major limitation of this study was that we could not control the amount and rate of urine output among the live pigs which would dilute the dye solution. It has been shown in the in vivo pig study by Otero et al. (26) that urine output varies

minute by minute but this variability was greatest under conditions of sepsis. In our study, we used healthy pigs of the same species and weight and the hydration conditions were similar so as to keep the variability of the urine output similar. The number of pigs used in each arm was also small due to financial restraints, limiting the power of our results.

CONCLUSIONS

In this limited, initial in vivo study, we demonstrated that retrograde infusion of a fluorescent dye solution (indigo carmine) to the UUT by an open ended ureteral catheter resulted in highest mean fluorescence detected at all 6 pre-defined points of the UUT urothelium (upper pole, mid pole, lower pole, renal pelvis, mid ureter and distal ureter) compared to antegrade infusion and vesico-ureteral reflux via indwelling ureteric stents indicating retrograde method results in the greatest staining of topical therapy throughout the UUT urothelium. More clinical studies are needed to demonstrate if retrograde method could lead to better clinical outcomes compared to the other two methods.

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CONFLICT OF INTEREST

None declared.

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Electron microscopic changes of detrusor in benign enlargement of prostate and its clinical correlation

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ABSTRACT

Aims: To study the ultra structural changes in bladder musculature in cases of BPE and their clinical relevance.

Material and Methods: In this descriptive longitudinal, controlled, observational study patients were enrolled into three groups, group 1, group 2A and group 2B. Control group (group-1) consisted of age matched normal male patients, who underwent surveillance or diagnostic cystoscopy for microscopic hematuria or irritative symptoms. Case group (group-2) comprised of patients with BPE, undergoing TURP. Case group (group-2) was further classified into: Category 2A (patients not on catheter) and category 2B (patients on catheter). All relevant clinical parameters like IPSS, prostate size, Qmax, PVR were recorded. Cystoscopy and bladder biopsy were performed in all patients. Various ultrastructural parameters like myocytes, fascicular pattern, interstitial tissue, nerve hypertrophy and cell junction pattern were analyzed under electron microscope and they were clinically correlated using appropriate statistical tests.

Results: Control group had significant difference as compared to case group in terms of baseline parameters like IPSS, flow rate and prostate size, both preoperatively and postoperatively, except for PVR, which was seen only preoperatively. There was statistically significant difference in ultrastructural patterns between case and control group in all five electron microscopic patterns. However, no significant difference was found between the subcategories of case groups.

Conclusions: BPE is responsible for ultra structural changes in detrusor muscle and these changes remain persistent even after TURP. Nerve hypertrophy, which was not thoroughly discussed in previous studies, is also one of the salient feature of this study.

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INTRODUCTION

Bladder dysfunction is often seen secondary to outlet obstruction in benign prostatic enlargement (BPE). These dysfunctions persist even after surgical correction and may be responsible for persistence of symptoms. However, the underlying mechanism for bladder dysfunction is not

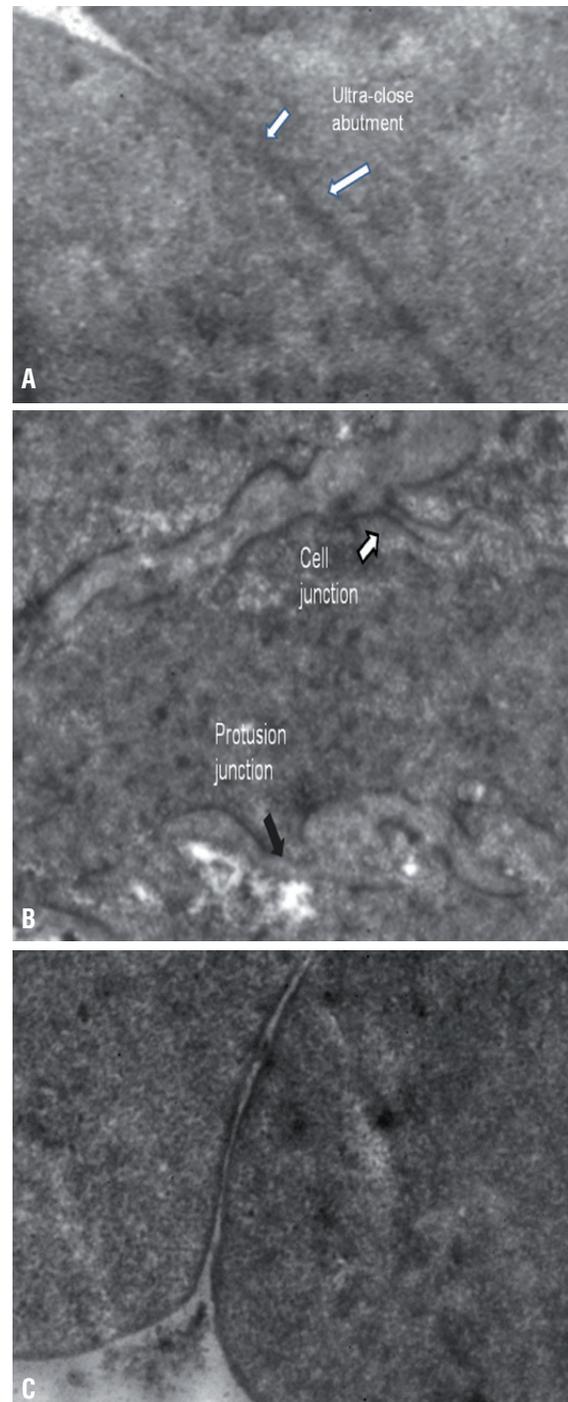
well understood. In clinical practice, urodynamic studies (UDS) can be used effectively to assess bladder function and degree of resistance, but its value in predicting the outcome of surgery has certain limitations. Earlier studies were done on animals to understand histological changes in bladder in cases of BPE (1), however, only little work had been done in this field on human beings. Aim

of this study was to study electron microscopic changes in bladder muscle in cases of BPE and its clinical correlation.

Normal bladder muscles are composed of fascicles. Fascicles in turn are made up of uni-directionally arranged four to twelve spindle shaped myocytes which are surrounded by interstitial microsepta, made up of collagen and occasionally by elastin (2). Hailemarium and Elbadawi graded fascicles as 1) Compact- Bundle of fascicles with occasional myocyte separation, 2) Intermediate- Mixture of compact and loose fascicles and 3) Loose- Moderate to severe myocyte separation or irregular arrangement with rarely seen uniform units (2). Together these muscle fascicles are compact and form muscle bundle and these too are also separated by collagen and elastin. Normal amount of collagen help in mechanical cell coupling, which help in complete bladder emptying. Collagen content in detrusor muscle is much varied and most studies are qualitative in nature, however Mirore et al. had showed that mean collagen content in normal detrusor muscle is <21% (3). For contraction, only a small proportion of myocytes are directly stimulated by nerve while majority of them receive the signals either through electrical or mechanical coupling via Intercellular junction (ICJ) that is why sparse axon bundles are seen in interstitium. Commonest ICJ is intermediate cell junction, which consists of two closely apposed cell wall (sarcolemma) lying parallel to each other for a length of up to 10 micro meters with paired symmetrical dense plaques. Other junction patterns are less frequently seen in normal detrusor. When myocytes are tightly opposed than that is known as “gap junction”. Other variants are “protrusion junction”, which are slender finger like projection between cells with tip to tip contact and “ultra close abutment” (Figure-1a), which are a tight opposition on a parallel surface in a shadow bomb impression configuration (4).

Although gap junctions are seen in normal detrusor, their ratio compared to normal ICJs increases in patients with detrusor instability, demonstrating a syncytium pattern of gaps between cell processes linking up to or more than ten myocytes. This leads to the summated detrusor contraction. So instead a low resistance pathway

Figure 1 - a) Variant of normal Intercellular Junctions (x-16400); 1b) Myocytes with normal intercellular junction and protrusion junction (X-17000); 1c) Normal Intercellular Junction (X-17600) this consists of two closely apposed 25-70nm wide gap sarcolemma lying parallel to each other for a length of up to 10µm with paired symmetrical dense plaques.



occur, thus mediating rapid electrical coupling. This ultimately results in the unstable contractions seen on urodynamic studies of subjects with an overactive detrusor (4, 5).

In pathological conditions, myocytes, interstitium and cell junction may show certain changes (6-8), these changes may be isolated or in various combinations. In BPE there may be changes in myocytes cell density, shape and content. Besides hypertrophy, myocytes may be empty or contain vacuoles and debris, their shape may be shriveled or disruptive. In dysfunctional bladder, myocytes can be breaded, branched, intertwined or bizarre shaped (9). In pathological conditions, fascicles may show marked separated arrangement. Abnormal fascicle arrangement and architecture is usually associated with abnormal interstitial tissue. Interstitium may have excessive collagen or elastin, loose fascicular pattern is more particularly associated with increased interstitial tissue and seen in hypocontractile bladder (6-8). When nerve is thickened over its length then it can be considered as hypertrophy. Although no study had exactly quantify this, in our study we considered >10 micrometer diameter as an abnormal finding.

MATERIAL AND METHODS

After institutional review board approval the descriptive type of observational study with control group and longitudinal design was conducted in our department. Informed written consent was taken from all the patients. Patients attending the treatment of lower urinary tract symptom (LUTS), retention of urine and hematuria were enrolled. Detailed history was noted and physical examination was done in all patients. International prostate symptom score (IPSS) was recorded in all catheter free patients. Besides routine investigations, prostate specific antigen (PSA) estimation, and ultrasonography (USG) of kidneys, bladder and prostatic regions were also done in all the patients. Uroflowmetry (UFM) and post void residual urine (PVR) estimation were carried out in all the catheter free patients, whereas, UDS was also done in selected patients only. Patients with significant LUTS or retention of urine undergoing

trans urethral resection of prostate (TURP) were enrolled in the case group (group-2). These patients were categorized into groups.

Control (group-1) comprised of age matched patients, who underwent cystoscopy for evaluation of microscopic hematuria or irritative LUTS without any evidence of BPE. Patients having IPSS >8, prostate volume>25mL, PVR >50mL, or peak flow rate (Qmax) <15mL/sec were excluded from this group.

Case (group-2) comprised of patients suffering from BPE. Only patients who had prostate volume >35mL with either retention of urine or having IPSS >15 and undergone TURP were included in this group. Patient having Qmax >15mL/sec or showing malignancy on TURP biopsy were excluded from this group. This group was further subcategorized into two groups: group 2A (catheter free) and group 2B (patients on catheter drainage).

Patients with past history of prostatic or bladder surgery, stricture urethra, neurological disorder, pelvic irradiation, prostatic/bladder malignancy, diabetes, renal impairment, prostatic or bladder abnormalities, active urinary tract infection, PSA>4ng/mL or on medical treatment (alfa blockers, 5 alfa reductase inhibitors, phosphodiesterase 5 inhibitors {PDE 5I}, anticholinergics and cholinergics) and those with follow-up duration of less than 3 months, were also excluded from the study.

All patients underwent cystoscopy as standard procedure under anesthesia and findings were recorded. Bladder biopsy was taken using cold cup biopsy forceps. Minimum of two biopsies were taken, approximately 2cm supero-lateral to the ureteric orifices (2, 4). Following bladder biopsy, patients in case group underwent standard TURP. Biopsy specimens were immediately fixed in chilled buffered 4% formaldehyde solution and kept refrigerated at 5 degrees C until it processed to the concerned electron microscopic histopathology department. After that, mucosa was carefully stripped and detrusor muscle was separated. Later on, by using standard techniques, staining and fixation done and bladder biopsy specimen were examined under electron microscope. Electron microscopic histological features were evaluated re-

garding myocyte changes like degenerative pattern, fascicular arrangement, interstitial tissue pattern, nerve hypertrophy and communication between myocytes and these findings were compared with patient's clinical findings. Collagen content >34%, nerve diameter >10 micrometer and in ICJ, gap junction ratio >50% (gap junction/normal ICJ) were labeled as abnormal parameter in our study (3, 5).

Post operatively at the end of 1st and 3rd month patients of both groups were assessed by IPSS, PVR, Qmax and prostatic volume. Endoscopy and UDS were performed in selected cases.

In statistical analysis, continuous variables were summarized as mean and standard deviation, while categorical/nominal variables as proportions (%). One way ANOVA test with Post Hoc Tukey HSD test were used for analysis of continuous variables where subgroups were more than two, while chi-square test and Fisher exact test were used for nominal/categorical variables as per their indications. P value <0.05 was taken as significant. SPSS 21.0 version was used for all statistical calculation.

RESULTS

In our patient series 50 patients met the inclusion criteria, with 21 patients in control group and 29 patients in case group. Case group 2A had 20 patients and group 2B had 9 patients. Table-1 shows the preoperative clinical parameters and comparison between various groups. Group-1 and group-2 were comparable in terms of their age but had statistically significant difference with respect to IPSS, flow rate and prostate size. In terms of PVR all three categories (group 1, group 2A and group 2B) had statistically significant differences.

Table-2 shows electron microscopic features of control and case group patients. In control group 18 (85.71%) patients had normal myocyte pattern (Figure-1b) whereas majority of patients in case group had varied morphological features. In case group, 9 (31.03%) patients had both myocyte hypertrophy and degenerative pattern (Figure-2a). Degenerative pattern (Figure-3) was seen in 17 (58.62%) case group patients and out of these, 12

Table 1 - Preoperative (Baseline) comparison between groups.

Characters	Group	N	Mean	Std. Deviation	'p' Value*	Significant difference from#
Age(in years)	Control (Group 1)	21	60.19	3.49	0.289*	
	Case (Group2 A)	20	62.35	5.70		
	Case (Group2 B)	9	62.56	5.66		
IPSS	Control (Group 1)	21	3.14	1.46	<0.001	
	Case (Group2 A)	20	20.45	3.90		
	Case (Group2 B)	9	-+	-+		
Flow Rate(Qmax)	Control (Group 1)	21	17.29	2.62	<0.001	A,B
	Case (Group2 A)	20	6.54	1.31		
	Case (Group2 B)	9	0.00+	0.00+		
Prostate Size (in grams)	Control (Group 1)	21	16.00	2.35	<0.001	A,B
	Case (Group2 A)	20	59.15	11.85		
	Case (Group2 B)	9	62.00	15.28		
PVR(in mL)	Control (Group 1)	21	5.29	6.48	<0.001	A,B
	Case (Group2 A)	20	88.35	50.14		
	Case (Group2 B)	9	400.00	242.38		

* ANOVA Test; # Tukey HSD; + For patients on catheter drainage; **Qmax** were underleveled as 0, similarly as these patients were not able to recollect their **IPSS**, hence it was not calculated in this group.

Table 2 - Comparison of electron microscopic findings between groups and statistical correlations.

Microscopic findings	Control (Group 1)		Case (Group 2)				Total Case (N=29)	
	(N=21)		Catheter free (A) (N=20)		On catheter (B) (N=9)			
	No.	%	No.	%	No.	%	No.	%
Myocytes								
Normal	18	85.71	4	20.00	1	11.11	5	17.24
Hypertrophy	3	14.29	5	25.00	2	22.22	7	24.14
Degeneration	0	0.00	5	25.00	3	33.33	8	27.59
Hypertrophy+Degeneration	0	0.00	6	30.00	3	33.33	9	31.03
Fascicles								
Compact	18	85.71	4	20.00	2	22.22	6	20.69
Intermediate	3	14.29	2	10.00	2	22.22	4	13.79
Loose	0	0.00	14	70.00	5	55.56	19	65.52
Interstitial tissue								
Normal	21	100.00	6	30.00	2	22.22	8	27.59
Collagen	0	0.00	8	40.00	5	55.56	13	44.83
Elastin	0	0.00	4	20.00	0	0.00	4	13.79
Collagen+Elastin	0	0.00	2	10.00	2	22.22	4	13.79
Nerve hypertrophy								
Absent	21	100.00	16	80.00	7	77.78	23	79.31
Present	0	0.00	4	20.00	2	22.22	6	20.69
IC Junction (Communication)								
Normal	21	100.00	9	45.00	5	55.56	14	48.28
Dysjunction Pattern	0	0.00	11	55.00	4	44.44	15	51.72

(41.37%) patients had either retention of urine or significant PVR before TURP 18 (85.71%) patients of control group had compact fascicular arrangement whereas, in case group loose fascicular pattern was more prevalent which was seen in 19 patients (65.52%) (Figure-2b). Normal interstitial pattern was seen in all control group patients whereas increased collagen (Figure 4a and 4b) was seen as the predominant interstitial pattern in case group in 13 patients (44.83%). Nerve hypertrophy (Figure-5) was absent in all control group patients while it was present in 23 (79.31%) patients of case group. Normal communication pat-

tern (Figure-1c) was present in all control group patients, whereas in case group 15 (51.72%) patients showed dysjunction pattern.

Table-3 shows that on application of chi-square test and Fischer exact test there was a statistically significant difference in all electron microscopic patterns in the control and case groups. However, there was no statistically significant difference in microscopic patterns among the subcategories of the case group.

Table-4 shows the correlation of IPSS and PVR with all five ultrastructural pattern and it is clear from this Table that if we subcategorize the-

se patients, into IPSS <19 and >19, then although abnormality is more evident in patients with severe symptoms (IPSS >19) it was not statistically significant. Similarly in cases of PVR, ultrastructural characteristics like myocytes and communication pattern are more evident in severe symptoms (PVR >300mL) but these were statistically insignificant.

Table-5 shows the postoperative outcome after surgery (TURP). After TURP, all patients except two, successfully voided after catheter removal. In these two patients, catheter was kept for longer duration (15 days), after that one of them was able to void. These patients also had urodynamic finding of hypotonic bladder and complete myocyte degenerative pattern on electron microscopy. On analysis, there was significant improvement in postoperative clinical parameters like IPSS, Qmax, decrease in PVR and prostatic size in the case group when compared to preoperative parameters. However, when compared to control group, the postoperative outcome of case group were found to be inferior.

DISCUSSION

Initial electron microscopic studies suggested that aging can lead to morphological changes in bladder musculature (4, 6, 10). However, recent studies failed to demonstrate these findings (11-13). In our study, we did not find any correlation between morphological changes and aging, as nearly all of our age matched control patients had normal muscular architecture in bladder on electron microscopy. Probably these observational differences among various studies might be due to the variation in selection criteria and lack of control group.

Collado et al. reported that increased outlet resistance may lead to compensatory myohypertrophy of detrusor muscles in bladder outlet obstruction (BOO) (14). These hypertrophied myocytes are responsible for increased collagen and elastin synthesis and deposition in interstitium (11, 14). However, there is no consensus regarding the amount

Figure 2 - a) Myohypertrophy (a-X-27600 and b-X-13000). Marked intercellular separation and cellular hypertrophy. Excessive collagen present between the cells as well as between the fascicles. Red arrow shows myohypertrophy and black arrow shows excessive collagen and 2b) shows myohypertrophy in obstructed bladder shown by red arrow and loose fascicle with increased intercellular space shown by black arrow (X-13200).

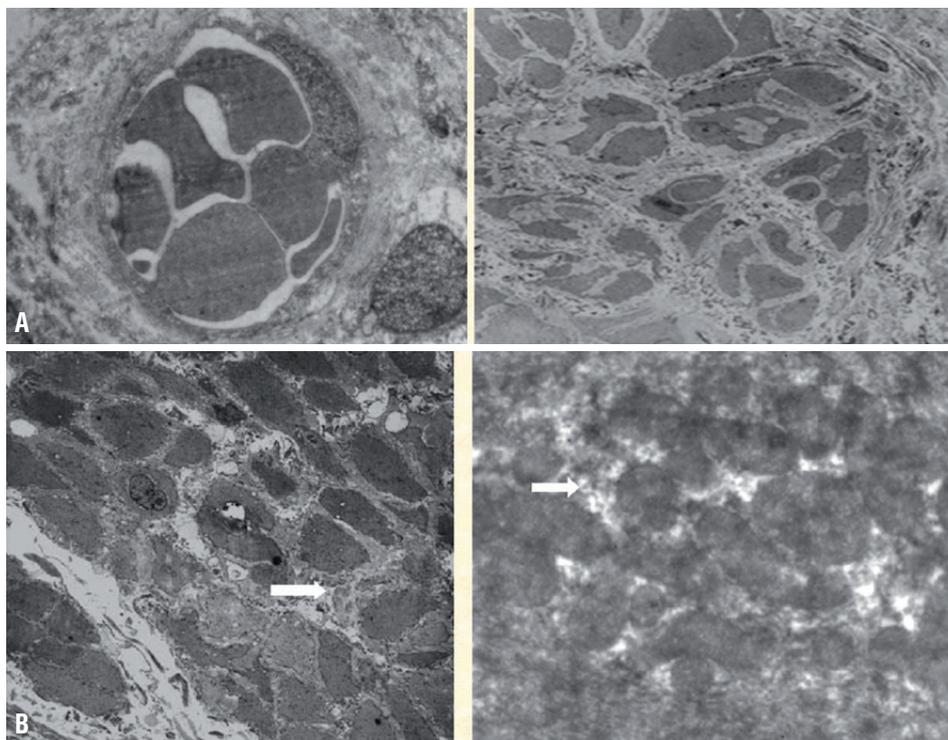


Figure 3 - Degenerative pattern (X-12800). Black arrow shows degenerative pattern.

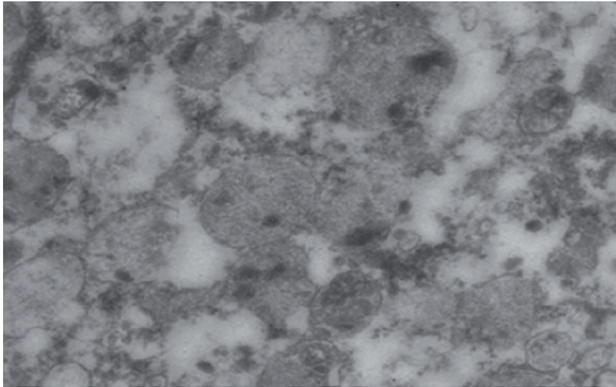


Figure 5 - Nerve hypertrophy (X-50220).

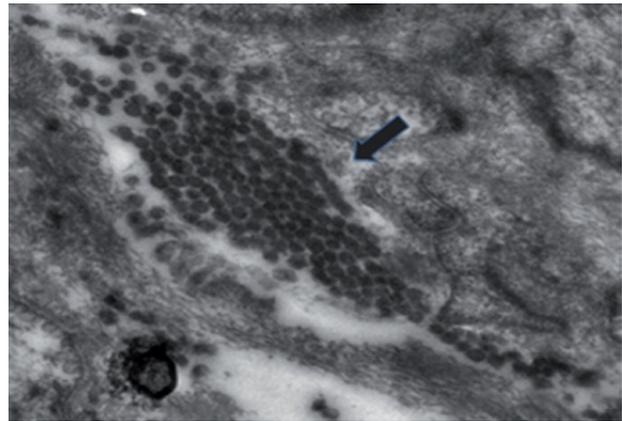
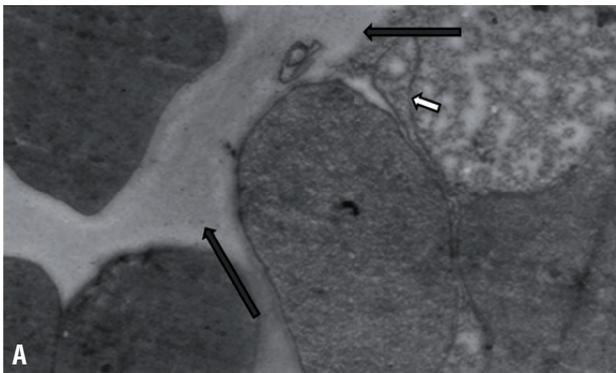
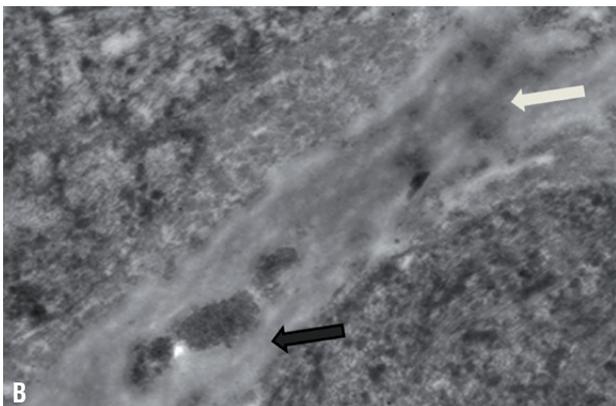


Figure 4 - a) Widened intercellular spaces with excessive collagen deposition along with degenerative myocytes (X-25860) and 4b) Excessive Collagen with Hyperelastosis (X-22380) with black arrow shows collagen and red arrow shows elastin.



 Degenerated myocytes
  Excessive collagen deposition



of collagen deposited in interstitium of obstructed bladder (15, 16). Mirone et al. showed that detrusor collagen content increases in symptomatic cases of obstructive BPE and this could be responsible for persistence LUTS (17). Our finding also supported the concept that myohypertrophy is responsible for increased collagen deposition in bladder outlet obstruction (BOO), as nearly all of our case group patients having myohypertrophy also had increased interstitial tissue content. However, whether excessive collagen deposition is more frequently seen in acute (14) or chronic retention (12), is still an unanswered question. Notably, we failed to find any difference in interstitial tissue content among sub-categories of case group.

Various studies had shown that degenerative changes in bladder muscle are responsible for increased residual urine, which persists even after TURP (9). In the present series, all patients having degenerative changes were able to void postoperatively, except two patients, without significant PVR. These two patients were found to have severe degenerative myocytes changes. This suggests that not only presence but severity of degenerative pattern also affects the outcome.

BOO induced growth factors regulate bladder remodeling by different mechanisms (17). The increased expression of nerve growth factor (NGF) is reported to be a key factor in the neuronal hypertrophy observed in BOO patients (18). In our study, nerve hypertrophy was seen in six patients although individual significance of this feature could not be

correlated with any clinical outcome. But we believe that along with muscle hypertrophy, it may be one of the adaptive change in response to outlet obstruction. In the present study, we also found significant correlation between increase in prostatic volume, PVR, IPSS and decrease in Qmax, with the morphological changes in bladder muscle, although this had a nonlinear relationship.

In an animal study, Kim et al. had shown that, changes in detrusor can regress after the correction of obstruction (1). Although there was significant improvement in IPSS, PVR, UFM after TURP in our case group, the outcomes were inferior when

compared to control group. These findings suggest that, in contrast to study by Kim et al. (1), these various ultrastructural changes could not revert to normal even after removal of obstruction. That is why patients having severe morphological changes continue to have bothersome IPSS and poor flow rate even after TURP. We believe that probably these patients had detrusor changes which did not revert back to normal state, even after relief of obstruction, and were responsible for persistent symptoms. So, morphological parameter can have a role in predicting outcome and future treatment for bladder dysfunction. Limitation of our study includes less num-

Table 3 - Statistical correlation of electron microscopic findings in various groups.

Abnormality of electron microscopic pattern	Control (Group 1) v/s Cases (Group 2)	Control (Group 1) v/s Case (Group 2 A)	Control(Group 1) v/s Case (Group 2 B)	Case A v/s B (Group 2A v/s Group 2B)
Myocytes*	<0.001	<0.001	<0.001	1.000
Fascicles*	<0.001	<0.001	<0.001	0.643
Interstitial Tissue*	<0.001	<0.001	<0.001	0.544
Nerve Hypertrophy#	0.033	0.048	0.083	1.000
IC junction (Communication)#	<0.001	<0.001	0.005	0.700

* Chi-square Test; # Fisher Exact Test

Table 4 - Showing correlation of ultrastructural characteristics with IPSS and PVR.

Ultrastructural characteristics		IPSS				PVR			
		<19	>19	Total	'p' Value*	<300mL	>300mL	Total	'p' Value*
Myocytes	Normal	2 (22.22%)	0 (0%)	2	0.099344	5 (22.73%)	0 (0%)	5	0.165377
	Abnormal	7 (77.78%)	11 (100%)	18		17 (77.27%)	7 (100%)	24	
Fscicles	Normal	2 (22.22%)	2 (18.18%)	4	0.82218	4 (18.18%)	2 (28.57%)	6	0.555599
	Abnormal	7 (77.78%)	9 (81.82%)	16		18 (81.82%)	5 (71.43%)	23	
Interstitial tissue	Normal	3 (33.33%)	3 (27.27%)	6	0.76857	6 (27.27%)	2 (28.57%)	8	0.946613
	Abnormal	6 (66.67%)	8 (72.73%)	14		16 (72.73%)	5 (71.43%)	21	
Nerve hypertrophy	Normal	8 (88.89%)	9 (81.82%)	17	0.65952	17 (77.27%)	6 (85.71%)	23	0.631069
	Abnormal	1 (11.11%)	2 (18.18%)	3		5 (22.73%)	1 (14.29%)	6	
IC Junction (Communication)	Normal	4 (44.44%)	6 (54.55%)	10	0.65309	11 (50%)	3 (42.86%)	14	0.741854
	Abnormal	5 (55.56%)	5 (45.45%)	10		11 (50%)	4 (57.14%)	15	

* Chi-square Test

Table 5 - Post operative (at 3rd months) comparison between groups.

	Group	N	Mean	Std. Deviation	'p' Value*	Significant difference from#
IPSS	Control (Group 1)	21	3.19	1.29	0.003	A
	Case (Group 2A)	20	6.65	4.40		1
	Case (Group 2B) +	8	4.75	1.67		-
Flow Rate(Qmax)	Control (Group 1)	21	17.37	2.45	<0.001	A,B
	Case (Group 2 A)	20	12.13	4.66		1
	Case (Group 2 B)	9	11.36	4.71		1
Prostate Size(in grams)	Control (Group 1)	21	16.00	2.35	<0.001	A,B
	Case (Group 2 A)	20	24.30	3.85		1
	Case (Group 2 B)	9	23.56	4.95		1
PVR (in mL)	Control (Group 1)	21	5.33	5.83	0.061	-
	Case (Group 2 A)	20	13.70	26.2		-
	Case (Group 2 B)	9	59.78	132.17		-

* ANOVA Test; # Tukey HSD; + one patient not able to void

ber of patients and lack of post TURP morphological data to confirm the residual detrusor changes. With enrolment of more number of patients and quantification of morphological changes, impact of these changes on various clinical findings could have been better explored.

CONCLUSIONS

In our study detrusor morphological changes have been seen in various combinations in cases of BOO. These ultrastructural patterns showed non-linear correlation with clinical measures of bladder dysfunction. Nerve hypertrophy, which was not thoroughly discussed in previous studies, is one of the key features of this study. Surgery definitively leads to improvement in various clinical parameters but these parameters could not reach up to the level of control group because of underlying morphological changes.

ABBREVIATIONS

BOO = Bladder outlet obstruction
 BPE = Benign prostatic enlargement
 ICJ = Intercellular junction
 IPSS = International prostate symptom score
 LUTS = Lower urinary tract symptoms

NGF = Nerve growth factor
 PDE 5I = Phosphodiesterase inhibitors
 PSA = Prostatic specific antigen
 PVR = Post void residual urine
 Qmax = Peak flow rate
 TURP = Transurethral resection of prostate
 UDS = Urodynamics studies
 UFM = Uroflowmetry
 USG = Ultrasonography

CONFLICT OF INTEREST

None declared.

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What is the quickest scoring system to predict percutaneous nephrolithotomy outcomes? A comparative study among S.T.O.N.E score, Guy's Stone Core and CROES nomogram

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ABSTRACT

Objective: To compare the application time and the capacity of the nomograms to predict the success of Guy's Stone Score (GSS), S.T.O.N.E. Nephrolithometry (STONE) and Clinical Research Office of the Endourological Society nephrolithometric nomogram (CROES) of percutaneous nephrolithotomy (PCNL), evaluating the most efficient one for clinical use.

Materials and Methods: We studied 48 patients who underwent PCNL by the same surgeon between 2010 and 2011. We calculated GSS, STONE and CROES based on pre-operative non-contrast computed tomography (CT) images and clinical data. A single observer, blinded to the outcomes, reviewed all images and assigned scores. We compared the application time of each nomogram. We used an analysis of variance for repeated measures and multiple comparisons by the Tukey test. We compared the area under the ROC curve (AUC) of the three nomograms two by two to determine the most predictive scoring system.

Results: The immediate success rate was 66.7% and complications occurred in 16.7% of cases. The average operative time was 122 minutes. Mean application time was significantly lower for the GSS (27.5 seconds) when compared to 300.6 seconds for STONE and 213.4 seconds for CROES ($p < 0.001$). There was no significant difference among the GSS (AUC=0.653), STONE (AUC=0.563) and CROES (AUC=0.641) in the ability to predict immediate success of PCNL.

Conclusions: All three nomograms showed similar ability to predict success of PCNL, however the GSS was the quickest to be applied, what is an important issue for routine clinical use when counseling patients who are candidates to PCNL.

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INTRODUCTION

Nephrolithiasis is a common condition, with high prevalence and recurrence, constituting one of the most common diseases of the urinary tract (1). The disease affects 5% to 15% of the

world population, with a peak incidence in young adults between the third and fourth decade of life (2, 3). The surgical treatment of nephrolithiasis has advanced substantially in recent years. Percutaneous nephrolithotomy (PCNL) remains the gold standard modality for treatment of complex renal

stone and/or high volume stone (4-6). Despite the establishment of PCNL as one of the most important methods for the treatment of kidney stones, currently there is no gold standard tool for predicting success and complications associated with this surgery (7). This is important because a scoring system could help the surgeon in planning surgical strategies, predict the success rate and complications, result in better patient counseling, and facilitate comparison of outcome between the different institutions (7).

There are a few scoring systems in the literature which assess pre-operative parameters and predict the success rate of PCNL (8-11). They have showed to positively correlate with outcomes or complications, but comparison among them is required in order to determine the most practical and applicable one in clinical practice. Currently, three nomograms have been more extensively studied: Guy's Stone Score (GSS) (8), the S.T.O.N.E. Nephrolithometry (STONE) (10) and the nomogram of the Clinical Research Office of the Endourological Society (CROES) (9). The GSS consists of a nomogram using as parameters the amount of stones, their renal location, history of spina bifida or spine injury, and the association with possible anatomical changes, as horseshoe kidney: nephrolithiasis burden is classified in 4 degrees related to different success rates in PCNL (8). The parameters used in the STONE include stone size, distance to the skin, the degree of obstruction in the urinary tract, the number of renal calices involved, and stone density (10). Finally, the CROES uses variables such as area, number and location of the stones, previous treatment, staghorn stone and number of cases treated per year in the institution (9).

Some studies have shown that all nomograms correlated well with success or complications (12-18) and that they have similar ability to predict surgical outcomes (19-22). Nevertheless, there are no studies assessing these nomograms in clinical practice, where time is an important factor. In this study, we aim to compare the acquisition times for the most used nomograms.

OBJECTIVE

Our primary goal was to compare the application time of GSS, STONE and CROES, eval-

uating which one is quickest to be applied in clinical practice.

Our secondary objective was to compare the ability of the nomograms to predict the immediate success rate of PCNL.

MATERIALS AND METHODS

We performed a retrospective review of medical record data from a prospectively collected database. In our institution, we perform >180 PCNL/year. We analyzed patients who underwent PCNL between February, 2010 and December, 2011 at our institution by the same senior urologist (FCV) under the same technique, as previously described (22). Briefly, under general anesthesia, all patients were positioned in the supine decubitus with the posterior axillary line located just outside the border of the surgical Table; the flank was extended to increase the space between the last rib and the iliac crest; all csPCNLs were performed without boosters under the flank and all patients were maintained in the same position during the entire procedure.

Tract dilation was performed with fascial dilators (numbers 10, 20 and 30Fr, sequentially) and the Amplatz® sheath was placed. Nephroscopy was performed with a 26Fr rigid nephroscope (Karl Storz, Munich, Germany) and we used an ultrasonic lithotripter for stone fragmentation and suction. The stone free status was verified with combined fluoroscopy and flexible nephroscopy. A 16Fr nephrostomy tube was placed at the end of the procedure in case of bleeding, residual stones, solitary kidney, suspected pelvic injury, or multiple tracts. A 6Fr ureteral catheter was routinely placed; in cases of ureteropelvic junction significant edema, extensive pelvic injury, or ureteral manipulation, a double-J stent was used instead. Operative time was considered from the beginning of cystoscopy for ureteral catheter insertion to the end of nephrostomy placement.

Complication was defined as any deviation from normality in the peri or postoperative period of 72 hours, using the Clavien Scale, validated for postoperative complications in PCNL (23). Complication rate comparison was not one of our objectives due to the fact that GSS was not

developed for this purpose. The study protocol had ethical review board approval.

Selection criteria

Exclusion criteria comprised patients younger than 18 years old and older than 70 years old, patients with inadequate analysis by preoperative CT (low resolution or not performed in our service) and without at least one follow-up consultation in a 60 days period.

Measurements

We calculate the GSS, STONE and CROES of all patients based on preoperative CT images as described by Thomas et al.(8), Okhunov et al. and Smith et al.(9), respectively. A single observer (FRS) reviewed all images and performed scoring according to each system. The reviewer was a 5th year medical student (of a total of 6 years), with basic knowledge in radiology, with no previous use of any of the scoring systems, who was initially trained to evaluate non-contrast CT scans by calculating the three scores for 20 different cases under supervision of two senior urologists before initiating the study. We did not use these cases for our study, just for training the observer. A concordance index among the calculations of the 3 observers for these 20 cases was 0.86, showing that the reviewer has been properly trained. We analyzed all the images on the computer screen and all parameters were acquired through the image display program. The high concordance index allowed us to make all the analysis based on the data calculated by only one observer. The observer also had a Table with the data regarding physical examination, history of previous surgery and presence of spina bifida or not. The time for clinical data analysis was not considered, because in a clinical setting this information usually is already known by the assistant when analyzing the CT images, according to a regular patient evaluation.

Acquisition Time

The time required for application of nomograms was individualized for each nomogram in each case. For this, we used a simple timer. The count began at the time the researcher started the

evaluation of the imaging exam and ended when the investigator obtained the final score of the nomogram. First, we calculated the GSS consecutively for all patients and the data inserted into an Excel Table (Microsoft, California). Then, in another day, we calculated the STONE parameters also for all patients. Stone burden was estimated in mm² using the formula, $\Sigma (0.785 \times \text{length}_{\text{max}} \times \text{width}_{\text{max}})$ and the value was automatically generated by the software (24). Finally, again in another day, we obtained the CROES from the same images.

We did not reuse the results of measurements taken for one nomogram for calculating the other one and we did all the measurements in different days in order to guarantee that the observer did not have memory of the image previously seen. Every nomogram had its proper Excel Table, with the patient's demographic data, history of previous surgery and presence of spina bifida or not.

Definition of success

We defined success as stone fragments $\leq 4\text{mm}$ on CT scan on the first postoperative day (POD1). Stone-free rate refers to no identification of any stone fragment on the POD1 CT. Final success rate was defined as the result of the last radiological exam performed after all the auxiliary procedures, consisting of revision PCNL, external shockwave lithotripsy or flexible nephroscopy.

Statistical analysis

We calculate the sample size based on an expected difference between GSS and the others nomograms of 50% in time acquisition, with a power of 80% and a significance level of 0.05, based on the initial findings of the 20 cases studied for training the observer. With these parameters, we reached a total number of 18 patients. We studied 48 trying to improve the success comparison among the nomograms.

To check the normality, we used the Wilk-Shapiro test. GSS distribution was non-normal. So, to verify if the differences between the acquisition times were significant, we performed an analysis of variance (ANOVA) for repeated measures and compared these analyses to others performed with Wilcoxon signed-rank test, showing that

they were similar, supporting our ANOVA use for this study. To check if they were all different, we did a multiple comparison by the Tukey method, comparing the scoring systems in pairs. We generated receiver operator characteristic (ROC) curves for each scoring system. We calculated the area under the curve and asymptotic 95% confidence intervals were calculated for each ROC curve. We performed all statistical analyses using SPSS 19.0 software for Windows (SPSS Inc., Chicago, USA). A p value of <0.05 was considered statistically significant.

RESULTS

Perioperative data

Demographic data are shown in the Table-1. The immediate success rate was 66.7% (29.2% of stone free and 92.4% for final success rate after a mean of 1.29 auxiliary procedures), and complications occurred in 16.7% of cases. The average operative time was 122 minutes (Table-1).

Nomograms application speed

Mean application time for the GSS was 27.5±30.0 seconds, significantly shorter than the 300.6±56.5 seconds for STONE and 213.4±59.4 seconds for CROES. There was a significant difference between all groups (p <0.001) (Figure-1).

Scoring Systems Reliability

After the two by two comparison of the AUC, there was no significant difference among the GSS (AUC=0.653), STONE (AUC=0.563) and CROES (AUC=0.641) in the capacity to predict immediate success of PCNL (STONE x GSS: p=.445; STONE x CROES: p=0.513; GSS x CROES: p=0.912). Figure-2: shows the percentage of success by groups of scores. Figure-3: shows the AUC and ROC curves for each of the scoring systems. All scoring systems demonstrated similar accuracy.

DISCUSSION

Instruments that aim to classify the surgical risk and estimate the percentage of success involve risk scales, nomograms, probability Tables and analysis by regression trees. They are very

Table 1 – Baseline characteristic of study patients.

No. pts	48
Mean ± SD age	46.4±14.3
% Male	29.16
Mean ± SD body mass index (kg/m2)	28.3±7.6
% Right kidney	56.3
% American Society of Anesthesiologist score:	
I / II / III / IV	50 / 43.7 / 6.3 / 0
% Ipsilateral Prior surgery(PCNL or SWL)	25
% of patients with spina bifida or spine injury	0
% Immediate success (fragments ≤4mm POD1)	66.7
% Stone-free (no fragments POD1)	29.2
%Final Success Rate (after all auxiliary procedures)	92.3
% Complications	16.7
Mean ± SD operative time (min)	122.3±46.1
% Guy’s Stone Score:	
1	10.4
2	39.5
3	33.4
4	16.7
% S.T.O.N.E. Nephrolithometry	
5-6	18.75
7-8	50
9-12	31.25
% CROES Nephrolithometric Nomogram	
130-169	16.75
170-219	39.5
≥220	43.75

useful because they help the surgeon in planning surgical strategies, result in better counselling of the patient and allow the comparison of outcomes between the experiences of different institutions.

The three nomograms evaluated in this study, GSS (8), STONE (10) and CROES (9) have been recently proposed as tools to predict success in PCNL. They use measurable and qualitative parameters, acquired from preoperative imaging studies and medical history. Despite the heterogeneity

between nomograms, the three aim to classify patients into groups with different graduations of success in PCNL.

We have demonstrated that the nomograms were not significantly different in regards to the ability to predict success from PCNL. In the ROC curve analysis for the three scoring systems, we found that the Area Under the Curve (AUC) of CROES and GSS were similar (0.641 and 0.653, respectively), while the STONE was lower (0.563).

Figure 1 – Time of application of the nomograms (in seconds).

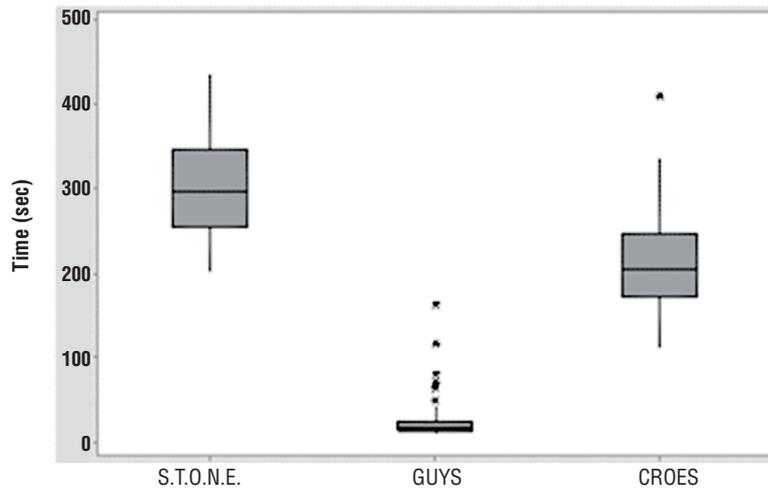


Figure 2 – Percentage of success by groups of scores.

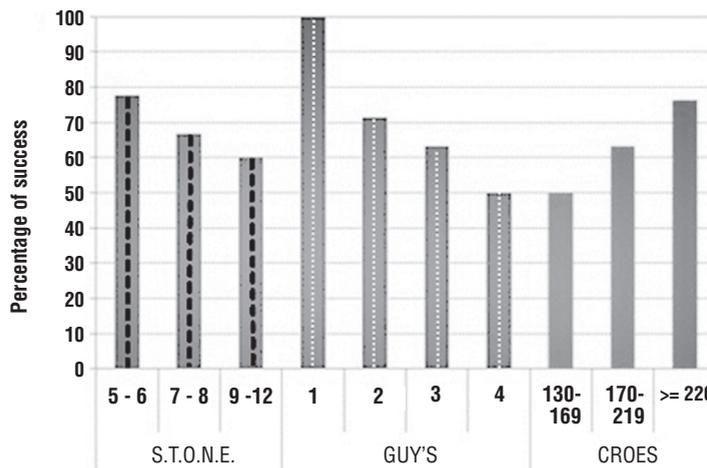
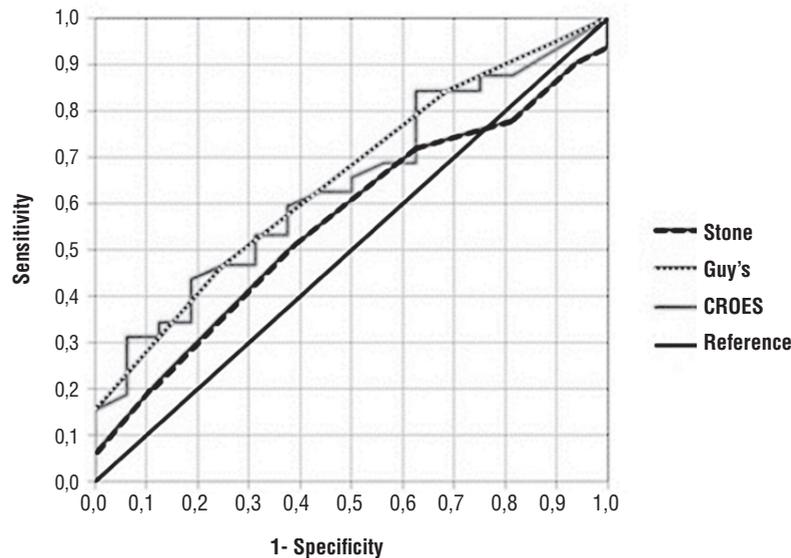


Figure 3 – ROC curve for STONE score, Guy's score, CROES nomogram and reference.

However, the two by two comparison between them revealed no significant difference (STONE x GSS: $p=0.445$; STONE x CROES: $p=0.513$; GSS x CROES: $p=0.912$). Other studies have compared the three nomograms and the results are similar to ours. In a study involving 246 patients, Labadie and Okhunov (19) found the area under the curve of GSS, CROES and STONE were 0.634; 0.671 and 0.670, respectively and also demonstrated that the nomograms were not significantly different. Noureldin et al. (20) also showed similar AUC between GSS and STONE. Sfoungaristos et al. (21) could demonstrate the correlation between higher complexity according to the three nomograms and use of fluoroscopy. It is important to note that the value of the AUC is relatively low, suggesting a low capacity of predicting success. In our point of view, the use of AUC actually does not reflect the real benefit of using any of these nomograms. When we evaluate the different groups created by the nomograms, we can clearly see that these groups are different among them regarding peri and postoperative data, according previous reports (10, 11, 13-15, 18). The information obtained is critical when counselling the patient about the expectations for the surgery. Moreover, in a group of urologists used to the nomograms, when one says that is going to operate a patient with

a GSS 4, everyone knows the difficulties that are expected, including the anesthesiologist and staff room, facilitating the operative planning. This is exactly what we observe in our institution, where we have been using the GSS for the last years. Similar effect can be expected if the team is used to another nomogram.

Withington et al. (22) in a recent systematic review of the literature could not firmly recommend one nomogram over the others, but found that the quality of evidence supporting validation of the GSS was marginally superior. If all scoring system are good for predicting outcomes and are similar among then in their capacity of doing that, which one should be used in a daily basis? The applicability of a nomogram depends on how easy it is to be used in a clinical setting. Hence, time used for calculating the score is an important factor when considering routine use. For that reason, we decided to study the application time for each nomogram. To the best of our knowledge, this is the first study to address this issue. In our study, we could verify that the GSS, a visual method that requires no measurement, was the fastest to be calculated. There was a large difference between application times of scoring systems, and GSS was the fastest, with an average of 27.5 seconds followed by CROES with 213.4 seconds and STO-

NE with 300.6 seconds ($p < 0.0001$). With these findings, it has been demonstrated that the GSS could become the most practical nomogram to be used for predicting outcomes for PCNL. This new information could be useful for urologists who want to start using a nomogram but are unsure which one to choose. If all nomograms have similar ability to predict success, then choosing the quickest seems logical.

Our study is not without limitations. Being retrospective is a weakness, but as we evaluated basically CT scans and the clinical data was prospectively acquired, we believe this characteristic does not compromise the results. The number of patients is relatively small compared to other recent larger multicenter series, but the results related to predicting success were similar to the others studies and the analyzed cohort was large enough to identify statistically significant differences between the nomograms in regards to the acquisition time. In addition, we performed a standardized pre- and postoperative evaluation of all patients with CT scan, increasing the reliability of the outcome assessment. Only one observer did all the measures, what could cause some bias. However, this observer was previously trained by two experienced urologists and as we had a high concordance index among them, we believe that one evaluator would be adequate. All patients underwent surgery performed by the same experienced surgeon using the same technique, reducing the potential biases in PCNL outcomes. Finally, the success evaluation was very early and rigorous, but we believe this was the better moment to have adequate and standardized evaluation for all patients. Certainly, this early evaluation causes a relatively low success rate, however as our final success rate after all secondary procedures was 92.3% the difference among the groups created by the nomograms probably would not be significant. Considering this, we believed that the immediate success evaluation with CT scan would provide the best information for comparison.

In our study, we found that all three nomograms showed similar ability to predict success of PCNL but the GSS was quicker to use than the others. Maybe these nomograms can be automated, making them easier to use, but at present this is not available. The relative low AUC of the three no-

mograms calls attention for necessity of continuing development and improvement of these tools.

CONCLUSIONS

All three nomograms showed similar ability to predict success of PCNL, however the GSS was the quickest to be applied, what is an important issue for routine clinical use when counseling patients who are candidates to PCNL.

CONFLICT OF INTEREST

None declared.

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A neural network – based algorithm for predicting stone – free status after ESWL therapy

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ABSTRACT

Objective: The prototype artificial neural network (ANN) model was developed using data from patients with renal stone, in order to predict stone-free status and to help in planning treatment with Extracorporeal Shock Wave Lithotripsy (ESWL) for kidney stones.

Materials and Methods: Data were collected from the 203 patients including gender, single or multiple nature of the stone, location of the stone, infundibulopelvic angle primary or secondary nature of the stone, status of hydronephrosis, stone size after ESWL, age, size, skin to stone distance, stone density and creatinine, for eleven variables. Regression analysis and the ANN method were applied to predict treatment success using the same series of data.

Results: Subsequently, patients were divided into three groups by neural network software, in order to implement the ANN: training group (n=139), validation group (n=32), and the test group (n=32). ANN analysis demonstrated that the prediction accuracy of the stone-free rate was 99.25% in the training group, 85.48% in the validation group, and 88.70% in the test group.

Conclusions: Successful results were obtained to predict the stone-free rate, with the help of the ANN model designed by using a series of data collected from real patients in whom ESWL was implemented to help in planning treatment for kidney stones.

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INTRODUCTION

Kidney and ureteral stones are the third most commonly encountered pathologies in urological practice after urinary infections and diseases of the prostate. The incidence of stones has been reported as 5-10% and they are observed three-fold more frequently in men than in women. The risk is high between the ages of 30 and 50 years, and patients with stones have been reported to experience stone development more than once throughout their lifetime. Most of the stones formed are eliminated through urination. The time

period for the elimination process depends on the location and size of the stone. Spontaneous passage of ureteral stones smaller than 5mm is at a rate of about 80% (1). Extracorporeal Shock Wave Lithotripsy (SWL), ureterorenoscopy (URS), retrograde intrarenal surgery (RIRC), percutaneous nephrolithotomy (PNL), and open surgery are used to treat active stones.

Extracorporeal Shock Wave Lithotripsy (SWL): Despite the vital place of surgical treatment in the management of urinary system stones, surgical treatment has the disadvantage of reducing the patient's quality of life for a certain period of

time, increases the length of hospitalization, and has a high-cost rate, thus favoring SWL (2, 3). The principle of SWL, where shock waves are directed over the stones, was first initiated in Russia in the 1950s; studies on the modality started in 1974, and it was first experimented on humans in 1980. SWL has become the first option in the management of urinary stones particularly those smaller than 2cm, since there is no need for surgery and anesthesia (3). The success rate in stones smaller than 2cm is 70-80%. Many factors have been reported to affect success including anatomic factors and age of the patient; type, opacity, and size of the stone; its location in the collecting system; and the anatomy of the kidney collecting system. In this study, we developed an algorithm which predicts the stone-free status of the patients in order to select the better treatment method and to notify patients.

The aim of this study was to develop a prototype artificial neural network (ANN) model from data obtained from real patients.

MATERIALS AND METHODS

Patients who presented for SWL treatment at the Urology Department, between January 2013 and December 2015, were included in the study. Data were collected from 203 patients including gender, single or multiple nature of the stone, location of the stone, infundibulopelvic angle (IPA), history of SWL treatment, status of hydronephrosis, stone size after SWL, age, number of SWL session, size, skin to stone distance, stone density and creatinine, for a total of eleven variables. The first seven of these variables were categorical; the remaining six were numerical variables. Accordingly, sizes of the stones after SWL were determined as an output, and the size of the kidney stone was used to demonstrate the success rate of treatment.

Alyuda NeuroIntelligence Software randomized the training set, test set and validation set in the order of 100 (69.44%), 22 (15.28%) and 22 (15.28%) respectively.

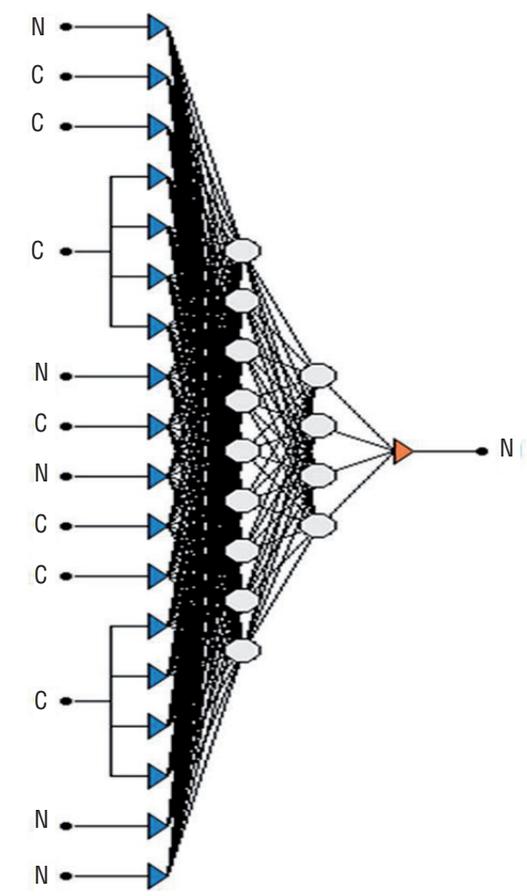
Artificial Neural Network

The Alyuda NeuroIntelligence 2.2 (Alyuda Research, Inc., Los Altos, California, USA) software

was used to create an artificial neural network (ANN). While creating the ANN, data were analyzed with regards to training, validity, and test. Data were analyzed according to numerical and categorical data, and also about what percentage of data was training, validity or test related. The second stage involved the transformation of all data to the numerical form for processing. In the third stage, the ANN structure was formed (Figure-1). A total of 16 neurone input values were introduced into the network structure; eight each for two intermediate neurone layers and one neurone was formed to express output. In this study, it was demonstrated that formation of two intermediate neurone layers was important to better understand the training set. The number of neurones was determined with trials. There are no particular rules in the literature expressing how to determine it. In the ANN model, all input and output values are determined according to logistic activation functions - values are transformed between the 0 and 1 interval. After determining the network structure, the training function is selected at the next training stage. This function would not normally prolong the duration of the procedure and the derivative should be an easily accessible function. Considering the features of our data, the Conjugate Gradient Descent function appeared to be the ideal training algorithm among the software programs used. Furthermore, this algorithm provided the best training means from the previous data. The classification Accuracy Ratio was considered a criterion for suspending the algorithm. This ratio was accepted as 95% for our training set. In order to prevent the duration of the training from being hindered by local minimal values, we designated the learning coefficient as 0.1 and the speed emission coefficient as 1.75. The program was designed to suspend the algorithm when the training data attained the required learning level. Weight values between the neuronal connections were constantly updated throughout the training period. An accurate prediction was said to be in place for a new case when these weights attained the most accurate value.

Regression Analysis

The same parameters used for the ANN procedure were implemented for the regression

Figure 1 - The architecture of the current algorithm.

analysis. The SPSS for Windows 22.0 (SPSS Inc, Chicago, IL) program was hence used. Significant values were calculated from coefficients obtained from the regression analysis. A value of 95% was considered significance level.

RESULTS

A total of 203 patients were included in our study (Table-1). The patients were aged between 1 to 77 years (mean: 33.87 ± 17.90 years). Of these patients, 121 (59.6%) were male and 82 (40.4%) were female. A hundred and thirty-eight patients (68%) were shown to have a single kidney stone, while 65 (32%) patients had multiple kidney stones. Localization of the stones in the patients was as follows: in 15 (7.4%) patients at the upper pole, in 132 (65%) patients at the middle

calyx group and pelvis, in 28 (13.8%) at the lower pole, and in 28 (13.8%) patients in multiple locations. SWL was performed on 11 (5.4%) patients in one session, 25 (12.3) patients in two sessions, 24 (11.8%) patients in three sessions, 45 (22.2%) patients in four sessions, 20 (9.9%) patients in five sessions, 76 (37.4%) patients in six sessions, and on two (1.0%) patients in seven sessions. The IPA was found to be $<45^\circ$ in 13 (6.4%) patients, and $>45^\circ$ in 190 (93%) patients. The sizes of the kidney stones varied from 10-489mm². They were between

Table 1 - Patient Characteristics.

Mean age, (range)	33.87±17.90 (1-77)
Gender, n (%)	
Male	121 (59.6%)
Female	82 (40.4)
Stone no, n (%)	
Single	138 (68%)
Multiple	65 (32%)
Stone localization	
1 Upper pole	15 (7.4%)
2 Middle calyx group and pelvis	132 (65%)
3 Lower pole	28 (13.8%)
4 Multiple locations	28 (13.8%)
Number of SWL Sessions	
1	11 (5.4%)
2	25 (12.3%)
3	24 (11.8%)
4	45 (22.2%)
5 and above	98 (48%)
Infundibulopelvic angle	
<45 degree	13 (6.4%)
>45 degree	190 (93.6%)
Mean Serum creatinine (range, mg/dL)	0.10-1.70
Pre-treatment stone size (range, mm ²)	10-489
Post-SWL stone size (range, mm ²)	0-100

0-100mm² following SWL. Two hundred (98.5%) patients were receiving SWL for the first time, whereas three (1.5%) patients were receiving it for the second time. Hydronephrosis was identified in 120 (59.1%) patients; Grade 1 hydronephrosis in 39 (19.2%) patients, Grade 2 hydronephrosis in 22 (10.8%) patients, and Grade 3 hydronephrosis in 18 (8.9%) patients. There is no information about the presence of hydronephrosis in the remaining patients (2%). The creatinine levels of the patients ranged between 0.1-1.7mg/dL.

Significant correlations were found between stone size, number of SWL session, stone location, infundibulo-pelvic angle, skin to stone distance and stone-free rate in the regression analysis (Table-2).

Patients were randomly divided into three groups in order to implement the ANN: the training group (n=139), the validation group (n=32), and the test group (n=32).

The ANN analysis demonstrated the prediction accuracy of the stone-free rate as 99.25% in the training group, 85.48% in the validation group, and as 88.70% in the test group.

DISCUSSION

Decision support systems (DSS), such as the ANN, can be used in the medical field, as computer generated algorithms, which help health care officials in clinical decision making. The basis of algorithms written in various forms is to mimic the learning style of the brain. These algorithms assist medical officials in decision making using specific clinical information of patients. As it is with other algorithms, real patient data should be registered in the computer. Data organization with the group of information at hand is useful, and with the help of various functions the computer is taught how to use data to make certain analysis. With the help of functions used on ANN, the computer is programed to predict certain parameters in a clever form, using training sets within the time frame. At the end of the training, the performance of the trained computer, which uses these real data is evaluated. The extent to which the computer has learned is evaluated, with regards to validity and test data. If the computer is concluded to have

Table 2 - Coefficients obtained from regression analysis.

	Standardized Coefficients	Significance
Age	-0.044	0.663
Sex	-0.107	0.291
Single/Multiple	0.268	0.007
Location	0.221	0.027
Number of sessions	0.245	0.014
IP angle	0.207	0.039
Stone size	0.394	0.001
Primary/Secondary	-0.149	0.140
Hydronephrosis	-0.019	0.848
Creatinine	0.088	0.382
Stone Density	0.056	0.578
Skin to stone distance	-0.200	0.046

learned enough to predict users, the program is deemed useful.

The ANN may be very beneficial in improving the quality of health services, help in the early diagnosis of diseases, prevent medical faults, and help health officials provide appropriate treatment to patients and to reduce costs. The decision making process is terminated by the selection of one of the alternative results from cognitive processes. The cognitive process is said to increase and the possibility of making mistakes also increases with an increase in the number of alternatives. At the end of the decision, an action or an idea is formed. An investigative subject is produced in different forms to demonstrate how individuals make decisions. The interaction of psychological factors and cognitive activities with the environment should be analyzed during the decision making process. A person's mind is expected to provide certain suggestions through logical filtering, and then make the right decision by itself. Despite much research, it is not yet well understood how the human brain functions in decision making. Solid and reliable information is needed by the brain to carry out the decision making function. All alternatives should be considered together in order to make the right decision. Since knowledge is a value against time, it

is necessary to provide the decision maker with the data in the shortest possible time, in order to make effective and fast decisions. As a result, many administrative and specialist organization currently require the ANN for effective and speedy decision-making.

Recently, the ANN has gained effective usage in urological practice. Most of these studies have been on diseases of the prostate, particularly on carcinoma, with acceptably successful results (4).

SWL, which has long been used in the management of urinary system stones, is especially effective in the treatment of small sized stones. However, SWL may produce poor results in a certain group of patients. From the patient's perspective, this accounts to time wasted, loss of kidney function, additional costs, and stress. As a result, it is important to know beforehand if certain treatment procedures would be successful in certain diseases. Clinicians actually use certain parameters to predict the success of treatment through discussion with their patients, and depending on their clinical experience. Studies that have been conducted to predict the success of SWL have identified certain effective parameters. However, the desired results have not been attained from these studies conducted using classical statistical methods. For example, Gomha et al. (5) used a logistic regression model to investigate stone-free status and demonstrated some significance only in the location of stones and the presence of a stent.

In the pilot study conducted by Hamid et al. (6) on 82 patients to predict the optimal fragmentation of stone on SWL, it was demonstrated that the prediction complete fragmentation was possible in a rate of approximately 75%, but reported that more advances studies are required for better results.

The current study predicted the rate of stone elimination by developing an algorithm aimed at directing the course of effective treatment, making sure that the patient received the right treatment method and providing the patient with the required information. Unlike in previous studies, our study included a larger sample

size and used ANN to attain a higher stone-free rate, proving that it was possible to predict with high accuracy (99%). By so doing, data collected before treatment and registered into the system were used with an already prepared algorithm to predict the success rate of treatment. As a result, treatment modalities that were predicted as unsuccessful were discontinued in order to save cost and time, and to examine other possible treatment measures.

CONFLICT OF INTEREST

None declared.

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Efficacy and safety of Elevate[®] system on apical and anterior compartment prolapse repair with personal technique modification

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ABSTRACT

Aim: To evaluate the effectiveness and safety of Anterior Elevate[®] mesh kit system (AES) in woman with symptomatic stage 3 or 4 anterior and/or apical pelvic organ prolapse (POP).

Materials and Methods: This retrospective, monocentric, single surgeon study enrolled between May 2010 and January 2013 fifty-six woman experiencing symptomatic anterior vaginal prolapse with or without apical descent (POP-Q stage 3 or 4). All women received a AES and 7 (12.5%) received a concomitant transvaginal hysterectomy. Primary endpoint was anatomic correction of prolapse; success was defined as POP-Q stage ≤ 1 or asymptomatic stage 2. Secondary endpoints were quality-of-life (QOL) results and patients' safety outcomes, which were assessed by 3 validated self-reporting questionnaires at baseline and annually: ICIQ-UI short form, ICIQ-VS and P-QOL. All patients completed 2-years and 28 women 3-years of follow-up. Surgical approach was modified in women with uterus, moving the two-propylene strips anteriorly around the cervix itself crossing one another, so the left will take place in the right side and the right on the opposite. This modification was made in order to better support the uterus. **Results:** Vaginal mesh exposure was present in 3 (5,3%) patients. Very good anatomical outcomes were seen, with one (1,8%) failure at 6-months, 4 (7,1%) at 1-year, 6 at 2-years (10,7%). Statistically significant improvements were seen in the ICIQ-VS and P-QOL questionnaires throughout follow-up.

Conclusion: Our data suggest that AES is a minimally-invasive transvaginal procedure to repair anterior and apical POP, with good evidence related to mid-term safety and efficacy.

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INTRODUCTION

Pelvic organ prolapse (POP) occurs when there is a disruption of the natural supporting structures of the pelvic organs, often with impaired function of the pelvic floor musculature. The loss of these normal attachments and the dynamic support of the pelvic floor result in the descent of one or more pelvic structures including the blad-

der, the rectum, the uterus and cervix, or the vaginal cuff and the small bowel in case of previous hysterectomy. Even if there is a lack of epidemiological studies of the natural history, incidence and prevalence of POP, it is widely accepted that 50% of women will develop prolapse, but only 10 to 20% of those seek evaluation for their condition (1). POP has become a major health concern, as it may affect 50% of women over age 50 (2) and

the lifetime risk of needing surgery for prolapse or urinary incontinence by 80 years of age has been reported as high as 19% (3). Many women with clinically evident POP on physical examination may be asymptomatic. When symptomatic, they complain of bothersome symptoms that can be divided in vaginal urinary, defecatory and sexual. Treatment for POP is based upon symptom bother, patient expectation, and quality of life impact. The direct costs of POP in the United States has been estimated at U.S. \$1billion, with similar costs expected throughout the developed countries (4). Given the increasing time and resources that will be required for POP surgery in the future, it is paramount to perform effective, durable, cost-effective interventions with minimal morbidity. The failure of traditional repairs has led to the use of graft materials, particularly synthetic mesh, to augment prolapse repair in an attempt to improve success and durability. In the last decade, several mesh kits have been developed and commercialized to repair POP through a vaginal approach (5). All these procedures have gained popularity, because of their minimally-invasive approach and low morbidity rate. Despite that, the Food and Drug Administration issued a Public Health Notification in October 2008 to inform physicians and patients of adverse events related to vaginal reconstructive surgical use of synthetic mesh and to provide recommendations on how to mitigate risks and counsel patients appropriately (6). A wide spectrum of potential complications exists with the use of transvaginal mesh in POP surgery, even severe complications, such as fistula formation, mesh erosion into adjacent organs, and death. In 2010, after a 15-years of experience on transvaginal POP and stress urinary incontinence (SUI) mesh surgery, we started using the Elevate® Anterior and Apical prolapse system (AES) kit (American Medical Systems, Minnetonka, MN) to repair anterior and apical compartment prolapse. In women with uterus we decide to make a change to the standard technique, moving the two-propylene strips anteriorly around the cervix itself crossing one another. This approach could give a better support to the uterus left in place. The aim of the study presented in this paper was to evaluate the effectiveness and safety of AES in

women with symptomatic stage 3 or 4 anterior and/or apical POP.

MATERIALS AND METHODS

This is a retrospective medium-term study on safety and efficacy of AES in apical and anterior compartment prolapse correction. The study had been reviewed and approved by a certified Ethical Board. Inclusion criteria were symptomatic primary or recurrent anterior and/or apical compartment prolapse stage 3 or greater, according to pelvic organ prolapse quantitative (POP-Q) system (7). Exclusion criteria were known hypersensitivity to synthetic materials, pelvic cancer or chemotherapy 1-year before enrollment, previous pelvic irradiation, previous mesh surgery, restricted leg motion, uncontrolled diabetes, immune suppression or use of immune modulators. All patients signed an informed consent form and the change in the licensed mesh technique have been entailed a specific consent form. All women were evaluated with medical history, clinical examination, cough test, 24 hour-pad test, smear test, urodynamics and multiple self-reported validated questionnaires. POP was staged by senior surgeon (C.V.) using the POP-Q system (7) and occult SUI was evaluated using a pessary placement (8) during urodynamics evaluation. Urodynamics were performed in accordance with International Continence Society (ICS) recommendation (8). Subjective and quality-of-life (QOL) outcomes were assessed by three validated self-reporting questionnaires at baseline and annually. Urinary incontinence was evaluated using the International Consultation on Incontinence questionnaire on urinary incontinence (ICIQ-UI) short form (9), vaginal and sexual symptoms using International Consultation on Incontinence questionnaire on vaginal symptoms (ICIQ-VS) (10) and prolapse-quality of life questionnaire (P-QOL) (11). All data were routinely collected for all patients. The primary outcome of this study was anatomic correction of prolapse; success was defined as POP-Q less than or equal to stage 1 or asymptomatic stage 2. Secondary outcomes were QOL results and patient safety outcomes. Complications were reported according to Clavien and Dindo Classification of surgical complications (12)

and to The International Urogynecological Association (IUGA)/ICS joint terminology and classification of the complications related directly to the insertion of prostheses and grafts in female pelvic floor surgery (13). Between May 2010 and January 2013 fifty-seven women met the criteria and were enrolled in the study, held at Urology Unit, University of L'Aquila, Teramo Hospital, Italy. One patient was lost at follow-up. Fifty-six patients were available for analysis. Slings were not performed at the same time in any patient with SUI, because we prefer a staged mesh surgery. Mid-urethral slings were offered subsequently only in patients who needed. Statistical analysis was performed using the Student t-test. Qualitative data are shown as mean \pm SD. Statistically significantly difference is considered as $p < 0.05$.

Surgical Technique

AES kit contains a shaped mesh with two-self anchoring tips and two-propylene strips with a self-fixating tip at their top. Distally the mesh has to be fixed in the obturator foramen using the attached self-fixating tips. Proximally two strips have to be attached to the sacrospinous ligament using the needle present in the kit, which are assembled with the mesh. All patients received antibiotics prophylaxis with ceftriaxone 1g intravenously 30 minutes prior surgery. All procedures were performed by single experienced pelvic surgeon (C.V.), in a dorsal lithotomic position and under spinal anesthesia. Surgery is different in woman with uterus respect of those with post-hysterectomy vault prolapse. In latter group the AES is placed in the standard fashion, according to the instructions for use. Conversely in patients with uterus in place, surgery begins with an incision at level of posterior vaginal wall, followed by a sharp and blunt dissection bilaterally towards the sacrospinous ligament, where the combined elements are placed in the standard fashion. After that, a second incision and dissection are performed in the anterior vaginal wall. After a blunt dissection of the cervix, the two-propylene strips are moved anteriorly around the cervix itself crossing one another, so the left will take place in the right side and the right on the opposite. This personal

technique modification can be watched in a video clip available online in the journal website. At this point, surgery is equal in both groups; the distal part of the mesh is anchored using the needle, which drives the self-fixating tips to the obturator internal muscle. The mesh is distally fixed with two tension-free vicryl 2/0 sutures at the level of the bladder neck and proximally to the uterosacral ligaments or their residual part in patients without uterus. Finally, both propylene strips are inserted to the open eyelets and adjusted in a tension-free manner using the locking eyelets. Cystoscopy is performed to rule out any bladder injury. Surgery ends with closure of vaginal wall incisions with a double vicryl suture, 2/0 internally and 0/0 externally, positioning a 16-Fr indwelling catheter and vaginal packing. In case of stage 2 posterior compartment prolapse a simple colpoperineoplasty is performed.

RESULTS

Fifty-seven women underwent transvaginal anterior POP repair using a polypropylene mesh AES between May 2010 and January 2013. One patient has been lost at follow-up. Statistical analysis was performed in 56. Patient's characteristics and demographics at baseline are shown in Table-1. Mean operative time was 47.3 (± 8) minutes. Twelve patients had previous surgery for anterior prolapse and 33 had a previous hysterectomy. Seven women underwent a concomitant hysterectomy for large uterine volume due to fibromatosis. No bladder injury was seen. Urethral catheter and vaginal packing were removed in the first post-operative day. Only one patient went to retention, most probably because she had a voluminous bladder diverticula; she was managed with self-intermittent catheterization and regained spontaneous micturition on day 5. Ultrasound post-voiding urine residual (PVUR) was performed in each patient twice daily; significant PVUR (≥ 100 mL) was not present in the remaining patients. All patients were discharged on post-operative day 2. No major bleeding was observed: mean drop in post-operative hemoglobin was 1.7 ± 0.6 g/dL. Post-operative complications, according to the Clavien-Dindo classification (12), in the first

Table 1 - Baseline demographics and characteristics.

	Number	%
Age, mean \pm SD	69 \pm 5.3	
BMI, mean \pm SD	26.5 \pm 4.5	
Vaginal deliveries, Mean \pm SD	2.6 \pm 1.4	
Menopausal status	56	100
Prior hysterectomy	33	58.9
Prior prolapse surgery	12	21.4
SUI before surgery	21	37.5
Urge incontinence	7	12.5
Sexually active	40	71.4
Dyspareunia	13	23.2
Sexually inactive	16	28.6
Stage 3 anterior prolapse	22	39.2
Stage 4 anterior prolapse	19	33.9
Apical prolapse		
Vault	23	41
Uterine	10	17.8
Enterocoele	5	8.9
Concomitant hysterectomy	7	12.5

month were grade I (intravenous analgesics and anti-emetics) in ten cases, and grade III-a (vaginal infected hematoma with wound dehiscence, requiring drainage in local anesthesia) in one. This complication can be classified as 3CbT2S1 according to IUGA/ICS terminology and classification of the complications related directly to the insertion of prostheses and grafts in female pelvic floor surgery (13). Transient buttock pain was reported in 4 (7.1%) of the patients in the first week post-operatively and it disappeared spontaneously. All patients were examined 6-weeks after surgery, 6 months and then annually with urine culture and pelvic examination according to POP-Q. ICIQ-UI-short form, ICIQ-VS and P-QOL questionnaires were self-administered annually. Post-operative results are shown in Table-2. All patients completed a 2-years and 28 3-years of follow-up. Anatomical results were excellent because failure (defined as symptomatic POP stage 2 or stage \geq 3) was seen in 1 (1.8%) woman at 6 months, in 4 (7.1%) at 1-year and in 6 (10.7%) at 2-years. At 3-years

of follow-up only 3 patients out of 28 (10.7%) had POP \geq 3 stage (data not showed in Table-2). Vaginal mesh exposure, defined as grade III-b (12) or 3BT3S1 (13) complication, was seen in 3 patients (5.3%) during the first year of follow-up. Questionnaires outcomes showed statistical significant improvement of symptoms and QOL domain except for incontinence (Table-3).

DISCUSSION

Traditional anterior colporrhaphy for repair of anterior prolapse has an estimate risk of recurrence between 30-50% (14, 15). Randomized controlled trials and recent meta-analysis showed superior anatomical outcomes in mesh repair compared to anterior colporrhaphy (14, 16, 17). Nowadays more than 40 implants are available on the market (18) even with little evidence on their safety and efficacy related to mid- and long-term. Indeed, the FDA warned in 2011 regarding serious complications associated with transvaginal placement of surgical mesh and reinforced the basis that surgeons should perform prolapse repair only if they are adequately subspecialized in this area (19). The AES is a relative new kit composed of a type I polypropylene mesh with bilateral anterior and posterior graft arms for anchoring them to the obturator foramen and sacro-spinous ligament, respectively. This kit has two major advantages; first its fixation is easy to perform via self-fixating tips, avoiding blind trocar passage through the obturator and perirectal fossa seen with alternative mesh kit techniques. Secondly it seems to fit perfectly anterior and apical prolapse surgical repair, because it is well known that most anterior-compartment prolapse is associated with apical prolapse (20). Crossing of the strips of the mesh anteriorly to the cervix is different from the standard technique. We made this change because we believe that it could better support uterus. Our data show results of the AES for the repair of anterior and apical vaginal wall prolapse with a minimum of 2-years of follow-up in 56 patients and 3-years in 28. It is possible, hence, to find several important findings; first of all, the AES appears to be a safe and minimally invasive procedure with very low incidence of associated adverse events.

Table 2 - Anatomical POP-Q results and complications at follow-up.

	6 weeks	6 months	1 year	2 years
	n (%)	n (%)	n (%)	n (%)
Stage 1 POP	3 (5.3)	6 (10.7)	9 (16)	12 (21.4)
Stage 2 POP	1 (1.8)	5 (8.9)	5 (8.9)	6 (10.7)
symptomatic	0 (0)	1 (1.8)	1 (1.8)	2 (3.5)
Stage 3 POP	0 (0)	0 (0)	2 (3.5)	3 (5.3)
Stage 4 POP	0 (0)	0 (0)	1 (1.8)	1 (1.8)
<i>De novo</i> SUI	5 (3)	4 (7.1)	5 (8.9)	5 (8.9)
Persistent SUI	10 (17.8)	10 (17.8)	10 (17.8)	11 (19.6)
<i>De novo</i> urgency	2 (3.5)	3 (5.3)	8 (14.3)	6 (10.7)
Urge incontinence	0 (0)	1 (1.8)	8 (14.3)	6 (10.7)
Persistent dyspareunia	2 (3.5)	5 (8.9)	4 (7.1)	5 (8.9)
<i>De novo</i> dyspareunia	3 (5.3)	3 (5.3)	5 (8.9)	5 (8.9)
Vaginal mesh exposure	0 (0)	1 (1.8)	2 (3.5)	0 (0)
Positive Urine Culture	8 (14,3)	10 (17,8)	7 (12,5)	9 (16)

Table 3 - Subjective ICIQ-UI short form, ICIQ-VS and P-QOL outcomes at baseline and after AES implant.

	Preop	1 year	2 years	3 years
	Mean ± SD (range)	Mean ± SD (range)	Mean ± SD (range)	Mean ± SD (range)
ICIQ-UI-short form (max=21)	10.2±2.1 (0-16)	6.3±1.8 (2-12) p>0.11	6.1±1.5 (3-11) p>0.1	6.5±1.8 (3-12) p>0.18
ICIQ-VS (max)				
Vaginal symptoms (53)	18.5±8.6 (9-37)	3.2±3.1 (0-20) p<0.011	4.2±5.9 (0-28) p<0.13	4.4±5.9 (0-21) p<0.14
Sexual matters (58)	18.9±16.5 (0-42)	2.5±7.4 (0-25) p<0.01	6±12.5 (0-26) p<0.02	5±11.8 (0-36) p<0.02
QOL (10)	4.7±3.9 (0-10)	0.8±1.4 (0-6) p<0.01	0.5±1.5 (0-7) p<0.01	0.7±1.5 (0-7) p<0.01
P-QOL (max)				
General health perception(100)	53±9.5 (50-75)	26 ± 3.2 (12-44)p<0.023	24±2.5 (11-35) p<0.022	23±3.4 (12-37) p<0.021
Prolapse impact (100)	93±3.2 (67-100)	5±2.3 (0-12) p<0.001	10±3.2 (0-17) p<0.004	12±2.5 (0-18) p<0.004
Role limitations (100)	61±7.4 (51-85)	2±2.3 (0-7) p<0.001	3±3.5 (1-5) p<0.001	2±3.2 (1-5) p<0.001
Physical limitations (100)	62±7.8 (33-83)	2±1.8 (0-17) p<0.001	3±2.4 (0-19) p<0.001	3±3.1 (0-20) p<0.001
Social limitations (100)	58±6.5 (22-56)	2±0.8 (1-11) p<0.001	3±1.2 (1-12) p<0.001	3±1.7 (1-12) p<0.001
Personal relationship (100)	67±12.1 (38-100)	1±0.5 (1-3) p<0.001	2±0.8 (1-4) p<0.001	2±0.7 (1-4) p<0.001
Emotions (100)	55±5.6 (45-89)	1±0.8 (1-3) p<0.001	2±1.2 (1-4) p<0.001	2±1.6 (1-4) p<0.001
Sleep/energy (100)	25± 5.7(17-41)	2±0.8 (1-5) p<0.001	3±0.9 (1-5) p<0.001	3±1 (1-5) p<0.001
Severity measures (100)	42±7.5 (34-65)	4± 4.2 (1-14) p<0.002	6±3.8 (2-15) p<0.003	6±4.1 (2-16) p<0.003

Preop = preoperative. Not statistically significant vs preoperative (p>0.05) Statistically significant vs preoperative.

There was no bladder and rectal injuries. Only 3 vaginal mesh exposure were identified and were treated surgically with excision of the exposed vaginal area. Transient buttock pain was reported in 4 (7.1%) of the patients in the first week post-operatively and it disappeared spontaneously. The mechanism of this transient pain is likely due to local entrapment of pudendal branches such as the perforating cutaneous nerve (21). Secondly, our mean operative times is shorter (47.3 min) than reports of abdominal (221–225 min) and robotic (226–328 min) sacrocolpopexy (22, 23). Third, our series demonstrate very good anatomical outcome, with one (1.8%) failure at 6-months, 4 (7.1%) at 1-year, 6 at 2-years (10.7%). At 3-years follow-up only 3 patients out of 28 (10.7%) were POP ≥ 3 stage. Our 1-year anatomic results were similar to other transvaginal mesh procedure; Vaiyapuri (24) reported in his series of Prolift® a cure rate of 92.1%, Jacquelin (25) 81.6% of success rate in TVM technique. The anatomic result remained stable for the next two years (89.2% at 2-year of follow-up and 87.4% at 3-year of follow-up). Our results are consistent with other AES series recently published (26–27). Both Rapp (26) and Huang (27) have 90% of anatomical success rate at 2-years follow-up. Anatomic failure is present in our series at one-year follow-up and it remains almost the same during the next two years; no patients required a second surgery so far. Last but not least, the current series demonstrates excellent subjective outcomes; ICIQ-VS and P-QOL questionnaires demonstrated statistically significant improvements not only in vaginal and sexual symptoms, but also in QOL at each follow-up visit. Regarding preoperative SUI we prefer, as previously mentioned, a staged surgery in these patients, because restoring pelvic organ support has cured SUI in 10 women out of 21 who had preoperative SUI as showed in Table-2 (11 patients with persistent SUI after 2-years of follow-up). We performed a secondary sling procedure only in patients asking for it (8 women) after at least one year of follow-up. Our results showed that AES is a minimally-invasive transvaginal procedure to repair anterior and apical POP, with good evidence related to mid-term safety

and efficacy. Further studies are indeed needed to confirm the long-term results.

ABBREVIATIONS

POP = Pelvic organ prolapse

AES = Elevate® Anterior and Apical prolapse system

SUI = stress urinary incontinence

POP-Q = pelvic organ prolapse quantitative

ICS = International Continence Society

QOL = quality-of-life

ICIQ-UI = International Consultation on Incontinence questionnaire on urinary incontinence

ICIQ-VS = International Consultation on Incontinence questionnaire on vaginal symptoms

P-QOL = prolapse-quality of life questionnaire

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CONFLICT OF INTEREST

None declared.

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Abobotulinum – a toxin injection in patients with refractory idiopathic detrusor overactivity: injections in detrusor, trigone and bladder neck or prostatic urethra, versus detrusor – only injections

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ABSTRACT

Purpose: To evaluate if the injections of abobotulinum-A toxin in trigone and bladder neck/prostatic urethra in addition to detrusor provides better symptoms relief and urodynamic findings in patients with idiopathic detrusor overactivity (IDO) refractory to medical treatment.

Materials and Methods: A total of 74 patients with IDO refractory to anticholinergics received injections in detrusor, trigone and bladder neck/prostatic urethra (Group A, N=36) versus detrusor only injections (Group B, N=38) of abobotulinum-A toxin. All patients were evaluated by a standard overactive bladder symptom score (OABSS) questionnaire and cystometrography before and 6 weeks after the operation. OABSS questionnaire was also completed 20 weeks after the operation.

Results: The magnitude of OABSS reduction from baseline to 6 weeks after operation in groups A and B patients was 13.4 ± 2.2 versus 11.7 ± 2.1 ($p=0.001$). Cystometry results were similar in both groups except for higher volume at urgent desire to void in Group B patients ($p < 0.001$). The mean \pm SD change in residual volume in Group A at 6 weeks after the operation was -4.8 ± 28.6 mL ($p=0.33$) compared to 21.3 ± 16.9 mL in Group B patients ($p < 0.001$).

Conclusions: In patients with IDO, adding trigone, and bladder neck/prostatic urethra as sites of abobotulinum- A toxin injection produces greater reductions in OABSS score and less residual urine volume but a lower volume at urgent desire to void in comparison with detrusor only injections.

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INTRODUCTION

Overactive bladder is defined as lower urinary tract symptoms including urgency with or without urge incontinence, sometimes accompanied by nocturia and frequency (1). It affects about 17% of adult European population (2). In patients with idiopathic detrusor

overactivity refractory to anticholinergic therapy, intravesical injection of botulinum toxin has emerged as a second line minimally invasive treatment (3, 4).

Botulinum toxin A (BoNTA) is a potent neurotoxin produced by *Clostridium botulinum* (5). The two commonly used products in urology are Botox (Onabotulinum toxin A) and

Dysport (Abobotulinum toxin A). BoNTA is an effective treatment in patients with idiopathic overactive bladder.

Currently there is no consensus about the exact dose or sites for injection of this toxin (6). The trigone had been spared of BoNTA injection fearing the theoretical risk of vesicoureteral reflux. However, Karsenty et al. and Mascarenhas et al. reported that trigonal injection of BoNTA will not induce reflux in patients with overactive bladder (7, 8). The first prospective randomized controlled trial by Abdel-Meguid displayed the superiority of trigonal BoNTA injection in neurogenic bladder patients over trigonal sparing injections (9). A randomized clinical trial by Manchesha et al. also pointed to the superiority of trigone including injections for IDO patients, but this study included few patients.

A recent meta-analysis on six studies by Davis et al. revealed that there is no significant difference between trigonal and extra-trigonal BoNTA injections in terms of adverse effects and short term efficacy and that more trials are needed to define the optimal injection techniques and sites for delivery of intravesical BoNTA (6).

The primary objective of this study was to evaluate the efficacy of BoNTA injections in detrusor, trigone, and bladder neck/prostatic urethra in comparison with detrusor only injections. Patient satisfaction and urodynamic findings in follow-up were used to assess the efficacy of injections. We introduced new sites of injection around bladder neck in women and prostatic urethra in men and evaluated them in distinction to the current literature.

MATERIALS AND METHODS

Studied population

This prospective study was performed between April 2012 and July 2015. Patients older than 18 years old with IDO proven by urodynamic study, refractory to anticholinergic therapy (for at least 3 months) were recruited. Neurology consult was requested in young patients and subjects with atypical clinical presentation or severe contractions on cystometrogram. Patients were excluded in case of coagulopathy or neurological

detrusor overactivity. Patients were also excluded if they had history of previous BoNTA injection or surgery of the genitourinary tract. Urinalysis and culture were performed to rule out patients with urinary tract infection prior to surgery. Male patients with clinical or urodynamic evidence of bladder outlet obstruction were excluded from the study.

All patients underwent voiding cystourethrography (VCUG) before surgery and reflux was not detected in any of them. Anticholinergic therapy was discontinued in all patients 7 days prior to injection.

Baseline Assessment

All patients were assessed at baseline by history, physical examination, the Overactive Bladder Symptom Score (OABSS) questionnaire, and urodynamic examination. Symptoms were evaluated according to the validated seven questions of OABSS questionnaire (score range: 0-28) (10). Studied urodynamic parameters consisted of maximum cystometric capacity (MCC), maximum detrusor pressure in filling phase (MDP), volume at first desire to void (VFDV), and volume at urgent desire to void (VUDV). Post void residue (PVR) was also assessed by abdominal ultrasonography.

Injection technique

Patients received deep IV sedation, and then spinal anesthesia. We have performed the last 8 injections under sedation. 200mg IV ciprofloxacin was given to all patients 1/2 hour before the operation. All injections were performed by an expert female urologist. We used 500U BoNTA and 20 injection sites in each patient. Botulinum toxin A (Dysport) (500U) was reconstituted with 20cc saline 0.9%. Cystoscopy was performed with 21Fr rigid cystoscope in lithotomy position and after filling bladder with 150mL of irrigation fluid, intradetrusor injections were performed by a 27G disposable needle. Choice of injection into trigone and bladder neck/prostatic urethra versus detrusor only injections was made at the discretion of the operating surgeon. Generally, patients with storage predominant symptoms were more likely to be included in the detrusor only group (Group B) and patients with emptying predominant symptoms

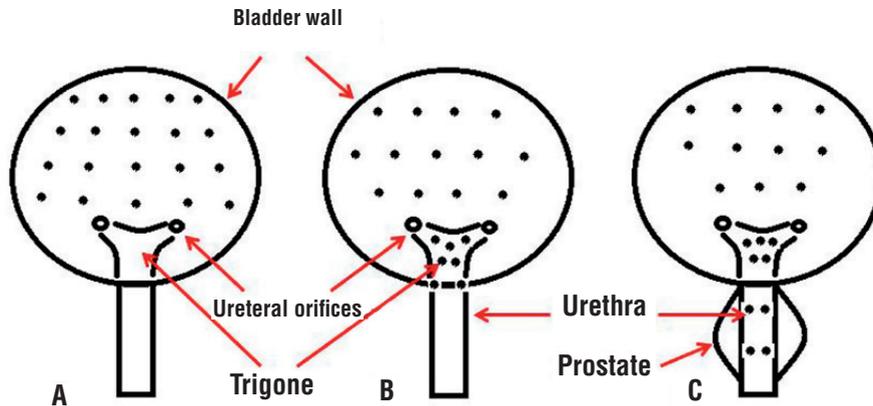
were more likely to be included in the detrusor, trigone, and bladder neck/prostatic urethra group (Group A). Group A patients received 2 injections in the bladder neck in female patients and 4 injections in male patients in proximal and distal prostatic urethra as illustrated in Figure-1, the remaining sites included 11 or 13 injections into detrusor and 5 trigonal injections away from ureteral orifices. Patients with detrusor only injections (Group B) received 20 injections of the same preparation at different parts of detrusor excluding trigone (Figure-1). The depth of injection in detrusor, trigone and bladder neck was about 2mm as estimated by insertion of half of the 4-mm needle. Bladder neck injections in female patients were performed at 5 and 7 o'clock positions (Figure-1). In male patients,

tinue anticholinergic medications with half of the original pre-treatment dose. Patients were re-evaluated at 6 and 20 weeks after treatment by history, physical examination, and OABSS questionnaire. Urodynamic study and PVR were reevaluated at 6 weeks after injections.

Statistical analysis

Student t-test was used to compare quantitative variables at baseline and also for comparing the magnitude of difference from baseline to follow-up visits between treatment groups. Fisher exact or chi-square tests were used to compare nominal data at baseline. Paired t-tests were used to compare follow-up data with their baseline values.

Figure 1 - Schematic diagram for sites of botulinum toxin A injection: A) Group B patients, male or female (20 detrusor only injections); B) Group A patients, female (13 injections into detrusor, 5 trigone injection and 2 bladder neck injections at 5 and 7 o'clock positions); C) Group A patients, male (11 detrusor injection, 5 trigone injections, and 4 injections in the proximal and distal prostatic urethra at 3 and 9 o'clock positions).



prostatic urethra injections were performed in the proximal and distal prostatic urethra at 3 and 9 o'clock positions (Figure-1) with the injection needle fully inserted into the prostatic tissue (4mm depth of injection).

Follow-up

Foley catheter was discontinued the day after surgery. Urinalysis and urine culture were requested two weeks after treatment and in this clinic visit PVR was assessed by urethral catheter. If PVR was less than 100mL, patients were allowed to con-

The ethics of this study was approved by the Hasheminejad Kidney Center ethics committee and are in accordance with the Helsinki declaration of 1964 and its later amendments. Patients were explained about the study objectives in their own language. Written informed consent was obtained from all patients.

RESULTS

A total of 74 patients (38 males and 36 females) aged 21 to 59 years (mean±SD: 39.1±14.0)

were studied. Thirty-six patients were included in Group A (18 females and 18 males) and 38 in Group B (18 females and 20 males). The mean±SD of patients' age in Groups A and B was 39.1±14.4 and 39.0±14.0. Table-1 summarizes OABSS scores at baseline and at 6 and 20 weeks after injections.

The magnitudes of reduction in OABSS scores from baseline to 6 weeks after injections in Groups A versus B were 13.4±2.2 versus 11.7±2.1 (p=0.001). The magnitudes of reduction in OABSS score from baseline to 20 weeks after injections in

achieve statistically significant difference between the treatment groups (Table-2).

8 patients in Group A and 10 patients in Group B needed a reinjection of BoNTA injection after follow-up at 20 weeks (p=0.77).

DISCUSSION

The efficacy of intravesical BoNTA as an alternative therapy for bladder overactivity refractory to anticholinergics has been proven (11,

Table 1 - Overactive Bladder Symptom Scores (OABSS) at baseline and at 6 & 20 weeks after Abobotulinum toxin injections.

	Group A*	Group B**	P-value
Number of patients	36	38	
Age, years; mean±SD	39.1±14.4	39.0±14.0	0.99
Total OABSS			
Baseline, mean (range)	22.3 (17-28)	21.8 (18-26)	0.41
6 weeks, mean (range)	8.9 (6-14)	10.1 (8-14)	0.006
Compared to baseline (P value)	<0.001	<0.001	
20 weeks, mean (range)	10.9 (8-17)	13.4 (9-17)	<0.001
Compared to baseline (P value)	<0.001	<0.001	

*Detrusor, trigone, bladder neck/prostatic urethra including injections

**Detrusor only injections

OABSS = Overactive Bladder Symptom Score

Groups A versus B were 11.4±2.2 versus 8.3±2.2. (p<0.001) The magnitude of reduction in OABSS score in both treatment groups was not related to gender or age of patients. Regarding PVR, no statistically significant change in PVR was observed in Group A patients relative to baseline while statistically significant increase in PVR was observed in Group B patients. The change in PVR was dependent on gender of patients. The mean±SD of PVR increase in male patients was 0.1±31.4mL versus 17.6±16.6mL for female patients. (p=0.004) This difference remained statistically significant after controlling for treatment Groups of A and B.

One patient developed urinary retention in each group and responded to clean intermittent catheterization.

Regarding urodynamic parameters, apart from VUDV other urodynamic parameters did not

12). Denys et al. in a double blind, dose rating, placebo-controlled study with 6 month follow-up observed >50% improvement in UII and urgency in 65% and 56% of patients who had received 100U and 150U of BoNTA (13). Kuo did not detect any difference in outcome relative to the injecting dose (14). In a randomized clinical trial, Manecksha et al. reported on the superiority of detrusor+trigonal injections of BoNTA in terms of reduction in OABSS score in comparison with detrusor only injections (15).

A problem with injections of BoNTA was the increase in residual urine volume which can potentially predispose to UTI (14, 16, 17). Some studies reported that patients with idiopathic detrusor overactivity (IDO) contract their bladder neck to prevent urinary incontinence. So that, during voiding funneling of bladder neck is incom-

Table 2 - Cystometric parameters at baseline and at 6 weeks after Abobotulinum toxin injections.

	Group A*	Group B**	P-Value
MCC, mL; Mean (range)			
Before injection	224.44 (160-400)	284.59 (160-520)	0.61
6 week after injection	385.69 (280-490)	409.82 (290-530)	0.11
P-value (6 week to baseline)***	0.08	0.07	
MDP, cm H₂O; Mean (range)			
Before injection	39.9 (14-63)	40.56 (17-73)	0.86
6 week after injection	14.75 (6-29)	16.3 (7-30)	0.53
P-value (6 week to baseline)***	0.8	0.9	
VUDV, mL; Mean (range)			
Before injection	211.45 (110-360)	205.68 (134-310)	0.73
6 week after injection	334.13 (200-456)	363.93 (254-476)	0.02
P-value (6 week to baseline)***	<0.001	<0.001	
VFUV, mL; Mean (range)			
Before injection	175.59 (100-330)	164.21 (110-290)	0.49
6 week after injection	288.06 (200-450)	298.24(200-404)	0.32
P-value (6 week to baseline)***	0.1	0.08	
PVR, mL; Mean (range)			
Before injection	64.6 (20-140)	69.3 (20-170)	0.61
6 week after injection	60.8 (43-920)	90.6 (44-190)	<0.001
P-value(6 week to baseline)***	0.33	<0.001	

*Detrusor, trigone, bladder neck/prostatic urethra including injections

**Detrusor only injections

***Within Group p-value: Comparison cystometric variables 6 weeks after injections with values before injection

PVR = Post void residue; MCC = Maximum cystometric capacity; MDP = Maximum detrusor pressure in filling phase; VFUV = Volume at first desire to void; VUDV = Volume at urgent desire to void

plete and this results in high residual volume in these patients. Abdel-Meguid et al. reported >40% reduction in maximal urinary flow rate after injection of BoNTA (9) leaving patients with bladder outlet obstruction in state of high residual urine volume. Previous reports point to the positive effects of alpha adrenergic blocking agents in improving lower urinary tract symptoms and residual urine volume in female and male patients with lower urinary tract symptoms (18, 19). Therefore, we hypothesized that injection of BoNTA into bladder neck and prostatic urethra can further improve the symptoms and probably residual urine volume. Consequently, we introduced new sites for injection at bladder neck and prostatic

urethra to address these issues in our study.

The results of our study show that Group A had a lower residual urine volume in comparison with Group B patients. Thus, we expect older patients with large PVR and a greater risk of acute urinary retention, to be fit for the effects of bladder base BoNTA injection. It is established that large PVR volume especially in elderly patients is associated with repeated episodes of UTI. This injection site is especially important in male patients in whom bladder neck has an important resistive role in the outflow of urine.

It has been demonstrated that BoNTA affects the afferent nerve endings and consequently influences symptoms especially frequency and

urgency, as studied by Schemid et al. (20). High concentration of nerve endings in the trigonal area indicates that injection of BoNTA in this area may ameliorate patient symptoms as shown by our study. Patients in Group A showed lower mean OABSS compared to Group B at 6 weeks after treatment and this difference remained statistically significant 20 weeks post treatment.

We observed a low rate of retention after the procedures (2 patients; 3%). Overall retention rate after the study by Manchesha et al. was 9% (14) while in the study by Kuo et al., this rate was near 6% (19). The type, brand, dose and injection sites of BoNTA injection have been proposed as possible reasons for observation of different adverse effects/efficacy endpoints (18). Furthermore, we inserted overnight urinary catheter which was performed in Kuo et al. study with lower retention rates but not in the study by Manchesha et al. with higher retention rates.

Nevertheless, there are studies which reported no superiority of detrusor plus trigonal injections in comparison with detrusor only injections. Lucioni et al. used onabotulinum toxin A with two injections in trigone. They reported no difference in the trigonal group compared with detrusor group (6). Kuo et al. used 100U onabotulinum toxin A with 10 injection sites in the bladder base in one group and 20 injection sites in bladder body or body and trigone and reported comparable outcomes (19). Part of this inconsistency can be explained by difference in total injected doses of BoNTA or its formulations as indicated by Davis et al. in a recent meta-analysis (18).

Urodynamic parameters in Groups A and B were similar at baseline and follow-up urodynamic studies including MCC, VFDV, and MDP except for a higher VUDV in Group B patients 6 weeks after injections. Manecksha et al. randomized patients to trigone-spared group with 20 sites of injection into the bladder wall, and trigone-including group with 5 injections into the trigone and 15 injections into the bladder wall. No obvious between groups difference was shown in VUDV at 6 weeks postoperative assessment (14).

We observed minor complications such as slight hematuria that stopped spontaneously 24 hour after procedure. Acute urinary retention was observed only in one patient in each group whi-

ch responded to temporary catheterization. Antibiotic therapy was continued up to 1 week after injections. During further follow-up none of our patients developed UTI.

There are some limitations in our study. This study was not a randomized study. Therefore, the possibility of bias in patient selection cannot be ruled out. All cystoscopies were carried out by rigid cystoscope. Using flexible cystoscopies can reduce trauma and will lead to a more tolerable cystoscopic procedure with light anesthesia.

CONCLUSIONS

Adding trigone, and bladder neck or prostatic urethra as sites of BoNTA injection in patients with IDO produces greater reductions in OABSS score and less residual urine volume but a lower volume at urgent desire to void in comparison with detrusor only injections.

CONFLICT OF INTEREST

None declared.

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Schooling impacts on the overactive bladder diagnosis in women

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ABSTRACT

Objective: To evaluate the overactive bladder (OAB) diagnosis using OAB-V8 and ICIQ-OAB questionnaires in women with different schooling and cultural levels.

Materials and Methods: Three hundred and eighty six healthy women answered a clinical questionnaire filling out information about schooling, demographic and gynecological data. The OAB-V8 and ICIQ-OAB questionnaires were used to evaluate OAB diagnosis and symptoms; and the QS-F questionnaire, to determine the sexual function. All questionnaires were validated in Portuguese.

Results: The mean age was 37.3 years-old. Regarding to schooling level, 23.1% had concluded primary education; 65.8%, secondary school; and 11.1% had higher education. Considering the OAB-V8 (score ≥ 8), 51.8% of evaluated women had OAB diagnosis. There was a positive linear correlation between the OAB-V8 and ICIQ-OAB questionnaires in its sections "a" ($r=0.812$, $p<0.001$) and "b" ($r=0.759$, $p<0.001$). There was a positive linear correlation between age and the amount of time used to answer the OAB-V8, ICIQ-OAB and QS-F questionnaires ($p<0.001$).

The ICIQ-OAB was the hardest to answer for all schooling levels when compared to the other questionnaires. Women who had concluded primary and secondary education significantly demanded more help to answer all questionnaires than those with higher education ($p<0.05$). Furthermore, women with higher education took significantly less time answering all questionnaires when compared to their less educated counterparts (primary and secondary schooling), since they were quicker to answer each individual question.

Conclusion: Educational level and ageing had an impact on women response using different questionnaires for OAB and sexual function evaluations.

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INTRODUCTION

Overactive Bladder (OAB) is defined by the International Continence Society (ICS) as urinary urgency usually accompanied by an increase in urinary frequency and nocturia, with or without urinary incontinence, in the absence of other local diseases (1).

The prevalence of this condition is high, as well as its impact on quality of life. It is estimated that in 2018, approximately 546 million people will suffer of this problem (2). In southern Brazil, it is estimated that approximately 18% of the population present symptoms of overactive bladder (3).

The use of specific clinical questionnaires can be an important tool in the OAB evalua-

tion and diagnosis. These questionnaires assess the symptoms severity, the discomfort degree, and the influence on quality of life (4). Additionally, different authors have proposed the use of specific questionnaires for the evaluation of sexual function in OAB patients (5).

The OAB-V8 is a simplified version adapted from the symptom Bother Scale of the overactive bladder questionnaires (OAB-q), validated to Portuguese that may be used in OAB diagnosis. It consists of eight questions with domains of 0 to 5 and OAB is considered a probable diagnosis when the score is equal to or higher than eight (6, 7).

Due to a lack of studies evaluating the agreement and accuracy between the questionnaires used in the OAB diagnosis, this study proposed to estimate the impact of OAB on sexual function, and the agreement between the OAB-V8 and ICIQ-OAB questionnaires in women, considering different social and economic status.

MATERIALS AND METHODS

Three hundred eighty-six healthy women, aged 18 years or older, were included from three different centers: 129 participants from the Physiotherapy Service; 129 from the Rehabilitation Center and 128 from the Hospital Estadual. As the study was developed in three different rehabilitation centers, we estimated the proportion of responders for each of them, taking into account the amount of care in each service.

All interviewed participants were undergoing physiotherapeutic treatment for non-urological complaints, most of them, as companions of patients who were followed in these centers. Women were simply approached in the waiting room of these physiotherapy centers while waiting for care.

All participants were informed about the research, and, if they agreed to participate, they signed the free informed consent approved by the Ethical Research Board. The women answered a clinical questionnaire, which filled out information about schooling, demographic and gynecological data. The OAB-V8 and ICIQ-OAB questionnaires were used to evaluate OAB diagnosis and symptoms; the QS-F questionnaire was used to evaluate sexual function. All questionnaires were validated in Portuguese.

The OAB-V8 questionnaire consists of eight questions, with domains range between 0 to 5, and if the result is equal to or greater than 8 there is a probable diagnosis of overactive bladder (6).

The ICIQ-OAB evaluates the OAB symptoms in the "a" questions, and quality of life in the "b" questions, and highest scores correspond to worse situation regarding to a particular disorder (8).

The QS-F consists of 10 questions that evaluate all stages related to sexual function in women; the higher the score was, more favorable the situation (9).

The participants were subsequently invited to answer three questions: 1- Which questionnaire did you find the hardest to answer?; 2- Did you need to ask for help answering them?; 3- Which questionnaire demanded the most time for you to answer, in terms of minutes? The response time of each questionnaire was always recorded by the same researcher (a physiotherapist) using a digital chronometer.

The categorization of the schooling level of participants was established according to the organization and structure of education established by the Brazilian Ministry of Education (MEC) considering the information provided by the respondents (10).

Statistical analysis

The sample size was determined using casual participation of the subject to the survey, considering the probability of 50% that each person would accept or not to participate in the study, estimating 10% standard error and 5% significance level for the conclusions.

Considering that the study was developed in three different rehabilitation centers, we estimated a proportion of responders for each of them, taking into account the amount of care in each service. The total sample size was calculated as 384 (11).

For clinical and demographic data analysis, the nonparametric chi-square test was used (12). The measurement of the strength of the linear relationship between the different variables was performed by the Pearson Correlation Coefficient

when the variables were quantitative, and the Godman's Test was applied to establish the contrasts between qualitative variables within multinomial populations.

The Bonferroni Method was used to evaluate the variance analysis for models of repeated measures in independent groups (13-15).

RESULTS

Clinical and demographic data of the study population are summarized in Table-1. Associated comorbidities were observed in 25% of participants: hypertension (10.6%), hypothyroidism (3.6%), Diabetes mellitus (2.1%), and other diseases (8.7%).

In 51.8%, the symptoms score was higher than or equal to 8 using OAB-V8, representing probable OAB diagnosis (6).

Average scores questions on the sections "a" and "b" of the ICIQ-OAB questionnaire was 3.08 ± 0.16 , and 7.19 ± 0.61 , respectively.

There was a positive linear correlation between the OAB-V8 and OAB ICIQ-scores in both

sections "a" ($r=0.812$, $p < 0.001$), and "b" ($r=0.759$, $p < 0.001$).

Average QS-F score was 61.48 ± 1.5 , demonstrating that sexual performance in the women varied from regular to good (9).

There was a positive linear correlation between age and response time in all questionnaires, suggesting that older women need longer time to answer these questionnaires (Table-2).

There was a negative linear correlation between the OAB-V8 questionnaire as compared to the QS-F ($r=-0.519$, $p=0.033$).

ICIQ-OAB questionnaire was significantly more difficult to answer when compared to other ones in all level of education. However, there was no statistical difference among the different levels of education considering each questionnaire separately (Table-3).

Women with higher educational levels needed less assistance to answer any of the questionnaires when compared to other educational levels. However, in higher level about 15% of participants required some degree of help during filling out of OAB-V8 (Table-4).

Table 1 - Clinical and demographic data of the study population.

Characteristics of the population studied				
Variable	Mean	Variable	Total	%
Age	37.3 (14.4)	Skin color*		
BMI	27.0 (5.6)	White	288	74.6
Pregnancies	1.6 (1.8)	Mulatto	69	17.9
Vaginal delivery	0.6 (1.3)	Yellow	9	2.2
C-sections	0.8 (1.1)	Black	20	5.2
		Civil status*		
		Married	196	50.8
		Separated/ divorced	24	6.2
		Widow	14	3.6
		Single	152	39.4
		Education*		
		Primary	89	23.1
		Secondary	254	65.8
		Higher	43	11.1

* Categorical variables of the population studied, divided into sample size and their respective percentage

Table 2 - Pearson linear correlation: age versus time to answer the questionnaires.

Association	Linear correlation	
	"r" Value	"p" Value
Age x Time OAB-V8	0.190	p<0.001
Age x Time ICIQ-OAB	0.281	p<0.001
Age x Time QS-F	0.291	p<0.001

Table 3 - Number and percentage of women considering ease to answer the different questionnaires according to educational levels.

Education	OAB-V8	ICIQ-OAB	QS-F	Total
Primary	20 (42.6%) aB	6 (12.8%) aA	21(44.6%) aB	47 (100%)
Secondary	66 (41.0%) aB	38 (9.8%) aA	57(35.4%) aB	161(100%)
Higher	10 (55.6%) aB	2 (11.1%) aA	6 (33.3%) aAB	18 (100%)
Total	96 (100%)	46(100%)	84 (100%)	226(100%)

Different lower cases mean statistically significant differences among educational level in the same questionnaire.

Different upper cases mean statistically significant difference considering different questionnaires in the same educational level.

Table 4 - Number and percentage of women who needed assistance to answer the questionnaires according to the level of education.

Education	OAB-V8	ICIQ-OAB	QS-F	Total
Primary	5 (38.4%) bA	4 (30.8%) bA	4 (30.8%) bA	13 (100%)
Secondary	33 (46.4%) bA	19 (26.8%) bA	19 (26.8%) bA	71 (100%)
Higher	3 (15.2%) aB	0 (0%) aA	0 (0%) aA	3 (100%)
Total	41 (100%)	23 (100%)	23 (100%)	87 (100%)

Different lower cases mean statistically significant differences among educational level in the same questionnaire.

Different upper cases mean statistically significant difference considering different questionnaires in the same educational level.

It was found a significantly shorter time to answer the questions in all questionnaires in higher education level when compared to other educational levels (Table-5). Similar results were observed when compared secondary and primary education levels (Table-5), demonstrating the importance of this parameter in the evaluation of patients using specific questionnaires.

DISCUSSION

According to AUA guidelines, OAB is not a disease; it is a symptom complex that generally is not a life-threatening condition. In this context, in pursuing a treatment plan the clinician

should carefully weigh the potential benefit to the patient of a particular treatment against that treatment's risk for adverse events and its severity and reversibility. However, if after the assessment has been performed to exclude conditions requiring treatment and counseling, no treatment is an acceptable choice made by some patients and caregivers (16).

Once OAB is determined by subjective and objective symptoms, the patient's perspective is very important in the proper management of this important condition. The diagnosis of this clinical syndrome is based on detailed history, physical examination, and urine analysis. Additionally, OAB may negatively affect sexual activity and or-

Table 5 - Response time (seconds) for each questionnaire according to education level.

Education	OAB-V8	ICIQ-OAB	QS-F
Primary	134.4±56.5 c	137.1±55.7 c	200.1±79.6 c
Secondary	107.6±40.0 b	107.2±35.8 b	146.6±46.3 b
Higher	89.7±34.7 a	91±28.5 a	123±40.6 a
p Value	p<0.001	p<0.001	p<0.001

Different lower cases mean statistically significant differences different educational levels.

gasm. Different authors have demonstrated decrease in the orgasm in these patients (5).

For this reason, several diagnostic tools evaluating patient reported outcomes are proposed in attempt to help diagnosis. In some patients, the voiding diary may be used, as well as the measures of post-void residual urine (4). More complex diagnostic procedures such as urodynamic study may be not necessary, and it is reserved only to specific cases such as in neurologic disease, high post-void residue or pharmacological treatment failure (17).

The ICIQ-OAB questionnaire has level “A” of evidence, and provides assessment of urinary symptoms such as frequency and urgency, and also measures their impact on quality of life. Bothersome related to OAB symptoms are worse when final questionnaires score is higher (8).

Some authors have reported that age, BMI, pregnancy, type of delivery, and ethnicity may influence the incidence of OAB diagnosed by validated questionnaires (18-20). Considering the World Health Organization (WHO) (21) classification, we noted that most of women in our study were white overweight mature adults.

In our population, the most common comorbidities were arterial hypertension and Diabetes mellitus (DM). Some authors had stated that these comorbidities and depression might be associated with OAB (22, 23).

We observed a probable OAB diagnosis in more than half of women using the OAB-V8 questionnaire. Davila et al. (24) reported similar results (51%) using the same questionnaire, and also questioned the reliability of isolated use of this instrument for OAB diagnosis. Thus, if we consider only the OAB-V8 questionnaire our outcomes

may be overestimated.

Lapitan et al. (25) identified a global prevalence of OAB of 53.1% in an Asian female population, using a questionnaire specific to the region. Other studies report a prevalence ranging from 11.8% to 18.9% using different questionnaires (2, 26, 27). Some authors attribute this variation in prevalence rates to the different definitions and questionnaires used for the diagnosis of OAB (28). Scafuri et al. (29) stated that it is difficulty to select the adequate tools for assessment and diagnosis of OAB, impacting the divergences of OAB prevalence among different studies.

In Brazil, the questionnaires frequently used for OAB prevalence are not specific. So, using a specific questionnaire, we found a high prevalence of this disorder. It's important to remember that in our protocol we evaluated only female participants.

In their study, Teloken et al. concluded that overactive bladder is a highly prevalent condition, even in young populations (3). It affects both genders, yet it is more frequently observed in women (14.0% in men versus 23.2% in women; overall 18.9%). According to the author, the lack of standardization in the diagnosis and utilization of different criteria in scientific papers may hinder a comparative analysis.

Additionally, they applied a different strategy to evaluate their patients, once they use a questionnaire developed by the combination of questions from the King's Health Questionnaire validated for OAB syndrome, the AUA Symptoms Score and original questions.

Although our study has shown a possible overestimation in the OAB prevalence, there was a positive linear correlation between the OAB-V8

and ICIQ-OAB questionnaires, demonstrating that there was concordance in the OAB diagnosis and the answers about worsening on OAB symptoms and quality of life.

In the present study, the longer response time in all questionnaires observed in elderly women may reflect a reduced cognitive capacity resulting from the physiological aging process and mild cognitive decline, characterized by memory and attention deficits (30).

According to Vallet (31), some alterations in perception and cognition may occur during the process of physiological aging, such as reduced cognitive processing ability, reduced attention in the execution of some functions, as well as reduction in the ability of free recall and in episodic memory.

We observed that women had more difficulty in answering the ICIQ-OAB questionnaire in comparison to OAB-V8. This fact is in disagreement with other authors that have described the ICIQ-OAB as a single and objective questionnaire to assess the storage symptoms related to OAB (32, 33), suitable to use in different groups, young or elderly patients.

The QS-F questionnaire was specifically developed for the Brazilian population to evaluate the female sexual function. Actually, the studies available so far do not discuss or compare the response time with the level of education (9, 34). We observed a clear influence of the education level and the response time, demonstrating that women with higher level of education needed short time to answer these questionnaires. To our knowledge this is the first report in the current literature to address this question.

Our results showed that women who had only primary and secondary levels of education needed more assistance to answer the questionnaires. Some authors have reported that individuals with higher levels of education could solve more easily some problems and specific cognitive functions (35, 36). These facts justify why women with higher levels of education had shorter response time and less assistance to answer the questions in our study.

There was a negative linear correlation between the specific questionnaire for OAB symptoms and sexual function (SF), where higher scores for

OAB-related symptoms had worst results in SF. This fact demonstrates the negative influence of OAB symptoms in sexual performance.

In conclusion, educational level and ageing had an impact on women response using different questionnaires for OAB and SF evaluations.

Ethics Committee

Approval Ethics Committee: 1013798/2015.

Ethics Committee: Faculty of Medicine of Botucatu – UNESP, SP, Brasil

CONFLICT OF INTEREST

None declared.

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Ultrasound detection of prostatic calculi as a parameter to predict the appearance of hematospermia after a prostate biopsy

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ABSTRACT

Purpose: We evaluated the correlation between prostate calculi and hematospermia in patients undergoing prostate biopsy, and its impact on sexual activity of patients.

Materials and Methods: A single-center prospective randomized study of 212 patients referred for transrectal ultrasound-guided prostate biopsy (TRUSBx) was performed. All patients were divided into two groups: Group A (GA), 106 patients with moderate/ marked presence of prostatic calculi visualized by TRUS; Group B (GB), 106 patients with absence/scarce of prostatic calcifications. Patients were handed questionnaires to obtain a validated data on the duration and impact of hematospermia on sexual activity. The anxiety scores were recorded using a visual analogue scale.

Results: No significant difference was noted between the two groups when comparing age, preoperative PSA level, prostate volume, and biopsy number, except for digital rectal examination (DRE) findings. Post-biopsy results of patients included in GA revealed that the complication of hematospermia was present in 65.1%, while in GB was present in 39.7% ($p < 0.001$).

On multivariate analysis for identifying significant preoperative predictors of hematospermia, which included variables of age, PSA, prostate volume, and prostate cancer were not shown to be significant predictors of hematospermia, except DRE and prostate calculi ($p < 0.001$).

The mean anxiety score was 3.7 ± 2.8 in GA and 2.3 ± 1.9 in GB, respectively ($p < 0.001$).

Conclusions: Prostatic calculi are an independent predictive factor of severe hematospermia after TRUSBx on the basis of multivariate analysis, but don't affect the positive rate of prostate cancer. Patients should be adequately counselled before TRUSBx to avoid undue anxiety and alterations in sexual activity.

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Prostate; Ultrasound, High-Intensity Focused, Transrectal; Biopsy; Hematospermia

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INTRODUCTION

Prostatic calculi are presumed to form by the precipitation of prostatic secretions and calcification of the corpora amylacea under inflammatory conditions (1, 2). Prostatic calculi are common in men who are evaluated for benign

prostatic hyperplasia or prostate cancer (3) and are discovered incidentally, usually by means of ultrasound investigation for other medical conditions. The study of prostate with transrectal ultrasound (TRUS) provides both axial and sagittal images and thus improves the evaluation of the number, location, and dimension of the

prostatic calculi. Transrectal ultrasound-guided prostate biopsy (TRUSBx) is one of the most common urological procedures, approximately 1 million prostate biopsies are performed each year in Europe (~650.000) and United States (~350.000) as diagnostic investigation of patients with clinical suspicion of prostate cancer (4). TRUSBx is generally a safe procedure with minimal haemorrhagic complications. However, hematospermia is a well-recognized complication of TRUSBx (18-31%) (5). Although it is classified under minor complication, its persistence causes distress to the patient and the partner in sexual activity (6). In this study we prospectively evaluated patients undergoing prostate biopsy for suspicion of prostate cancer, what effect prostatic calculi will have on appearance of hematospermia after prostate biopsy, and its impact on sexual activity of patients.

MATERIALS AND METHODS

A single-center prospective randomized study of 212 consecutive patients referred for TRUSBx to our Department was performed between May 2010 to November 2015.

All patients underwent an initial TRUSBx for abnormal digital rectal examination (DRE), high prostate-specific antigen (PSA) levels (≥ 4 ng/mL), or both. Patients with a history of biopsy, on anticoagulation/antiplatelet therapy, surgical treatment of prostatic disease, neoadjuvant therapy, or no sexually active men were excluded from our study. No patient had any history of bleeding disorders. None had any history of hematospermia within 2 years before. Patients were instructed to take antibiotics, usually levofloxacin 500mg orally, for 5 days starting the evening before the procedure and a small evacuative enema starting two hours before the procedure. All patients enrolled in the study signed a consent form for the procedure. TRUSBx was performed with the patient in the left lateral decubitus using a General Electric Logiq 7 machine (GE Healthcare, Milwaukee, WI, USA) equipped with a 5-9MHz multi-frequency convex probe "end-fire". Each TRUS performed included an assessment of the prostatic diameter, the volume of the whole prostate, the transition

zone, capsular, seminal vesicle characteristics, presence/absence of prostatic calcification, and a morphological description of potential pathological features. The prostate volume was invariably calculated using prostate ellipse formula ($0.52 \times \text{length} \times \text{width} \times \text{height}$).

All patients were divided into two groups, as it follows: Group A (GA) included 106 patients with moderate/marked presence of prostatic calculi visualized by TRUS; Group B (GB) included 106 patients with absence/scarce of prostatic calcifications.

We defined moderate/marked presence of prostatic calculi as multiple (≥ 3 in number) hyperechoic foci, with significant area (≥ 3 mm in the largest diameter) with coarse shadow detected in both dimensions (Figure-1). Mild calcifications were defined as 1 or multiple small foci without coarse shadow (Figure-2). All measurements were made by an experienced urologist. After having images of the prostate, sampling was carried out with a 18-Gauge Tru-Cut (Bard Biopsy Systems, Tempe, AZ, USA) needle powered by an automatic spring-loaded biopsy disposable gun. A 14-core biopsy scheme was performed in each patient, as first intention, including 2 basal samples (lateral and medial), 2 parasagittal samples (lateral and medial), 2 apical samples (lateral and medial), and 1 transitional zone sample on each side.

The patients were treated under local anesthesia with Lidocaine Spray 10gr/100mL (ECO-CAIN®, Molteni Dental, FI, Italy) applied two minutes before the procedure (6). All patients received a detailed information guide before the procedure and a questionnaire where information of the participant's age, PSA, prostate volume (PV), DRE findings, use of anticoagulants/antiplatelet, and the number of cores biopsy taken were recorded. The patients were called about 20 days after the procedure to deliver histopathological examination. In this time, we gave to the patients a questionnaire to obtain data on post-biopsy experience. Patients were asked questions about complications occurred (yes/no), how many times (hours/days), severity of hematospermia on a scale 0-4, which was designed with 0 representing absence of bleeding and 4 severe bleeding (5). The presence or absence of each bleeding for its duration and

Figure 1 - Transrectal ultrasound in axial section of prostate shows marked calcifications with multiple hyperechoic foci and with significant area ($\geq 3\text{mm}$) with coarse shadow.

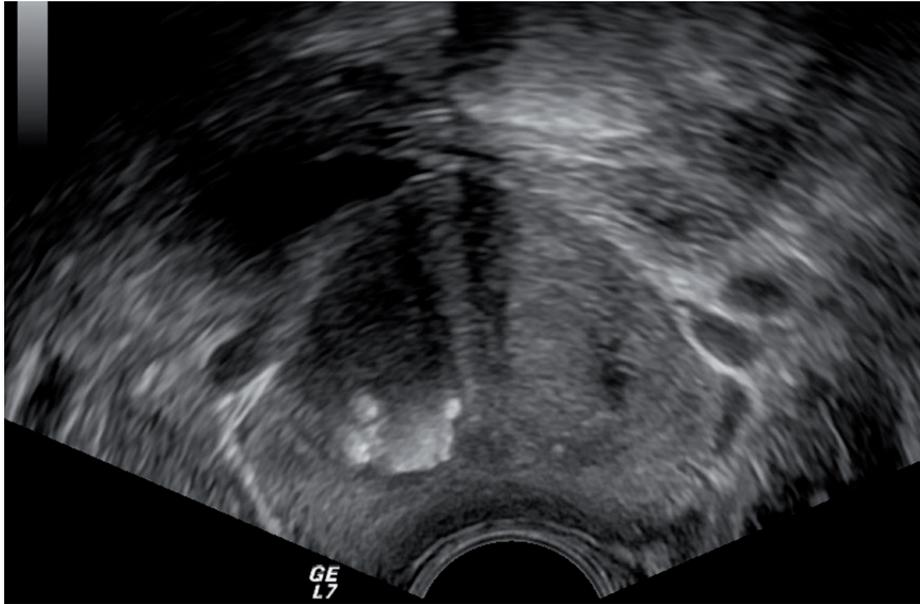


Figure 2 - Transrectal ultrasound in longitudinal section of prostate shows mild calcifications with 1 or multiple small foci without coarse shadow.



severity was reported but amount was not quantified. Values of 1-2 were classified as low severity and values of 3-4 were classified as high severity. Patients were handed questionnaires to obtain a validated data on the duration and impact of hematospermia on emotions and sexual activity. The anxiety scores were recorded using: 0-no anxiety, 10-extreme anxiety.

Statistical analysis

Comparisons between the two groups were performed using the Mann-Whitney U test for continuous variables and the chi-square test or Fisher’s exact test for categorical variables. Univariate logistic regression analysis was used to identify the individual clinical factors predictive of appearance of hematospermia. A p<0.05 was considered to indicate statistical significance.

RESULTS

The mean±standard deviation age of enrolled patients was 62.4±6.8 years, with a prostate

PV of 47.5±19.9mL, initial PSA of 7.2±5.8ng/mL. The number of biopsy cores was 10.7±4.5. No significant difference was noted between the two groups when comparing age, preoperative PSA level, prostate volume, and biopsy number, except for DRE findings. In Group A more frequent abnormal DRE findings were observed (Table-1). In GA, 3 patients (2.9%) received 5/6 core biopsies, 8 patients (7.5%) received 7/8 core biopsies, 11 patients (10.4%) received 9/10 core biopsies, 16 patients (15.1%) received 11/12 core biopsies, 43 patients (40.5%) received 13/14 core biopsies, and 44 patients (23.6%) received 15/16 core biopsies. In Group B (GB), 4 patients (3.8%) received 5/6 core biopsies, 9 patients (8.5%) received 7/8 core biopsies, 8 patients (7.5%) received 9/10 core biopsies, 13 patients (12.3%) received 11/12 core biopsies, 49 patients (46.2%) received 13/14 core biopsies, and 23 patients (21.7%) received 15/16 core biopsies. Prostate cancer was detected in 74 patients (34.9%) and their Gleason score were ≤6 (42 patients, 56.7%), 7 (23 patients, 31.1%), and ≥8 (9 patients, 12.2%). Post-biopsy results of the 212 patients included in GA revealed that

Table 1 - Demographic and clinicopathologic features of patients undergoing transrectal prostate biopsy.

Characteristics	Group A (n:106)	Group B (n:106)	p value
Age (years), mean ± SD	63.2±6.2	62.7±6.9	NS
PSA (ng/mL), mean ± SD	7.1±5.9	7.8±5.2	NS
PV (mL), mean ± SD	46.8±19.5	47.4±19.3	NS
Abnormal DRE, n (%)	43 (40.5)	28 (26.4)	<0.001
N° biopsy cores, mean ± SD	10.6±4.9	10.9±4.3	NS
Prostate cancers, n (%)	38(35.8)	36(33.9)	NS
Gleason score, n (%)			NS
≤6	23(60.5)	19(52.7)	
7	10(26.3)	13(36.2)	
≥8	5(13.2)	4(11.1)	
Hematospermia, n (%)	69(65.1)	42(39.7)	<0.001

SD = standard deviation; PSA = prostate-specific antigen; DRE = digital rectal examination; PV = prostate volume; NS = not significant

the complication of hemospermia was present in 65.1% (69/106) of patients, while in GB was present in 39.7% (42/106) of patients (p<0.001).

In Table-2 shows the incidence and severity of hemospermia between the two groups. Grade 1, 2, 3 and 4 complications occurred in 27 (24.3%), 33 (29.7%), 34 (30.6%), and 17 (15.3%) patients, respectively. Two patients in GA were admitted to hospital having developed urinary retention. The study revealed significant difference in the incidence in terms of hemospermia (p<0.002) and its severity (p<0.001) between both groups. Moreover, a statistically significant difference was found in the duration of hemospermia between two groups. Forty-two patients ejaculated in the first week. The duration was longer in GA (16.9 days) than in GB (11.3 days; p<0.002). The number of patients still reporting hemospermia at 4 weeks after TRUSBx was 12 (17.4%) in GA, and 4 (9.5%) in GB (p<0.001). On multivariate analysis for identifying significant preoperative predictors

of hemospermia, which included variables of age, PSA, PV, and prostate cancer were not shown to be a significant predictor of hemospermia, except DRE and prostate calculi (p<0.001) (Table-3).

The mean anxiety score was 3.7±2.8 in GA and 2.3±1.9 in GB, respectively (p<0.001). Forty-nine patients in both groups reported less sexual activity due to hemospermia, its duration and recurrence.

DISCUSSION

One of the most frequent and embarrassing complications of TRUSBx is hemospermia. Hemospermia is the presence of blood in seminal fluid (4). It is almost always caused by nonspecific inflammation of the prostate and seminal vesicles. In patients without coagulopathy, the incidence of this complication varies with patient’s factors as prostate size, anticoagulant medications, and procedural factors such as the number of biopsy cores

Table 2 - Incidence of complications among the two groups.

	Group A (n:106)				Group B (n: 106)				Total
	G1	G2	G3	G4	G1	G2	G3	G4	
Complication grade									
Hemospermia	9	18	27	15	18	15	7	2	111

Table 3 - Results of multiple logistic regression analysis examining the correlation between clinicopathological variables and hemospermia risk after prostate biopsy.

Characteristics	Adjusted Odds ratio	95% CI	p value
Age	0.703	(0.251-1.843)	NS
PSA	1.314	(0.614-3.132)	NS
Prostate Volume	1.22	(0.542-3.102)	NS
Abnormal DRE	2.128	(0.703-3.691)	<0.001
Prostate calculi	3.461	(2.308-5.624)	<0.001
Prostate cancer	2.015	(0.601-3.142)	NS

PSA = prostate-specific antigen; DRE = digital rectal examination; CI = confidence interval; NS = not significant.

taken (7). Hematospermia can cause extreme concern and anxiety in the patients and their sexual partners (8).

Prostatic calculi are commonly diagnosed by TRUS and their incidence is believed to begin after puberty and increase with age (9). TRUS is safe, cost-effective, radiation free and an excellent imaging modality for the prostate and seminal vesicles. Several articles reported on the findings of TRUS in patients with prostatic calcifications (10-12). At TRUS, prostatic calculi appear as well circumscribed focal foci of increased echogenicity with or without posterior acoustic shadowing, situated in the prostate gland or seminal tract (12). The mechanism of prostatic calculi formation is unknown. Proposed contributing factors include infections, urinary retention or reflux into the prostate, penetration of spermatozoa into prostatic glands, and desquamation of prostatic epithelium (13). Prostatic calculi have also been showed in association with various systemic diseases, prostatic diseases, and treatment modalities. Examples include hyperparathyroidism, hypercalciuria, prostatic hypertrophy and carcinoma, status after radiotherapy, and status after adenomec-tomy or transurethral prostate resection (14, 15). To our knowledge this study is the first to evaluate the effect of prostatic calculi on the outcome of TRUSBx in terms of appearance of hematospermia and its impact on emotions and sexual activity in patients. Gu M et al. (16) reported that patients with prostatic calculi, after the prostate biopsy, experienced more uncomfortable feelings and enjoyed higher urinary retention probability. Most studies have been showed that the degree of urinary retention may be relative to the presence of large prostatic calculi (2, 3, 17). Ludwig et al. (18) concluded that prostatic calculi are typical signs of inflammation. Their study showed a significant difference in the duration of symptoms of chronic prostatitis between prostatic calculi and non-calculi groups, but did not show a significant difference in the white blood cells count in the prostatic secretions. Abdelkhalek et al. (19) referred that after biopsy, the bacteria in calcifications may be disseminated by biopsy needle, then produced local inflammation, further quickly leading to more severe edema. The local inflammation and more

severe edema can cause hematospermia. However, unlike the previous studies, in our analysis we have attributed homogeneity in the method of enrollment patients. We prospectively evaluated and enrolled a cohort of patients using an uniform protocol. For one thing, there were no significant differences in the clinical variables among the patients in the two groups, except for DRE findings. In Group A more frequent abnormal DRE findings were observed ($p < 0.001$), probably also due to an increase in the thickness or abnormal shape of glandular prostatic tissue associated with the greatest number of prostatic calcifications. There was not a lack of measuring the sizes and numbers or locations of prostatic calculi when TRUS was performed because the criteria for classifying calcifications have been well established. Moreover, the rate of hematospermia has been calculated from the number of sexually active patients who pledged in sexual activity after TRUSBx and not from the overall number of patients included in the study. Nevertheless, this study had some limitations. The patients were recruited from a single center, the majority of the participants are white, therefore results might not be generalizable to other races. There may be different incidences of prostatic calculi with different distribution in diverse populations. In one autopsy study, the incidence was 70.1% and 29.1% in black men from USA and Nigeria, respectively. They suggest that dietary pattern was an important factor for determining the incidence of prostatic calcifications (20). In addition, although the prostatic secretions cultures were more likely to be positive in patients with prostatic calculi in the present study, the number of those men was unknown, and hence we did not separately assess if positive prostatic secretion culture had an impact on their hematospermia. In fact, Zhao et al. (21) reported in a study of 358 patients that prostatic calcifications are significantly associated with the presence of positive prostatic secretions cultures and erectile dysfunction in chronic prostatitis syndrome males. We also suggested that prostatic calcifications are common and not associated with any significant pathology as prostate cancer ($p = 0.326$). Although some publications as Suh et al. (22) and Smol-ski et al. (23) showed that interface calcification

is common and not associated with any particular pathology, peripheral zone calcification appears to be strongly associated with prostate cancer. However, further studies are required to assess if peripheral prostatic calculi are directly associated with an increased incidence of prostate cancer.

CONCLUSIONS

Hemospermia is a well-recognized complication of TRUSBx and is mostly self-limited. Although it is classified under minor complications, its persistence causes immense distress to the patient and the sexual partner. In this study we showed that prostatic calculi are an independent predictive factor of severe hemospermia after TRUSBx on the basis of multivariate analysis, but don't affect the positive rate of prostate cancer. This study result would be useful for predicting the uncomfortable feeling before prostate biopsy. Patients should be adequately counselled before prostate biopsy to avoid undue anxiety and alterations in sexual activity.

ABBREVIATIONS

TRUS = Transrectal ultrasound
 TRUSBx = Transrectal ultrasound prostate biopsy
 DRE = Digital rectal examination
 PSA = Prostate-specific antigen
 GA = Group A
 GB = Group B
 VSA = Visual analogue scale

CONFLICT OF INTEREST

None declared.

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Urethral duplication type influences on the complications rate and number of surgical procedures

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ABSTRACT

Introduction: Urethral duplication is rare. Characterized by the presence of two urethral channels. This anomaly presents a great variety of clinical findings that depend on the type of duplication that often is associated with other anomalies.

Material and Methods: We report thirteen boys with urethral duplication managed in our institution between 1988-2015. Clinical findings, associated anomalies, treatment of urethral duplication and our results are described. Patients were classified according to Effmann classification.

Results: Mean patient's age was 38.3±34.7 months (3-136 months). Mean follow-up was 7.7±3.4 years (3y8m-14y2m). Type II A2 was the most common pattern (8/13 patients, 61.5%), followed by type IA (3/13 patients, 23%) and IIA1 (2/13 patients, 15.3%). The most frequent clinical manifestations were urinary tract infections (UTI) observed in 11/13 patients (84.6%) and anal urinary leakage, found in 7/13 patients (53.8%). Associated anomalies were found in 9/13 patients (69.2%).

Required surgeries were 3.53±2.84 procedures per patient. Considering groups: Type IIA2 4.25±3.28, type IIA1 4±1.41 and type IA 1.33±0.57 needed procedures per patient. Complications rate were 0% for type IA, 50% for type IIA1 and 75% for type IIA2.

Conclusions: Patients with incomplete duplication (type I A or I B) can totally be asymptomatic, with no need of surgical correction. Type IIA2 is the most complex form of duplication to correct and multiple procedures might be required because of the very hypoplastic orthotopic dorsal urethral tissue. Surgical treatment should be individualized and parents should be advised on complications and need of multiple surgeries according to urethral duplication type.

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INTRODUCTION

Urethral duplication is a rare congenital anomaly characterized by two urethral channels, whose location and extension present variations. It is more common in males, occurring usually in the sagittal plane. In females, this anomaly is rare

and most often associated with bladder duplication (1-4).

Because of the diversity of clinical manifestations, diagnosis is difficult, as well as its classification. Patients can be either asymptomatic or symptomatic, most common clinical findings being incontinence, obstruction, recurrent urinary

infection, and occasionally double urinary stream (1-4).

The objective of this study was to review our experience in the management of urethral duplication anomalies and to determine whether the type of urethral duplication influences on the number of surgical procedures needed for repair and complication rates.

MATERIALS AND METHODS

Medical records of patients with urethral duplication anomalies were analyzed retrospectively. We searched in our hospital database for urethral duplication cases submitted to surgical treatment. Urethral duplication cases without surgical management were not included in this study. Clinical characteristics such as age at presentation, type of urethral duplication, clinical findings, associated anomalies, surgical treatment, complications and results were reviewed. Patients were classified according to Effmann et al., as shown in Table-1 (5).

Thirteen male patients with urethral duplication were managed surgically at our institution between 1988 and 2016. All patients were carefully assessed preoperatively by clinical

history, physical examination, kidney and bladder ultrasonography and voiding cystourethrography (VCUG). For surgical planning, an urethroscopy was performed at the beginning of the operation to aid the surgical decision.

Uroflowmetry and urodynamics studies were only performed in cases of associated dysfunctional voiding (irritative or obstructive lower urinary tract symptoms). Follow-up was done by regular clinic visits (every 6 months to 1 year) that included physical examination and ultrasonography of urinary system. VCUG was carried out only if there was recurrent urinary tract infection or suspicion of urethral obstruction.

After collection of analytical data, urethral duplication type was correlated to the number of surgical procedures needed for repair and complications rates.

Statistical analysis

All values were presented as mean \pm standard deviation with the ranges. Statistical analysis was done using ANOVA (Bonferroni) for categorical comparisons. Results were considered significant when p value was equal or less than 0.05.

Table 1 - Classification of urethral duplication (Effmann et al., 1976).

Type I	Incomplete urethral duplication (accessory urethra)
A - Distal:	The meatus is on the dorsal or ventral surface, but it does not have communication with the urethra or the bladder.
B - Proximal:	The accessory urethra originates from the normal urethra and ends blindly.
Type II	Complete Duplication
A – Two meatus:	1 – two non-communicating urethras originating independently from the bladder 2 – the second urethra originates from the first, with an independent channel until the second meatus
B – One meatus:	1 – two urethras originate from the bladder or of the subsequent urethra joining later in a single channel
Type III	Urethral duplication, part of a complete or incomplete caudal duplication

RESULTS

Mean age at surgical intervention was 38.3 ± 34.7 months (range: 3 to 136 months). Mean age \pm standard deviation (range) was 72.3 ± 55.1 (38 to 136 months) for group IA, 43 ± 26.8 (24 to 62 months) for group IIA1 and 24.3 ± 19.3 (3 to 61 months) for group IIA2. Mean follow-up was 7.7 ± 3.4 years (3y8m-14y2m).

Type IIA2 was the most common pattern, found in 8/13 patients (61.5%), followed by type IA (3/13 patients, 23%) and IIA1 (2/13 patients, 15.3%). The most frequent clinical manifestations were urinary tract infections (UTI) observed in 11/13 patients (84.6%) and anal urinary leakage, found in 7/13 patients (53.8%). Associated anomalies were found in 9/13 patients (69.2%). The most common associated anomalies were vesicoureteral reflux (6/13 patients, 46%) and renal abnormalities (4/13 patients, 30.7%), as demonstrated in Table-2.

Required surgeries were 3.53 ± 2.84 procedures per patient (1 to 12 procedures). Considering groups: type IA needed 1.33 ± 0.57 procedures per patient (1 to 2 surgeries), type IIA1 needed 4 ± 1.41 procedures per patient (3 to 5 surgeries) and type IIA2 need 4.25 ± 3.28 procedures per patient (2 to 12 surgeries). Children with types IIA1 and IIA2 of urethral duplication underwent significantly more surgical procedures than type IA (p values 0.05 and 0.05, respectively). Complications rate were 0% for type IA, 50% for type IIA1 (1/2 patients had urethral stenosis) and 75% for type IIA2 (6/8 patients, with 6/8 developing urethral stenosis and 2/8 with a defunctionalized bladder that required augmentation). However, no statistically significant difference was found when number of surgeries for types IIA1 and IIA2 were compared (p=1.0)

DISCUSSION

Urethral duplication is a rare anomaly, with great diversity of clinical presentations. Some explanations of its morphogenesis have been proposed and as a consequence, several hypotheses were formulated aiming to explain failures in its embryogenesis.

The occurrence of urethral duplication with the accessory urethra epispadic, as in cases 2 and 3 can be associated with the same embryology theory of the exstrophy-epispadias complex, in which it might occur failure of the fusion of lateral mesoderm in the midline, between the ectoderm and the endoderm of the cloacal membrane (5).

In cases of duplication in which one of the urethral meatus is located in the anal or perineal region (cases 4 to 13), these are probably secondary to failure of the urorectal septum normal development (6).

In the type III urethral duplication, associated with the syndrome of caudal duplication, that can present duplications of the uterus, vagina, rectum, colon, among others, the morphogenetic defect occurs due to the division of the notochord in earlier phase of the embryonic development (5). In cases of collateral urethral duplication, that is, when both urethras are side by side, the urethral groove could be divided before forming the urethra in the medium line, originating two urethras (7).

In this study, Effmann et al. (5) classification was used because it is considered the most complete, as described in Table-1.

Patients' clinical findings with urethral duplication are variable, depending on the duplication type. According to Bogaert, (8) an accessory urethra ends blindly (type I A) and can cause few symptoms as elimination of purulent secretion or be asymptomatic. Type IB can also be asymptomatic, many times difficult of being differentiated of urethral diverticulum. In cases of duplication with epispadic accessory urethra, a dorsal curvature of the penis can occur; in the cases of hypospadiac accessory urethra, ventral curvature can occur.

Types I B and IIB1 can be asymptomatic (8). Probably this is the reason why these are considered rare (difficult diagnosis due to the lack of clinical manifestations).

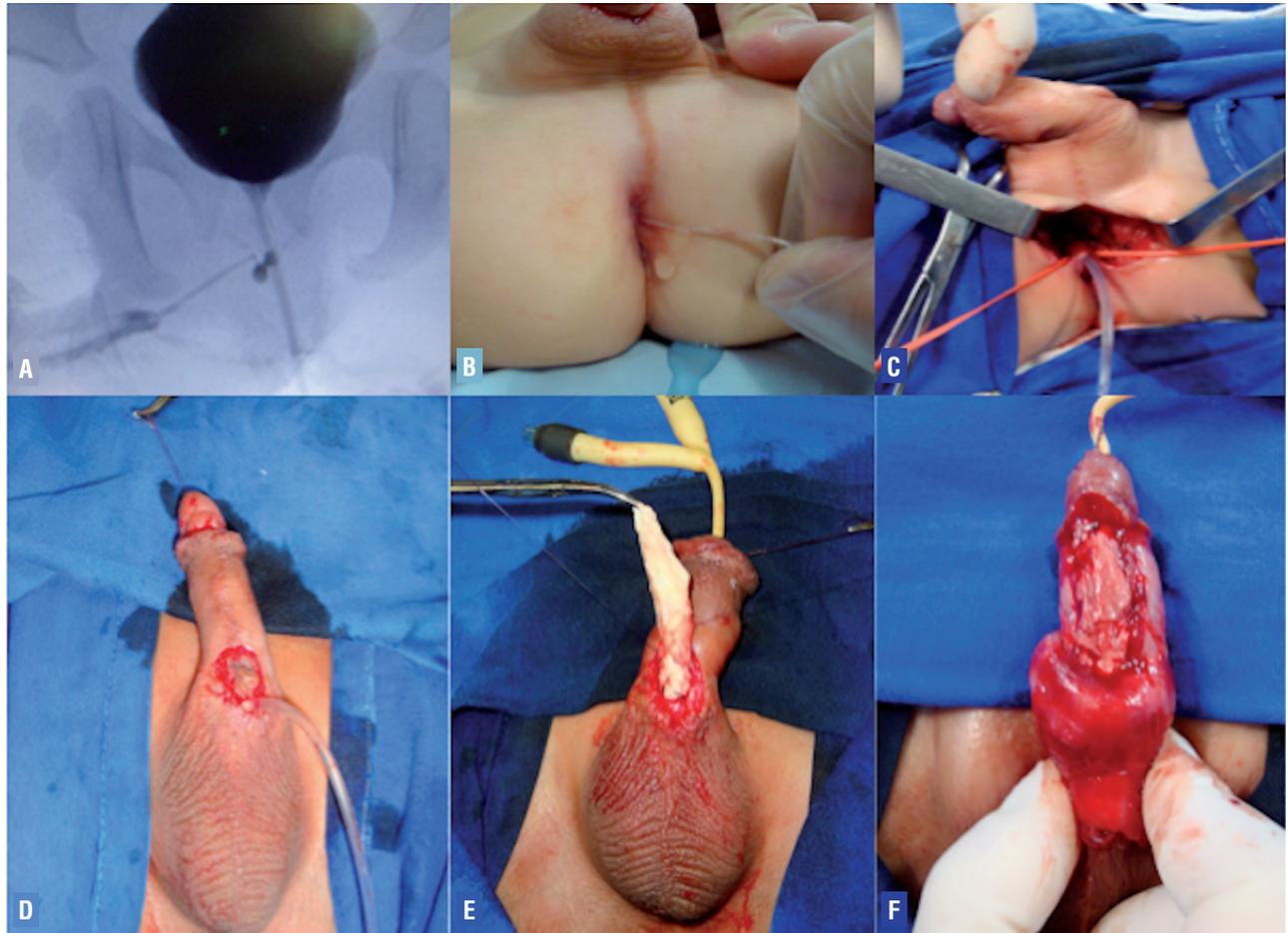
In cases of complete duplication, types IIA1 and IIA2, the most common clinical manifestations include recurrent urinary tract infections, double urinary stream and urinary obstruction. Type IIA2, with a perineal urethra or

Table 2- Clinical features of our cohort.

Pt	Age	Effmann	Clinical finding	Associated anomalies	Treatment # 1	Treatment # 2	Treatment # 3	Treatment # 4	Treatment #5	Complications	Follow-up
1	3y2m	IA	Hypospadic subcoronal urethral meatus	No abnormalities	Ventral incision in the accessory urethra	Denis Browne technique (hypospadia repair)				No	6y6m
2	11y4m	IA	UTI +purulent discharge from epispadic meatus	Left VUR grade III	Accessory urethral resection					No	3y8m
3	3y7m	IA	UTI + purulent discharge from epispadic meatus	No abnormalities	Accessory urethral resection					No	5y1m
4	2y	IIA1	Anal urinary leakage + UTI	No abnormalities	Vesicostomy	Perineal urethrostomy	Second perineal urethrostomy + vesicostomy closure	Preputial island flap neourethroplasty	2 nd stage urethroplasty	Urethral stenosis (treated successfully)	7y11m
5	5y2m	IIA1	Anal urinary leakage + UTI	Tetralogy of Fallot/ Left solitary kidney / Absence of the second right costal arch / Hemivertebra between the fourth and fifth lumbar vertebrae	Colostomy performed in another institution	Perineal urethrostomy + colostomy closure	Preputial island neourethroplasty			No	12y
6	1y6m	IIA2	Anal urinary leakage + UTI	Posterior urethral valves / Right VUR grade III / Right solitary kidney	Endoscopic transurethral valve resection	Resection of the accessory urethra + urethral stenosis dorsal urethra	T-T anastomosis dorsal urethra			No	6y3m
7	5m	IIA2	Anal urinary leakage + UTI	Left kidney exclusion / Right vesicoureteral reflux grade IV	Vesicostomy	Resection of the accessory urethra + urethral stenosis dorsal urethra	Urethrotomy + Neourethroplasty (distal correction + meatoplasty)			Urethral stenosis (treated successfully)	14y2m

8	5y1m	IIA2	UTI	Anal imperforation / Left vesicoureteral reflux grade V / Spina Bifida	Vesicostomy	Left nephrectomy + cystostomy	Bladder augmentation + Monti	Urethral stenosis + low capacity bladder (treatment: augmentation and Monti)	4y9m
9	2y4m	IIA2	Anal urinary leakage + UTI	Right dysplastic kidney/ Left vesicoureteral reflux grade III/ Congenital megacolon	Vesicostomy + sigmoidostomy	Perineal urethrostomy	Preputial island neourethroplasty	Urethral stenosis (treated successfully)	10y3m
10	3y3m	IIA2	Anal urinary leakage + UTI	Right ectopic crossed fused kidney	8 surgeries to correct the urethra/bladder in another institution	Vesicostomy	Perineal urethrostomy	Urethral stenosis (treated successfully)	8y5m
11	2y5m	IIA2	Anal urinary leakage	No abnormalities	Perineal urethrostomy	Preputial island neourethroplasty	Prepuce urethroplasty + vesicostomy closure + cystostomy	No	5y4m
12	3m	IIA2	UTI (sepsis)	Bilateral ureteral duplication	Cystostomy	Vesicostomy	Vesicostomy closure (obstruction)	Urethral stenosis (ended up with Mitrofanoff)	12y5m
13	1y	IIA2	Multiple UTI	Anal imperforation / Left vesicoureteral reflux grade IV	Vesicostomy	Bladder augmentation + Mitrofanoff		Urethral stenosis + low capacity bladder (treatment: augmentation and Mitrofanoff)	11y7m

Figure 1- A) Voiding cystourethrogram revealed urethral duplication with a narrow caliber dorsal penile orthotopic urethra and the ventral urethra meatus opening in the rectum (type IIA urethral duplication); B) Catheterization of the ventral urethra by its opening in the rectum (type IIA urethral duplication); C) Intraoperative picture showing dissection of the ventral urethra; D) Intra-operative view depicting catheterization of the accessory urethral meatus located at penoscrotal junction; E) Onlay acellular matrix urethral transplantation; F) Final aspect of onlay acellular matrix urethral transplantation (Patient 10 of Table-2).



in the anal channel, also called duplication in Y or H, usually presents a more functional ventral urethra. In those children, clinical findings may include urine elimination along with stool. However, in this presentation, some patients can present normal dorsal orthotopic urethra also called congenital urethroperineal fistula. It is considered as a separated entity of the urethral duplications by some authors.

Some patients with complete duplication can present urinary incontinence. Gross and Moore reported this manifestation in seven of 19 examined patients. In our cases, no patient presented

urinary incontinence. In most of the cases, the ventral urethra crosses the sphincter, also containing the accessory glands and the verumontanum (3). These patients can also present stress urinary incontinence. This clinical manifestation is due to the accessory urethra, which is not usually surrounded by the urinary sphincter (9).

In cases of collateral duplication, the double flow is the most common clinical manifestation. These patients usually present several other associated congenital anomalies, such as colon and bladder duplications, hemivertebrae, dysplasia and renal agenesis, among others (9).

No study about fertility and ejaculation in patients with urethral duplication was reported in the literature. The association between urethral duplication and posterior urethral valves was described by Lorenzo et al., (10) Fernbach et al. (11) and Ramanujam et al. (12). This association presents more difficulty to provide an accurate diagnosis of urethral duplications, as verified in case 6.

Other anomalies associated with urethral duplication include bifid scrotum, cryptorchidism, hypospadias, megalourethra, micropenis, vesicoureteral reflux, agenesis and renal ectopy, dysplastic-multicystic kidney, vertebral anomalies (sacral agenesis, thoracic hemivertebra), ano-rectal anomalies, trachea-esophageic fistula and penile, vagina, uterus, bladder and colon duplications (1, 2, 6, 7, 13).

The diagnosis of urethral duplication is performed by clinical history, physical exam and imaging methods, especially voiding cystourethrography. Kidney and bladder ultrasonography is recommended to investigate associated anomalies. Urethrocystoscopy is important for surgical planning. Magnetic resonance urography or excretory urography might be useful to further depict upper tract abnormalities.

Treatment of this anomaly depends on the duplication type and its clinical manifestations. Before any surgical decision, it must be identified which urethra is more functional. Patients with incomplete duplication (type I A or I B) can totally be asymptomatic, with no need of surgical correction. If those patients present purulent secretions or local infection, the accessory urethra should be resected (8). Other option is the opening of the ventral wall of the accessory hypospadiac urethra and posterior neourethroplasty, as described by Podesta et al. (14) to treat patients with hypospadias and incomplete urethral duplication. In these cases, it was observed fewer surgeries to proper surgical repair and no complications in our cohort.

In cases where the patient presents normal dorsal urethra and ventral urethra interfering in the anal canal (anorectal junction) or in the rectum (urethroperineal fistula) – type IIA2, our surgical approach aimed the initial urethros-

tomy of the ventral urethra and preservation of the dorsal orthotopic urethra. After that, a neourethroplasty using flaps or grafts was usually performed. In difficult redo cases, even an acellular matrix transplantation was performed for one of our patients (case 10) as shown in Figure 1.

These patients with urethral duplication of hypospadiac type, in which the ventral urethra is more functional and located in the perineal or anal area (type IIA2), are challenging to surgical correction and more commonly they present complications such as neourethral dehiscence and stenosis, which are common in these neourethroplasty types. In our study, a significant rate of surgical procedures per patient and complications were observed for type IIA duplications. Type IIA1 needed 4 ± 1.41 procedures per patient (3 to 5 surgeries) and type IIA2 need 4.25 ± 3.28 procedures per patient (2 to 12 surgeries) and rate of complications were 50% for type IIA1 and 75% for type IIA2 which should be informed for patients and families preoperatively. Type IIA2 is the most complex form of duplication to correct, and in such cases the orthotopic urethra usually has an extensive hypoplastic segment. Hence, it is recommended to mobilize extensively the ventral functional urethra to the perineal-scrotal junction to prevent complications and anticipate that multiple procedures might be required because of the very hypoplastic orthotopic dorsal urethral tissue.

CONCLUSIONS

Urethral duplication is a rare anomaly, with several forms of clinical presentation, often accompanied by other anomalies, and sometimes with difficult diagnosis. The treatment of urethral duplication should be individualized, according to its type. Significantly higher rates of surgical procedures per patient and, possibly complication rates were observed for type IIA duplications, which should be informed for patients and families preoperatively.

CONFLICT OF INTEREST

None declared.

Ethical approval

The Institutional Review Board at Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo approved this study.

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Transition to adulthood with a bladder augmentation: histopathologic concerns

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ABSTRACT

Aim: To investigate the histopathologic changes in native bladder and gastrointestinal segment, the relation between histopathologic changes, type of operation and the period passed over operation in patients with bladder augmentation.

Materials and methods: Twenty consecutive patients were enrolled in this study. Histopathologic evaluation of the cystoscopic mucosal biopsies from native bladder and enteric augment was performed in all patients.

Results: Active or chronic non-specific inflammation of various degrees was found in all specimens except two. Metaplastic changes were detected in 3 patients. Two patients had squamous metaplasia (one focal, one extensive) and one patient had intestinal metaplasia. All metaplastic changes were found in native bladder specimens. The type of augmentation in patients with metaplastic changes were ileocystoplasty and sigmoidocystoplasty. No signs of malignancy were detected in any patient.

Conclusion: The complexity of the disorders requiring bladder augmentation does not let the surgeons to draw a clear line between different groups of complications including malignancy formation. However, due to challenging course of the augmentation procedure itself, surgeons should be well aware of the possibility of malignancy development.

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INTRODUCTION

Bladder augmentation gained a widespread acceptance especially after implementation of clean intermittent catheterization procedure by Lapides in 1972 (1). This simple procedure was a revolutionary step in medicine and reasonably increased the popularity of bladder augmentations.

The main indications for bladder augmentation include decreased bladder capacity and compliance, increased intravesical pressure and detrusor overactivity. The operation itself is com-

plex and requires high surgical skills. Early and late postoperative courses are not innocent either. Each phase after surgery has a different set of complications like perforation, mucus and stone formation, metabolic disorders, urinary incontinence, hematuria-dysuria, gastrointestinal system related complications, growth alteration and tumor formation.

Traditional belief that augmented bladder possesses the risk for tumor formation is still debated by scientific authorities. Due to complexity of the diseases requiring bladder augmentation it

is hard to tell whether tumor formation in neobladder is directly related to the procedure itself. Several studies with various results have been conducted by different authors (2-9).

The aim of this study was to investigate the histopathologic changes, the relation of histopathologic changes with type of operation and the period passed over operation in augmented bladders during adolescence and early adulthood.

MATERIALS AND METHODS

Institutional Review Board approval was obtained prior to the study. The targeted population involved all patients who have undergone bladder augmentation in our clinic in the period between 1987-2009. The patients on routine follow up were called by phone and asked to be enrolled in the study. Informed consent was obtained and standard preoperative blood tests including renal function tests were performed in all patients. History of chronic bacteriuria, recurrent urinary tract infections and calculi were questioned prior to the procedure.

All cystoscopic procedures were performed under general anesthesia. The neobladder was evaluated thoroughly for calculi and the mucosa was examined for suspicious regions or lesions. Mucosal biopsies were planned to be obtained from 8 points (3 from native bladder, 3 from augmented tissue and 2 from anastomotic border) (Figure-1). The biopsies from native bladder and augmented segment were planned to be taken from 3 points each forming a virtual triangle. This was done to address the maximum possible area in each segment. Visual emphasis was made on the anastomotic border.

The specimens were sent to the Pathology Department where standard staining procedures (hematoxylin-eosin) were applied. The biopsy samples were examined for inflammation, fibrosis, metaplasia dysplasia and signs of malignancy.

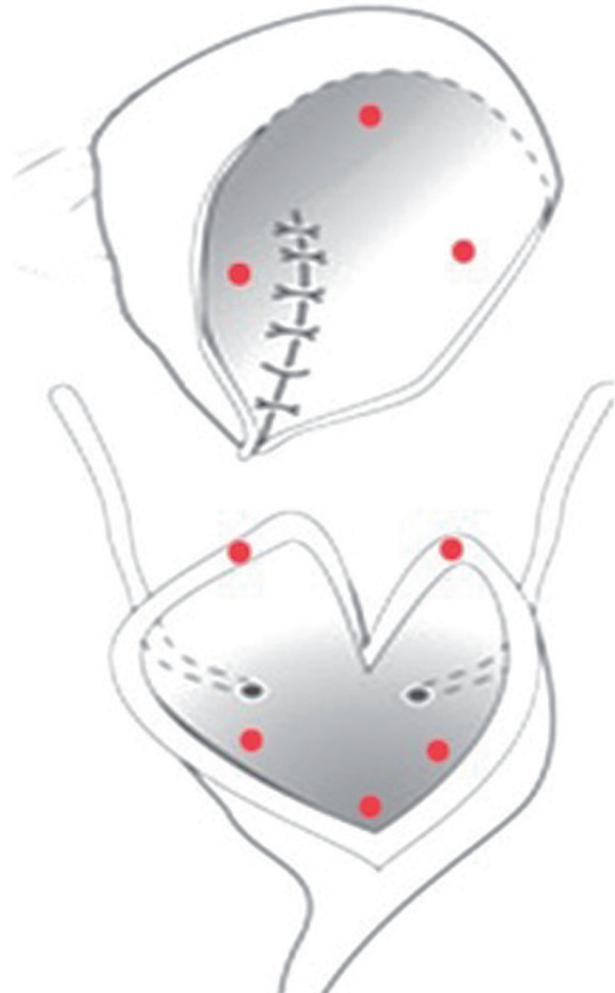
RESULTS

Seventy-seven patients have undergone bladder augmentation in our clinic in the period between 1987-2009. Patients who were lost du-

ring follow-up, patients who could not be reached and who rejected to participate were excluded, leaving 20 patients who were considered eligible for the study.

The study group consisted of 13 male and 7 female patients with the mean age of 20 (range: 11-29) who have undergone bladder augmentation for bladder exstrophy (8) and neurogenic bladder (10). Two other patients with long gap urethral injuries and severe bladder neck ruptures due to pedestrian type traffic accident also had neurogenic type bladder problems which were related with the severity of trauma or the side effects of correctional surgical procedures. The distribution of the patients according to the interval passed over the augmentation was as follows:

Figure 1 - Planned sites for biopsies (triangular manner).



1. Less than 5 years after the augmentation: 4 patients (4 ileal augmentations);
2. 5-10 years after the augmentation: 5 patients (2 ileal, 1 ileogastric, 1 gastric, 1 sigmoid augmentations);
3. More than 10 years after the augmentation: 11 patients (8 ileal, 1 ileogastric, 2 sigmoid augmentations).

Preoperative complete blood count and coagulation panel results were normal in all patients. Two patients had previously diagnosed chronic renal failure at the time of evaluation. Three patients had bladder stones and 4 patients had chronic asymptomatic bacteriuria. Twelve patients have experienced recurrent urinary tract infections either prior to or after the augmentation procedure.

The biopsies were obtained by cystoscopy in 17 patients and through open approach during planned surgery in 3 patients (2 bladder stone extractions and 1 bladder neck disconnection). The rigid cystoscope was introduced either via urethra (1 patient) or via Mitrofanoff stoma (16 patients) in patients with reconstructed or disconnected bladder neck. No complications associated with the biopsy procedure were noted postoperatively.

There weren't any suspicious macroscopic lesions in any patient. The intestinal mucosa is usually easily distinguished from native bladder by its color and villous appearance. However, it was impossible to differentiate native bladder mucosa from intestinal mucosa visually during cystoscopy in 6 patients. The biopsies were taken from the area closest to bladder neck in those patients presuming that it would be the native bladder mucosa but all those biopsies came out to be belonging to augmented intestinal tissue. Additionally, 3 patient's biopsies taken from "visually confirmed" native bladder mucosa revealed augmented intestinal tissue (Table-1).

The histopathology was evaluated using hematoxylin-eosin staining. All specimens except two showed various degrees of active or chronic non-specific inflammation. Different types of metaplastic changes were detected in 3 patients. All three patients were in exstrophy-epispadias complex group and had staged repair prior to aug-

mentation. All three patients had Mitrofanoff procedure during the augmentation. Additionally, two of them have undergone multiple operations after the augmentation (bladder neck procedures, post epispadias fistula repair etc.). Two patients had squamous metaplasia (one focal, one extensive) and one patient had intestinal metaplasia. All three metaplastic changes were found in the native bladder. Squamous metaplasia was detected in patients with ileocystoplasty and sigmoidocystoplasty. Interestingly the patient with extensive squamous metaplasia had undergone ileocystoplasty just four years prior to current evaluation. The patient with intestinal metaplasia had ileocystoplasty as procedure of choice (Figure-2).

The patient with previous sigmoidocystoplasty who had squamous metaplasia formation, had an additional stone formation as well (2x2.5x2cm). Pediatric Pathology Division commented that those histopathologic changes could be related with irritation process which was created by the stone. After the removal of stone patient had no clinical problem. The parents did not accept another biopsy examination after the stone removal, since they did not want another intervention under general anesthesia. Ultrasound exams and routine urine analyses and cultures revealed no major problem in this patient. In other two patients, which were treated for stone formation, histopathologic exam of the biopsy specimens revealed only chronic non-specific inflammatory changes as in the other 18 patients. For these two patients, it was decided to perform the routine follow-up with urine analysis, cultures and ultrasonographic exams. No additional problem was detected in these 2 patients.

No neoplastic changes or active tumor formation were detected in any patient (Table-1).

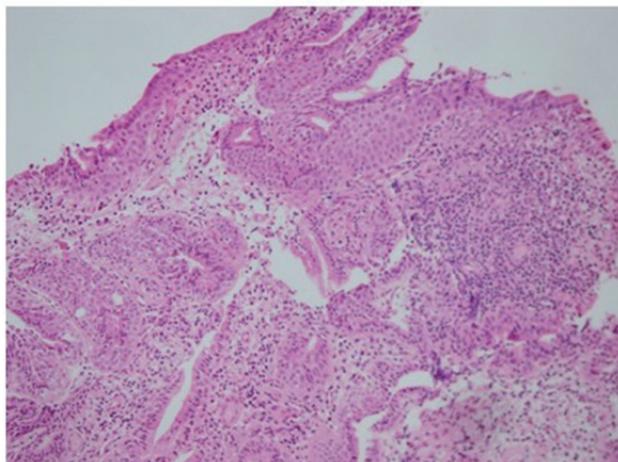
DISCUSSION

Malignancy development incidence in the published series ranges from 1.2 to 4.5%. Several hypotheses were proposed to explain tumor development. Inflammation secondary to chronic bacteriuria yielding cancerogenic nitrosamines, chronic contact of urine with the intestinal epithelium resulting in chronic irritation and interaction

Table-1 - Histopathologic findings (Table separated according to years passed over augmentation). “*”: biopsies with presumed native bladder tissue coming out as intestinal tissue (possible intestinalization)

Age-Gender	Period over augment (years)	Segment	Macroscopy	Native Bladder	Augment
28-♂	1	Ileal	No significant feature	Intestinal tissue*	Chronic inflammation
21-♂	4	Ileal	No significant feature	Intestinal tissue*	Chronic inflammation, fibrosis
15-♀	4	Ileal	No significant feature	Active inflammation	Active inflammation
23-♀	4	Ileal	No significant feature	Extensive squamous metaplasia	Active inflammation
21-♀	6	Ileal	No significant feature	Intestinal tissue*	Active inflammation
19-♀	6	Ileogastric	No significant feature	Intestinal tissue*	Chronic inflammation
11-♂	8	Ileal	No significant feature	Chronic inflammation	Chronic inflammation
22-♂	8	Gastric	No significant feature	Intestinal tissue*	Active inflammation
19-♀	9	Sigmoid	No significant feature	Intestinal tissue*	No inflammation
20-♂	10	Ileal	No significant feature	Active inflammation	Active inflammation
26-♂	10	Sigmoid	No significant feature	Chronic inflammation	Chronic inflammation
12-♂	11	Ileal	No significant feature	No inflammation	Active inflammation
29-♂	11	Ileal	No significant feature	Focal intestinal metaplasia	Active inflammation
17-♂	11	Ileal	No significant feature	Intestinal tissue*	Active inflammation
23-♀	12	Ileal	No significant feature	Intestinal tissue*	Active inflammation
17-♂	12	Ileal	No significant feature	No inflammation, fibrosis	Chronic inflammation
29-♀	13	Ileogastric	No significant feature	Intestinal tissue*	Active inflammation
22-♂	14	Ileal	No significant feature	Active inflammation	Active inflammation
16-♂	15	Ileal	No significant feature	Active inflammation	Active inflammation
25-♂	23	Sigmoid	No significant feature	Focal squamous metaplasia	Active inflammation

Figure 2 - Focal intestinal metaplasia (H-E staining, 200 magnification).



between two different types of epithelia leading to changes in intercellular interactions may contribute to malignant degeneration. Of course, many other factors like irritation from chronic catheterization, chronic asymptomatic bladder stones and mucus impaction may lead to development of chronic changes in the epithelial lining of both surfaces (2-9).

Several individual case reports and reviews on tumor development after bladder augmentation raised the concern on this issue (10-18). There are not many series in the literature regarding malignancy development following bladder augmentation. Thus, the series that have been published before were of extreme importance to us (Table-2).

Routine surveillance in patients with bladder augmentation is still a debate among scientific authorities. From the published literature, it seems that malignancies in patients with augmentation cystoplasty have aggressive progression and have to be diagnosed early. At the same time, some authors claim that malignancy rates are low and routine surveillance is a burden for both patient and government.

Soergel et al. in 2004 published a study where the authors detected 3 transitional cell carcinomas among 260 patients with bladder augmentations (3). The tumor development could not be associated to other predisposing factors. The

authors in the study proposed that bladder augmentation alone could be the cause of neoplastic development.

In 2008 Husmann et al. published another series where the authors detected 7 malignant tumors in 153 patients with bladder augmentation. The augmented enteric segment born adenocarcinomas were predominant in that study. There were 5 adenocarcinomas and 2 transitional cell carcinomas in total. In this study, direct correlation between augmentation and malignancy could not be proved due to various other predisposing factors like tobacco use and long term immunosuppression. The other probable predisposing factor stated in that study was bladder exstrophy (4).

Higuchi et al. proposed that annual surveillance of bladder tumors after enteroplasty is not cost effective due to low incidence of tumor formation. In a well-constructed long term study including 250 surveillance endoscopic evaluations, only 4 (1.6%) lesions were identified and none of them came out to be malignant (5).

More recently, Kispal et al. surveilled 54 patients with augmentation cystoplasty and detected metaplasia in 3, dysplasia in 6 and colonic segment adenocarcinoma in one patient. Metaplasia was seen in native bladder, enteric patch and anastomotic line. Dysplasia was detected mainly in the anastomotic region. The malignancy was detected 11 years after the augmentation procedure (6).

The difference in malignancy rates, study designs and low number of cases does not let the proposal of a consensus on malignancy surveillance of the patients with augmentation cystoplasty. Staying on the safe side urges pediatric urologists to perform routine cystoscopic evaluation especially when more than 10 years are passed over augmentation. The need for cystoscopic surveillance is not a debate itself, but planning of its frequency is.

The main emphasis of our study was made on histopathologic changes in neobladder in patients who were in the age group with no apparent risk for malignancy development. Our early and late complication rates in 77 bladder augmentation patients, regarding urinary incontinence, urolithiasis, metabolic issues, hematuria dysuria

Table-2 - Published routine cystoscopic surveillance series.

Author	Number of patients	Number of malignancies	Types of malignancies
Soergel et al. (3)	260	3	Transitional cell carcinoma
Husmann et al. (4)	153	7	Transitional cell carcinoma, enteric adenocarcinoma
Higuchi et al. (5)	65	None	-
Kispal et al. (6)	54	1	Enteric adenocarcinoma

syndrome, bladder perforations, Mitrofanoff stoma problems, capacity and compliance problems have been reported previously (19).

In our study, we did not detect malignancy in any of the 20 consecutive patients. However, we have shown different metaplastic changes in 3 patients. Two patients had squamous metaplasia and one had intestinal metaplasia. Intestinal metaplasia in bladder has always been a debate regarding whether it is precancerous or not. Some authors state that intestinal metaplasia is a precancerous change leading to adenocarcinoma. In contrary, Corica et al. published a series of 10 years-follow-up of 53 patients with intestinal metaplasia with zero malignancy development rate (20). Still we try to stay at the safe site of this debate and approach the patients with metaplasia with extra attention.

Two patients in our series with squamous metaplasia in native bladder raised a different concern in our minds. Both patients were bladder exstrophy patients and have undergone several operations including bladder exstrophy and epispiadias repair and bladder neck reconstruction procedures. One patient had several interventions for bladder stones at different times of disease course and this patient also developed vesicocutaneous fistula and was operated several times for that pathology. Even the problematic disease course of those two patients may have been the reason for metaplastic changes.

Regarding stone formation, all 3 stone patients developed during teenage period. In this age group, we noted a resistance against routine catheterizations and irrigations which we advise routinely for clearance of mucus. Thus, most of

the stone formations and problems related with mucus accumulations were mainly seen in these young patients. This problem usually disappeared when they became 16-17 years of age.

The type of augmentation in patients with metaplastic changes was ileocystoplasty and sigmoidocystoplasty. There was no obvious relation with the type of intestinal patch and underlying problem (like exstrophy or neurogenic bladder) as far as histopathologic changes were concerned. Also, literature data is not enough to conclude if the type of augmented tissue has an impact on metaplastic changes or malignancy formation.

The other interesting observation during this study was the inability to detect native bladder mucosa histologically or visualize it during cystoscopy. This fact was observed in 9 of 20 patients. One possible mechanism for that issue might be a very small bladder (as in bladder exstrophies) during the augmentation or multiple bladder neck operations which eventually reduced the size of native bladder. The other, yet unproven hypothesis which came to our mind during the study was the "mucosal changes in bladder mucosa to enteric mucosa" or "intestinalization of neobladder". This hypothesis only raised a scientific interest during the study but the proof of this issue seemed impossible because a full thickness biopsy would be needed to prove that. Yet this hypothesis may be a beginning for a design of in vitro cell and tissue culture study.

There were several limitations of our study. The most important was the number of the patients that could be enrolled in the study. The uneven distribution of patients in each group regarding period passed over augmentation made it

impossible to form a statistically healthy cohort. The other important limitation of the study was the rigid cystoscope that we had to use due to unavailability of the flexible cystoscope. To solve this issue, we planned the biopsy sites in triangular manner both in native bladder and enteric augment to address the widest area. However, inability to visualize periphery of the Mitrofanoff insertion point stayed as a drawback that we couldn't solve.

Chronic inflammatory changes were seen almost in all our patients even in late period. Stone formation may be an initiating factor, as far as metaplastic changes are concerned. However, we did not find an obvious relation between those two issues and at the same time there was no obvious relation between the intestinal or colonic patch which was used for augmentation and the type of inflammatory and metaplastic changes. Also, the Mitrofanoff procedure doesn't seem to have impact on secondary histopathological changes. However, exstrophy-epispadias complex may be a predisposing risk factor for metaplastic changes and potential malignancy. Still, we need more data to prove this hypothesis.

This is the first long-term histologic evaluation study of bladder augmentation in Turkey. In past decade, all patients in our clinic whose bladder augmentations were performed even less than 10 years ago were followed on a standard protocol which included annual radiologic screening and cystoscopic evaluation. However, during the progress of the study and with the new scientific data from the literature the annual cystoscopic evaluation in patients with less than 5 years after the augmentation was reserved to the patients with clinical complaints.

CONCLUSIONS

We think that malignant transformation in native or augmented patch of the neobladder is a serious long-term complication. Our initial strategy was to investigate those patients with cystoscopic examinations in late postoperative period. However, due to recent publications and our experience, we changed our approach (5, 6). Our recent protocol is to perform cystoscopy only

in symptomatic patients (e.g. hematuria, dysuria and any suspicious lesion in imaging studies). This kind of follow-up seems to be more comfortable to the patients and less costly to the government. At the same time, a better follow-up education program for adolescent patients aiming to solve the problem of "resistance against catheterization and irrigation" will decrease the mucus and stone formation problems. For that reason, a careful individualized follow-up of this group should be the strategy of choice for the next couple of decades.

CONFLICT OF INTEREST

None declared

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Medical ozone therapy reduces oxidative stress and testicular damage in an experimental model of testicular torsion in rats

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ABSTRACT

Objective: Testicular torsion (TT) refers to rotation of the testis and twisting of the spermatic cord. TT results in ischemia-reperfusion (I/R) injury involving increased oxidative stress, inflammation and apoptosis, and can even lead to infertility. The aim of this study was to investigate the effect of ozone therapy on testicular damage due to I/R injury in an experimental torsion model.

Materials and Methods: 24 male Sprague-Dawley rats were divided into 3 groups; sham-operated, torsion/detorsion (T/D), and T/D+ozone. Ozone (1mg/kg) was injected intraperitoneally 120 minutes before detorsion and for the following 24h. Blood and tissue samples were collected at the end of 24h. Johnsen score, ischemia modified albumin (IMA), total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI) levels were determined.

Results: Levels of IMA, TOS, OSI, and histopathological scores increased in the serum/tissue of the rats in the experimental T/D group. Serum IMA, TOS, and OSI levels and tissue histopathological scores were lower in the rats treated with ozone compared with the T/D group.

Conclusion: Our study results suggest that ozone therapy may exhibit beneficial effects on both biochemical and histopathological findings. Clinical trials are now necessary to confirm this.

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Keywords:

Ischemia; Oxidative Stress; Spermatic Cord Torsion

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INTRODUCTION

Testicular torsion (TT) results from the impairment of testicular and epididymal blood flow following rotation of the testicular spermatic cord and blood vessels (1). Progressive interruption of testicular venous flow then occurs. This subsequently leads to interstitial edema. Increasing and persis-

tent edema halts arterial blood flow, thus resulting in parenchymal ischemia. Prolonged ischemia may conclude in organ loss. This is generally frequently seen in the newborn, children, and adolescents and can lead to acute scrotum and require emergency intervention (1, 2). Time intervals between torsion and detorsion and degree of spermatic cord torsion are the main factors determining the severity of

testicular injury. In association with this loss of function, a decrease in fertility occurs in the ipsilateral testis, with testicular atrophy occurring in severe cases. Since blood flow in the testes is limited, these are particularly sensitive to ischemic injury (3). Although the basic pathological mechanisms are not yet fully understood, reactive oxygen species (ROS) resulting from ischemia and reperfusion (I/R) are known to play a role in tissue injury deriving from TT. I/R injury is characterized by neutrophil accumulation and increased pro-inflammatory cytokines, adhesion molecules, lipid peroxidation, and apoptosis (3-5). Oxidative phosphorylation is compromised due to insufficient oxygen caused by ischemia. Additionally, the Na⁺-K⁺ ATPase pump is inhibited as a result of an associated decrease in ATP levels. Intracellular Na⁺ and Ca²⁺ ion concentrations therefore increase. Intra and extracellular ion imbalance causes Ca²⁺ leakage into the mitochondria. An increase in mitochondrial Ca²⁺ activates various proteases and phospholipases, and cell lysis occur. These changes resulting from I/R injury trigger biochemical mechanisms, such as oxidative stress and inflammation (3). Various substances (phosphodiesterase inhibitors, vitamins, selenium, N-acetylcysteine, ethyl pyruvate, flavonoids, plant extracts, etc.) have been used in experimental studies to prevent this injury that can emerge following detorsion, and therefore the development of infertility (5-8). However, despite all the researches that have been performed, no additional therapeutic methods with easy clinical adaptation and proven utility have to date been obtained.

Medical ozone therapy is used in a wide spectrum for therapeutic purposes due to its antioxidant, anti-inflammatory, and antimicrobial effects. In contrast to treatment with pharmacological agents, ozone therapy provides defense against diseases by activating the body's antioxidant and anti-inflammatory pathways through the alarm reaction it creates, rather than through the classic drug-receptor relationship. The use of ozone therapy has been strongly emphasized in the treatment of diseases, such as chronic cutaneous ulcers, peritonitis, infected wounds, ischemic diseases, and joint problems (9, 10). In recent years in particular, studies have investigated the

protective effect of ozone therapy against testis injury induced by various means. Aydos et al. demonstrated that intraperitoneal ozone therapy exhibits a protective effect against I/R-induced testicular injury in a rat TT model by reducing levels of apoptosis and oxidative stress (11). Recently, Salem et al. reported that ozone therapy exhibits protective effects against adriamycin-induced testicular toxicity in an experimental rat model by reducing levels of oxidative stress and nitric oxide (NO) (12).

The purpose of this study was to investigate the effects of medical ozone therapy on experimental testicular I/R injury in biochemical and histopathological terms using such traditional biochemical parameters as ischemia modified albumin (IMA), total antioxidant status (TAS), total oxidant status (TOS) and the oxidative stress index (OSI).

MATERIALS AND METHODS

The experimental procedures in this research were approved by the Animal Care Ethical Committee of Karadeniz Technical University and were conducted in conformity with US National Institutes of Health guidelines. The experiments involved 24 male Sprague-Dawley rats (aged 4-6 months and with a mean weight of 250 g) fed on a standard chow pellet diet and with ad libitum access to tap water. These animals were housed in steel cages until the time of the study, under controlled lighting (lights on between 8:00 and 20:00h) at a temperature of 21-23°C. Water only was provided for the last 12h before the experiments.

Twenty-four rats were randomly assigned into one of three groups of eight members each. General anesthesia was induced with intramuscular injection of 10mg/kg of xylazine and 50mg/kg ketamine. The control group was subjected to a sham procedure (scrotal incision) only. In the other groups, the left testis was rotated 720 degrees clockwise to establish torsion. This was then maintained by fixing the testis. In the T/D group, detorsion was performed following 2 hours of torsion and then maintained for the subsequent 24 hours. These T/D procedures were repeated in the medical ozone group, but 1mg/kg ozone (Evozone

BasicPlus, Germany), was administered intraperitoneally (IP) immediately prior to detorsion for 2 hours. Blood samples were collected from the abdominal aorta of all rats 24 hours after detorsion. Table-1 provides a summary of the procedures performed in the different experimental groups.

Blood specimens were placed into separator tubes without anticoagulant and centrifuged at 2000×g for 10 min. The serum specimens obtained were divided into small volume tubes and stored

me. These were subjected to deparaffinization and then stained with hematoxylin-eosin (H&E). The preparates were analyzed under a light microscope (Olympus BX 51, Tokyo, Japan). The Johnsen Testicular Biopsy Score system was used to evaluate testicular tissue injury. Under that system, testis tissues were evaluated semi-quantitatively in five different areas at high magnification (200×) under light microscopy (14). A pathologist evaluated the testicular tissues using standard light

Table 1 - A summary of the procedures in the experimental groups.

	Groups		
	Control	T/D	Medical Ozone Plus T/D
Torsion 0 min		+	+
Immediately before detorsion			1mg/kg ozone
Detorsion +2 hours		+	+
Blood and Tissue Samples +24 hours	+	+	+

T/D = Torsion/Detorsion

at -80°C until biochemical measurements.

The colorimetric method described by Bar-Or et al. was used to determine IMA levels (13). The results were expressed as absorbance units (ABSU). Commercial colorimetric kits (Rel Assay Diagnostics, Gaziantep, Turkey) were used to determine TOS and TAS levels in rat sera. TOS results were expressed as µmol H₂O₂ equivalent/L and TAS results as mmol trolox equivalent/L. The TOS:TAS ratio was used as the OSI. For that purpose, the unit of TAS, mmol trolox equivalent/L, was converted to µmol trolox equivalent/L, and OSI was calculated using the formula:

$$OSI = [(TOS, \mu\text{mol H}_2\text{O}_2 \text{ equivalent/L}) / (TAS, \mu\text{mol trolox equivalent/L}) \times 10].$$

The testis tissue specimens obtained were fixed for 72h in Bouin’s solution for histopathological analysis. Care was taken to collect tissue specimens from approximately the same sections. The fixed tissue specimens were dehydrated by passing through 70%, 90%, 96% and 100% alcohol series. They were then rendered transparent by being passed through xylene solution. Following preparation of paraffin blocks, sections 5µm in thickness were taken using an automatic microto-

microscopy. This examination was completed in a random order and a blinded fashion. The histological sections were graded for testicular injury and spermatogenesis using the Johnsen score (JS). A minimum of 50 tubules were evaluated, with each tubule being scored from 1 to 10. Ten points expressed complete spermatogenesis with regular tubules; 9 points, many spermatozoa and irregular germinal epithelium; 8 points, presence of few spermatozoa; 7 points, no spermatozoa, many spermatids; 6 points, no spermatozoa, few spermatids; 5 points, no spermatozoa or spermatids; 4 points, few spermatocytes; 3 points, presence of spermatogonia; 2 points; sertoli cells only; and 1 point, complete absence of germ cells and spermatogenesis (6).

Statistical analysis was performed on SPSS 23.0 software. Kruskal-Wallis variance analysis (the Mann-Whitney U test with Bonferroni correction as post hoc) was used to compare the study groups. Statistical significance was set at p <0.05.

RESULTS

Oxidative stress markers and histopatho-

logical scores were the principal parameters for evaluating the degree of I/R damage and the effectiveness of medical ozone treatment in this study. No complication related to the T/D model or the administration of ozone therapy was observed. No mortality was observed in any group until the end of the experiment. Comparisons of group's biochemical parameters (IMA, TAS, TOS, and OSI) and histopathological scores are summarized in Table-2. Results are expressed as medians (interquartile range).

Serum IMA, TOS, and OSI levels were significantly higher in the T/D group compared to the control group ($p=0.006$, 0.0001 , and 0.0001 , respectively), but the levels of these parameters were significantly reduced by medical ozone therapy ($p=0.0001$ for all parameters). However, no significant difference was determined between the groups in terms of serum TAS levels ($p > 0.05$).

The histopathological score was significantly higher in the T/D group compared to the control and medical ozone therapy groups ($p=0.0001$, and 0.001 , respectively). The histopathological score in the medical ozone therapy group was also significantly lower compared to the score in the T/D group ($p=0.001$). In the control group, regular seminiferous tubular morphology with normal spermatogenesis were detected. In the T/D group, seminiferous tubule germinal epithelial structure was completely

poured. Spermatozoa were not available in the lumen and vasoconstriction was partly observed in the intertubular area. The seminiferous tubule epithelial structure was more regularly in the medical ozone therapy group compared to T/D group. In the medical ozone therapy group, germinal epithelial cells showed regular alignment in the lumen and spermatozoa were partly observed (Figure-1).

DISCUSSION

Testicular torsion is one of the emergency conditions frequently seen in the newborn and adolescent periods, and one that can lead to testicular injury or even subfertility. Since the testis is one of the most sensitive organs to hypoxia, even short-term torsion may lead to significant injury, such as hypoxia in testicular tissue, cell damage, and cell death. Oxidative stress and the inflammatory process associated with ROS are involved in the etiology of I/R injury observed during TT-detorsion. Irrespective of the etiological factor and despite research into alternative medical treatment models, emergency surgical intervention remains a valid and the most commonly applied treatment modality (7). Many pharmacological agents, such as phosphodiesterase inhibitors, vitamin C and E, selenium, flavonoids, NSAID, ethyl pyruvate, and N-acetyl cysteine have been investigated in animal

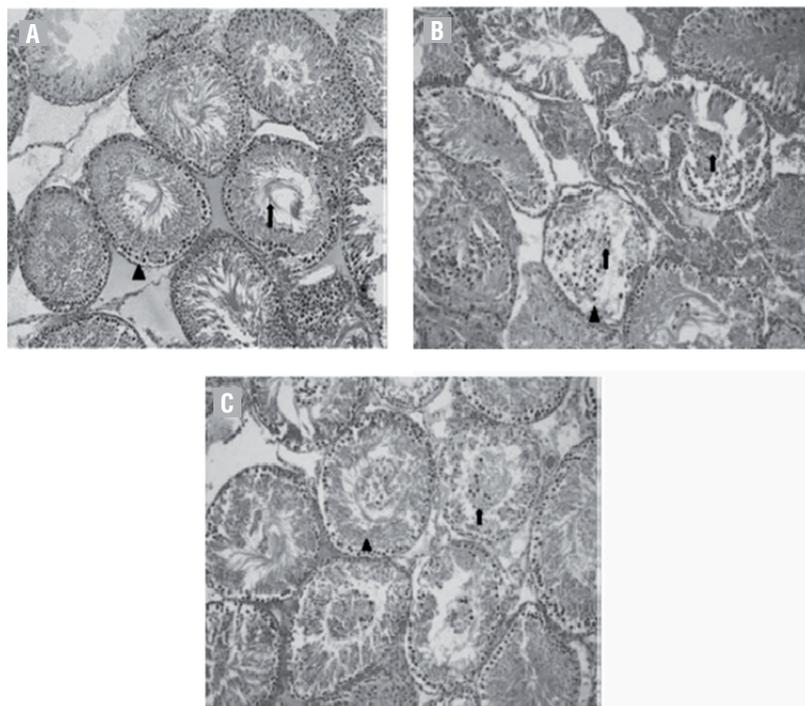
Table 2 - A comparison of biochemical parameters and histopathological scores in the groups.

	Groups		
	Control	T/D	Medical Ozone Plus T/D
IMA	0.794 (0.783-0.806)	0.853 ^a (0.834-0.861)	0.737 ^b (0.710-0.778)
TAS	0.437 (0.421-0.472)	0.436 (0.300-0.478)	0.375 (0.357-0.389)
TOS	16.0 (13.7-18.8)	42.5 ^c (32.7-55.4)	18.0 ^b (15.3-21.5)
OSI	3.9 (3.0-4.4)	9.9 ^c (7.4-16.6)	4.9 ^b (3.6-6.4)
HS	10 (10-10)	5.20 ^c (4.90-5.35)	8.20 ^{c, d} (7.85-8.35)

Values are expressed as median (Percentiles 25-75). **IMA** = Ischemia Modified Albumin (Absorbance Unit: ABSU); **TAS** = Total Antioxidant Status (mmol trolox equivalent/L); **TOS** = Total Oxidant Status ($\mu\text{mol H}_2\text{O}_2$ equivalent/L); **OSI** = Oxidative Stress Index; **HS** = Histopathological score; **T/D** = Torsion/Detorsion.

^a $p=0.006$ compared with control group; ^b $p=0.0001$ compared with T/D group; ^c $p=0.0001$ compared with control group; ^d $p=0.001$ compared with T/D group.

Figure 1 - Ipsilateral testis (x200, hemotaxylin and eosin stain [H&E]). A) A section from the sham with group, normal seminiferous tubule epithelial structure (Δ) and spermatozoons (\uparrow) were observed. B) A section from the T/D group, seminiferous tubule germinal epithelial structure was completely poured (Δ), and luminal irregular germinal cells (\uparrow) were observed. C) A section from the medical ozone therapy group, the germinal epithelial structure was regular (Δ) and regular lineage germinal epithelial cell (\uparrow) observed in the lumen.



models for their potential as adjunctive therapies to the surgical repair of TT. These chemicals generally have anti-inflammatory, antioxidant or ROS-scavenging properties (5-8). However, these chemical agents are little employed in routine clinical practice for reasons, such as insufficient effectiveness, safety concerns, and a lack of information concerning dosages (7). In recent years in particular, ozone therapy has been shown to exhibit positive effects on wound healing and pathological conditions, such as age-related macular degeneration and ischemic and infectious diseases. These effects of medical ozone therapy have been attributed to more than one mechanism (such as increasing 2, 3-bisphosphoglycerate levels in erythrocytes, providing platelet activation, and raising antioxidant enzyme levels) (9, 10). The purpose of this study was therefore to determine the protective effect of medical ozone therapy against I/R injury induced in the rat testis using oxidative stress markers and his-

topathological scoring. The measurement of changes in IMA, TAS, TOS, and OSI is often used as an index of oxidative stress in biological systems (15). These markers were therefore employed to evaluate oxidative stress in this study. Our results show that medical ozone therapy significantly reduced IMA, TOS, and OSI values that normally rise in a TT model. Histopathological analysis also revealed that medical ozone therapy significantly reduced scores that increase as a result of torsion.

Previous studies have also investigated the protective effect against testicular injury of medical ozone therapy. Ekici et al. reported that ozone therapy protected against I/R damage in an experimental unilateral TT model in rats. Ozone therapy significantly suppresses and induces malondialdehyde (MDA) and glutathione (GSH) levels, respectively. It has also been shown to significantly protect testicular tissue against I/R injury measured on the basis of Johnsen scores (16). Aydos et al.

determined that medical ozone therapy exhibited a protective effect against TT-induced I/R injury by reducing apoptosis and iNOS and increasing catalase enzyme activity (11). Salem et al. recently evaluated the protective effect of ozone treatment on adriamycin-induced testicular toxicity. They showed that medical ozone therapy exhibited positive effects on sperm numbers, motility, and viability in an induced model of testis injury. That study also reported that medical ozone therapy suppressed oxidative stress by reducing MDA and NO levels (12).

Recent studies have shown that ozone pre-conditioning is an effective means of preventing I/R damage in various organs, such as the liver, lung, intestine, ovary, and kidney. Chen et al. demonstrated that ozone therapy inhibits inflammation and apoptosis after renal ischemia/reperfusion injury in rats. They observed that increased levels of oxidative stress and inflammation (myeloperoxidase activity and the expression of interleukin-1 beta, tumor necrosis factor alpha, and intercellular adhesion molecule-1) markers were reduced by ozone therapy (17). Di Filippo et al. reported that acute oxygen-ozone therapy protects rats against the I/R damage in an experimental acute myocardial infarction model. Infarct size and levels of 3-nitrotyrosine (a product of protein oxidation), interleukin-6, interleukin-8, and caspase 3 are reduced by medical ozone therapy in a concentration-dependent manner (18). Haj et al. observed that treatment of I/R rats with ozone/oxygen mixture resulted in a significant decrease in intestinal injury scores and numbers of apoptotic cells in the ileum (19). Onal et al. reported that ozone administration increased the levels of superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), and TAS and reduced the level of TOS in an experimental intestinal I/R model. No difference was observed between the groups in terms of MDA or protein carbonyl levels in that study. Histopathological evaluation showed that pre-treatment with peritoneal ozone prevented intestinal mucosal injury caused by I/R (20). Sayar et al. recently reported that medical ozone therapy exhibits a protective effect on rat ovaries in an I/R injury model by reducing oxidative stress (21).

ROS derive from normal metabolic reactions and are involved in a wide range of processes, including apoptosis and cell signaling. They also oxi-

dize lipids contained in the cell and mitochondrial membranes, thus modifying membrane permeability and compromising cellular integrity. Ozone therapy is associated with effective regulation of oxidative stress at the cellular level. Previous studies have identified numerous beneficial biochemical effects of ozone therapy that raise antioxidant activity, which is believed to ready tissues for exposure to oxidative stress. The pathophysiology of the anti-inflammatory and antioxidant characteristics of ozone administered at therapeutic doses is still unclear, since ozone decomposes numerous components of blood. Ozone has been reported to increase the activity of antioxidant enzymes, such as GPx, SOD, and CAT. These enzymes ready the host for ROS-induced physiopathological conditions (2, 18). In the present study, serum IMA, TOS and OSI levels increased in untreated rats but, decreased in those administered ozone therapy. This suggests that one potential beneficial effect of ozone may be to minimize tissue damage via improved antioxidant enzyme activity.

Ozone therapy may prevent injury if oxidant status is dominant. However, if there is no challenge to the oxidant/antioxidant balance, then ozone may be deleterious. In order to obtain maximum benefit from the biological effects, the dose of ozone applied should be calculated very carefully (22). The concentration of ozone in medical therapy in previous reports varies between 0.1 and 4mg/kg, and it was administered IP. Both concentration of ozone (1mg/kg) and treatment time (2h) in this study were therefore compatible with previous studies (2, 11, 16, 18, 22).

CONCLUSIONS

Our data suggest that ozone therapy reduces the severity of I/R injury in an experimental model of TT by inhibiting oxidative stress. Our findings indicate that outcomes of TT can be improved by employing ozone therapy as an adjuvant therapy. However, further studies involving well-designed experimental models are now needed to clarify the mechanisms of action by which ozone exerts its effects.

Ethics Committee Approval

Ethics committee approval was received.

ved for this study from the ethics committee of Ethical Committee for Experimental Research on Animals.

CONFLICT OF INTEREST

None declared.

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Lysozyme gene treatment in testosterone induced benign prostate hyperplasia rat model and comparasion of its' effectiveness with botulinum toxin injection

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ABSTRACT

Objectives: To compare the effects and histopathological changes of botulinum neurotoxin type A and lysozyme gene injections into prostate tissue within a testosterone induced benign prostate hyperplasia rat model.

Materials and Methods: 40 male Wistar rats were randomized into four Groups. Group-1: Control, Group-2: Testosterone replacement, Group-3: Testosterone+botulinum neurotoxin type A, Group-4: Testosterone+plazmid DNA/liposome complex.

Results: Estimated prostate volume of the testosterone injected Groups were higher than the control (p <0.05). Actual prostate weight of the testosterone injected Groups was higher than the control Group (p <0.05). Testosterone undecanoate increased the prostate weight by 39%. Botulinum neurotoxin type A treatment led to an estimated prostate volume and actual prostate weights decreased up to 32.5% in rats leading to prostate apoptosis. Lysozyme gene treatment led to an estimated prostate volume and actual prostate weights decrease up to 38.7%.

Conclusion: Lysozyme gene and botulinum neurotoxin type A treatments for prostate volume decreasing effect have been verified in the present study that could be a new modality of treatment in prostatic benign hyperplasia that needs to be verified in large randomized human experimental studies.

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Keywords:

Prostatic Hyperplasia; Botulinum Toxins; Testosterone

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INTRODUCTION

The most frequent cause of lower urinary tract symptoms (LUTS) is benign prostate hyperplasia (BPH). Increase in BPH incidence with age raises concern for both health and economy policies (1). The current medical treatments of BPH have some adverse effects such as dizziness, asthenia, postural hypotension in patients taking α -adrenergic antagonists, and decreased libido

and impotence in patients taking 5 α -reductase inhibitors. Therefore, researches are still in progress leading to constantly increasing costs of medical companies and health organizations.

Intra-prostatic botulinum neurotoxin type A (BoNT-A) injection is among novel treatment options of BPH in several rat, dog, and human studies (2-6). The prostate is a secretory organ with several molecules in its secretion including lysozyme (Lys). Lys has besides its antibacterial

effects also antitumor and immunomodulation effects (7). In animal studies, testosterone replacement is reported to cause BPH (8-10).

OBJECTIVE

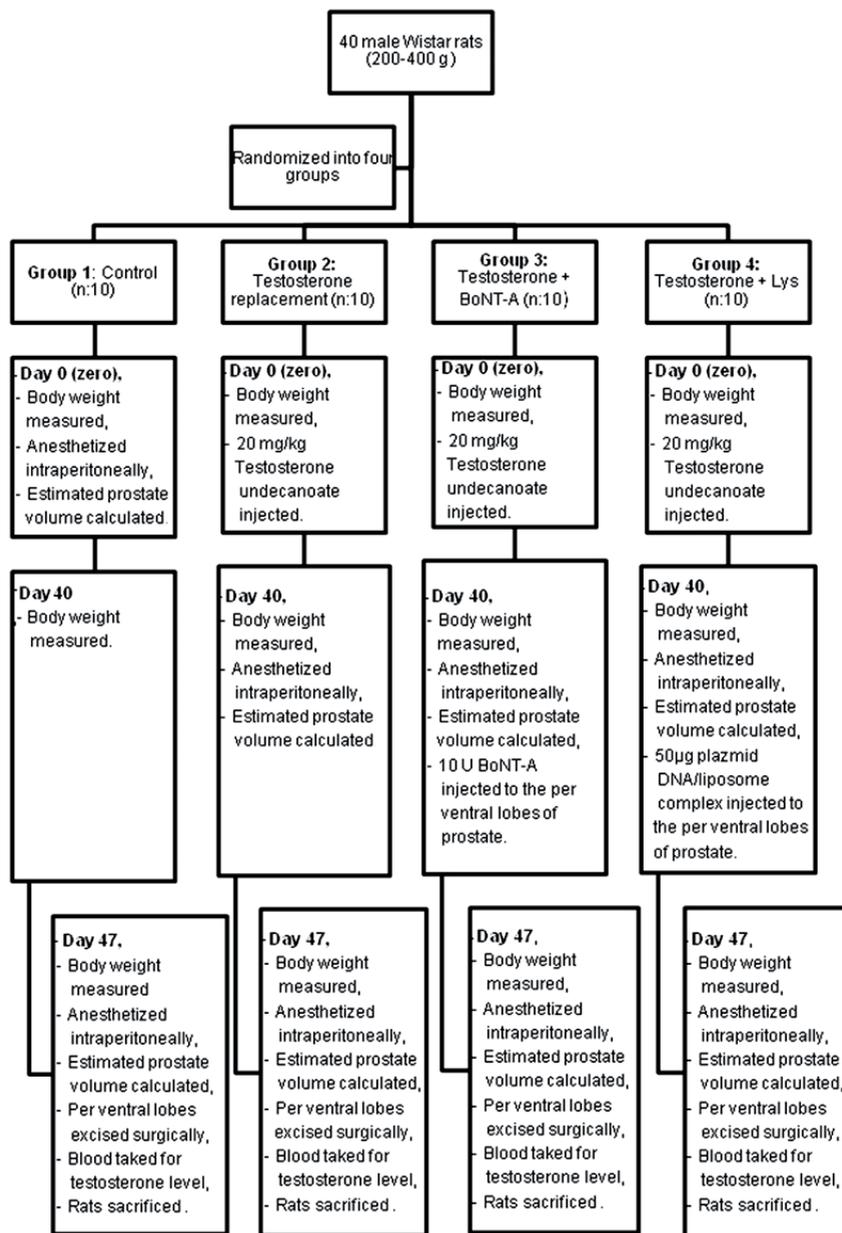
The aim of the present study is to compare the effects and relevant histopathological changes of intra-prostatic BoNT-A and Lys gene injection

into BPH tissue in a prospective controlled testosterone induced BPH rat model.

MATERIALS AND METHODS

Upon the approval of Süleyman Demirel University Animal Experiments Local Ethic Board, 40 male Wistar rats of 200-400g were included in the present study (Figure- 1). In each cage 5 rats were

Figure 1 - Experimental protocol.



kept in a room temperature of 22.4o Celsius. These were randomized into four Groups as following: Group 1: Control (n: 10), Group 2: Testosterone replacement (n: 10), Group-3: Testosterone+BoNT-A (n: 10), Group 4: Testosterone+Lys (n: 10).

At the beginning of the experiment, body weight of all rats was measured using a Pioneer made precision scale. This day was labeled as day zero. In order to anesthetize the rats in Group-1, 10mg/kg xylazin hydrochloride (Rompun®; Bayer Healthcare, Leverkusen, Germany) and 90mg/kg ketamine hydrochloride (Ketalar®; Pfizer, Istanbul, Turkey) were administered intraperitoneally. In supine position, right and left ventral lobes of the prostate were accessed via a midline abdominal incision under anesthesia. A Hart made mechanic compass was used for the three dimensions of the prostate. Estimated prostate volume (EPV) was calculated using ellipsoid formula “width x length x height x 0.5236”. Into the right gluteal muscle of Groups 2, 3, and 4 rats, 20mg/kg Testosterone undecanoate (Nebido®; Bayer Schering Pharma) was injected. A waiting period of 40 days was considered for BPH development.

40 days subsequent to testosterone injection, the body weight of all the rats was measured again. The dimensions of rat prostate lobes in Groups 2, 3, and 4 were measured via a midline abdominal incision access under anesthesia. 10U BoNT-A (BOTOX®; Abdi İbrahim, Istanbul, Turkey) with 0.2mL isotonic saline was injected under anesthesia to the per ventral lobes of the rats in Group 3. 50µg plasmid DNA/liposome complex in 10µL solution was injected to the per ventral lobes of the rats in Group-4 under anesthesia.

Forty seven days following testosterone injection, the body weight of all rats was remeasured. As previously described, the prostate volumes of the rats were recalculated under anesthesia. Per ventral lobes of all animals were excised surgically, weighted using a precision scale, and recorded. Following blood sampling to measure testosterone levels, all rats were sacrificed via exsanguination. All tissues obtained were embedded in 4% paraformaldehyde (Merck) and stained with hematoxylin-eosin before histological evaluation and scoring using a light microscope. In order to obtain an objective comparative evaluation of the

main histopathological findings a chart score protocol was used, which was described by Scolnik et al. (9). This protocol took into account the acinar morphology, such as crowding, intraluminal villosities, loss of basal nuclear polarity, and hyperplastic nodules, which were scored according to their degree of severity and distribution pattern. Tissues were categorized as normal and BPH. All animal's samples were analyzed immunohistochemically using Rabbit Active Caspaz-3 (Santa Cruz, sc-44976) and rabbit ABC Staining Kit (Santa Cruz, sc-2018). The active caspase 3 activity was used for apoptosis indicator. Asinus photos were taken at 400x enlargement in randomly chosen areas and 100 epithelial cells were counted for each sample. It was determined how many of these epithelial cells were positive for active caspase-3 staining. Rat blood testosterone levels were evaluated using Bmassay rat testosterone Elisa Kit.

Lysozyme gene preparation: pHM6 mammalian expression vector is a plasmide shuttle vector at 5442bp length. It can reproduce in many *E. coli* strains. Vector and *E. coli* XL1-Blue MRF bacteria had been obtained from Rize University, Medical Faculty, Department of Medical Biology and Genetics.

As the DNA molecules of the present pHM6 plasmide vector were limited in number, they were first transferred to *E. coli* and transformed recombinant bacteria stocks were formed. Using endotoxin-free Plasmide Midiprep kit, plasmide DNA isolations were achieved. The ideal form for transfection was plasmide DNA in supercoil form. DOTAP/DNA complex (for 1µg DNA 5-10µg DOTAP) was used for liposomal transfection in line with the suggestions of the manufacturer. Lipid vesicles and pHM6m Lys DNA complexes were hence formed.

Data obtained were analyzed using SPSS 15.0 package program (Statistical Package for the Social Sciences for Windows). Measures of central tendency and data distribution were evaluated. The normality and homogeneity of the data were evaluated using Kolmogorov Smirnov Test and One-Way ANOVA (homogeneity of variance). In the comparison of independent Groups, Mann Whitney U Test, Friedman variant analysis and in dependent Groups Wilcoxon T Test and Kruskal-Wallis variant analyses were employed.

RESULTS

The mean body weight of all the rats at day zero was 305.3 ± 43.7 and at the 47th day 327.2 ± 47 gr. There was no statistically significant difference among the Groups in terms of same day body weight ($p=0.149$). However, statistically significant differences in terms of body weight were present on comparing day zero and 47th day within the groups ($p<0.05$). The rats had gained weight during the experiment. Only one rat died throughout the study (Group 4 no: 6). The mean testosterone level of the rats in Group 1 was 0.46 ± 0.11 ng/mL, Group 2 0.74 ± 0.43 ng/mL, Group-3 0.76 ± 0.32 ng/mL, and Group-4 0.77 ± 0.29 ng/mL. Blood testosterone levels of the testosterone injected Groups were significantly higher than Group 1 ($p < 0.05$).

The mean EPV values of Group 1 on day zero and 47th day and of Groups 2, 3, and 4 on the 40th and 47th day are presented in Table-1. There was no difference in the EPV of the rats in Group 1 at the beginning and at the end of the study (Table-1). No statistically significant difference was present in the testosterone injected Groups in terms of prostate growth on the 40th day (Table-2). A statistically significant growth was seen in the EPV of the testosterone injected Groups on the 40th day compared with Group 1 at the 47th day.

EPV of Groups 2, 3, 4 on the 47th day were compared. There was a significant decrease in the

EPV of the Groups that underwent a medical treatment compared with Group 2 (Table-3). 40th and 47th day intragroup EPV comparison revealed a statistically significant decrease in both the BoNT-A and the Lys treatment group ($p=0.012$).

Mean weight of the actual prostates upon excision is presented in Table-4. The comparison of Group 1 and 2 per ventral prostate lobe weight revealed a statistically significant difference ($p=0.028$, $p=0.006$). Testosterone leads to an increase in the prostate weight of the rats.

A statistically significant difference was present in the comparison of the actual per ventral prostate lobes of Group 2 with Groups 3 and 4 ($p<0.05$, $p<0.05$). On comparing the actual per ventral prostate lobe weights of Groups 3 and 4, no statistically significant difference was seen ($p>0.05$, $p>0.05$). Both BoNT-A and Lys treatment led to statistically significant losses in actual prostate sizes.

The histological examination of Group 1 revealed that the acini preserved their lumen, structure, and space layout between acini. The acinar lumen was filled with homogenous acidophilic material. In some cases, very slight villous projections were observed. The glandular epithelium consisted of a single layer and was composed of small, round core, largely cubic or low columnar cells. The acini were surrounded with a thin stroma and the base membrane was thin and uninterrupted (Figures 2A

Table 1 - EPV measures made at different times and analysis of intra-group data.

	Gruplar	0 day	40 day	47 day	p
Group 1	RVPL	93.5±39.7	-	94.4±39.5	0.674
	LVPL	80.4±49.9	-	90.8±34.8	0.237
Group 2	RVPL	-	162.3±60	162.8±59.5	0.651
	LVPL	-	154.2±51.9	154.5±52.2	0.697
Group 3	RVPL	-	148.6±34.2	101.4±33	0.012
	LVPL	-	123.3±22.3	93.2±18.4	0.012
Group 4	RVPL	-	163.3±57.8	97.1±42.2	0.008
	LVPL	-	147.9±41	95.3±27.7	0.008

± = Standard deviation; **P** = *Wilcoxon T Tes*

RVPL = Right ventral prostate lobes

LVPL = Left ventral prostate lobes

Measurement = mm³ (cubic millimeter)

Table 2 - EPV Comparative analyses of Group 1 at the 47th day and Groups 2, 3, and 4 at the 40th day.

	RVPL	LVPL
	p	p
Group 1-2	0.007	0.002
Group 1-3	0.007	0.009
Group 1-4	0.018	0.009
Group 2-3	0.583	0.754
Group 2-4	0.656	0.863
Group 3-4	0.713	0.614

P = Mann Whitney U Test

RVPL = Right ventral prostate lobes

LVPL = Left ventral prostate lobes

Table 3 - EPV Comparative analyses of the groups at the 47th day.

	RVPL	LVPL
	p	p
Group 2-3	0.026	0.002
Group 2-4	0.014	0.006
Group 3-4	0.735	0.923

P = Mann Whitney U Test

RVPL = Right ventral prostate lobes

LVPL = Left ventral prostate lobes

Table 4 - Actual prostate weight of the groups.

	Group 1		Group 2		Group 3		Group 4	
	Right	Left	Right	Left	Right	Left	Right	Left
1	263	500	372	330	280	223	236	159
2	245	210	312	310	182	130	149	88
3	208	238	590	504	276	255	357	197
4	197	216	469	326	235	212	214	213
5	135	126	432	371	334	316	275	213
6	394	207	344	385	490	156	*	*
7	292	293	264	296	185	201	192	203
8	361	313	370	320	252	174	240	203
9	501	•	340	333	220	210	355	363
10	156	227	393	373	276	223	218	224

* = Ex; • = Single lobe

Measurement = g (gram)

and B). In Group 2, there were histological changes in line with BPH (Figure-2C). The epithelium was either cubic or cylindrical. The nucleus was either round or ovoid. There was mitotic activity in some of the cells. In some cases, there were either isolated or at multiple sites cell groupings in piling up formations. The basal membrane was thin and continuing. However, the H&E stained sections of the Groups 3 and 4 revealed some alteration at histological changes that was seen in Group 2.

Mean apoptotic number of cells in Group 1 was 26.1 ± 7.3 , in Group 2 22.9 ± 5.2 , in Group 3 34.3 ± 5.6 and in Group 4 33.3 ± 5.2 . The number of apoptotic cells increased significantly comparing Groups 1 & 2 with the groups receiving treatment ($p < 0.005$).

ally, in BPH studies the ventral lobe and in prostate cancer studies the dorsal and lateral lobe are preferred. The ventral lobe is a common site for prostatic hypertrophy and normal epithelial cells in the ventral lobe from aged rats are low cuboidal or flattened as compared to those in the dorsolateral lobe; recognition of a proliferative lesion by light microscopy is easy under low magnification (11). In the present we preferred the ventral lobes.

To form BPH model in rodent prostate, generally testosterone propionate, testosterone enanthate, estradiol, phenylephrine, growth factors, fetal urogenital sinus implants etc. are used (8-10, 12-15). In forming BPH model, the rat strain is also important. Scolnik et al. studied benign and atypical hyperplasia in the ventral prostate lobes of Wistar, Sprague-Dawley, Fischer and ACI/Ztm

Figure 2 - A) Normal prostate tissue. Regular acinus forms, homogeneous lumen secretion and connective tissue density. Hematoxylin-eosin (HE) stain, magnification 200X. Scale Bar: 500µm.; B) The acini were surrounded with a thin stroma and the base membrane was thin and uninterrupted. Hematoxylin-eosin (HE) stain, magnification 400X. Scale Bar: 100µm.; C) Hyperplastic prostate tissue. Columnar and overly curved acinus epithelium, irregular acinus and fibrotic connective tissue. Hematoxylin-eosin (HE) stain, magnification 200X. Scale Bar: 500µm.



DISCUSSION

The morphology and pathology of the dog prostate resembles human prostate most. However, dogs are in the category of higher vertebrates and limited in number in experimental animal laboratories and thus present increased costs. Therefore, in prostate studies rats and mice are preferred due to morphologic similarities despite the lack of an obstructive pattern. In rodent animals, the prostate lobes are classified as dorsal, ventral, lateral, and anterior (coagulating glands) lobes. Gener-

adolescent male rat strains in 1994 (9). They reported that Wistar is the most appropriate strain for the induction of prostate hyperplasia. In the present study, we preferred Wistar rats. In order to induce BPH 20mg/kg Testosterone undecanoate was used.

Comparison of rat prostate weight is among the different methods suggested in the literature to demonstrate BPH induction. In this method, the prostate weights are compared with the control Group or the Groups that were sacrificed at different times (8, 16-18). In the present study,

we measured EPV (calculated using length, width, and height of per ventral prostate lobes) and APV (measured following surgical excision). EPV statistical analyses revealed no statistically significant difference in the initial and final values of control Group. We determine that the EPV of the testosterone injected Groups were higher than the control Group. The actual prostate weight of the testosterone injected Groups was higher than the control Group enabling us to attribute the increase to testosterone injection. Using 20mg/kg testosterone undecanoate increased the prostate weight of the rats 39%.

Another method used to demonstrate BPH induction, suggested in the literature, is histological evaluation (9-11, 19). The findings of the present study were in line with the findings in the literature.

Botulinum neurotoxin, discovered first in 1897, has been used as a therapeutic agent since 1977. BPH impact has been studied since 2003. BoNT-A injection to the urethra or bladder provides long term relief, 6 months or more, in lower urinary system dysfunctions (20). It was reported to inhibit urethral norepinephrine release and to cause atrophy in the prostate glands through selective denervation (20, 21). Human studies reported a decrease up to 40% in prostate size and a recurrence to the initial treatment size within approximately 18 months (2, 22, 23). 10 units of BoNT-A were used in the present study. BoNT-A treatment led to a EPV and actual prostate weights decrease up to 32.5% in rats leading to prostate apoptosis.

Lys is an important antimicrobial enzyme of the defense system. Besides its antimicrobial activities, it is also known to inactivate certain virus Groups, preserve cell membrane of mammals, to increase polymorphonuclear leukocytes, macrophages and monocytes phagocytic/cytotoxic activity, to stimulate monocytes for analgesic, anti-tumoral, anti-metastatic, and anti-inflammatory activity, immunoglobulin production, and the induction of fosfolipid vesicular unity and thus increasing tumor cell immunogenicity (7, 24). The prostate is a secretory organ with several molecules in its secretion including Lys. Recent research has shown that lysozyme was a immunohistoche-

mical marker in prostatic ductal adenocarcinomas (25, 26). In the present study, in order to evaluate the impact of Lys to BPH model intra-prostatic ~50µg plazmid DNA/liposome complex was applied. At the end of the study, Lys treatment led to a decrease in both EPV and actual prostate weight. The decrease in the prostate weight was determined as 38.7%.

Androgen effects on the prostate are mediated by dihydrotestosterone, which is converted from testosterone by the enzyme 5α-reductase. Two 5α-reductase inhibitors are available for clinical use: dutasteride and finasteride. 5α-reductase inhibitors act by inducing apoptosis of prostate epithelial cells leading to prostate size reduction of about 18-28% and a decrease in circulating PSA levels of about % 50 after 6-12 months of treatment (27).

Pharmacological therapies with 5α-reductase inhibitors have gained widespread acceptance as safe and effective treatments for BPH. But they have adverse effects such as reduced libido, erectile dysfunction and less frequently, ejaculation disorders such as retrograde ejaculation, ejaculation failure or decreased semen volume (27-29). Their effect on the serum PSA concentration needs to be considered for prostate cancer screening. Also, due to the slow onset of action, they are suitable only for long-term treatment. At this point, BoNT-A and Lys may be an alternative treatment option because of their safety, effectiveness, and quick onset effect. However, further studies are needed especially on humans to determine their safety and effectiveness.

CONCLUSIONS

The present study was planned to answer the need for more effective non-invasive BPH treatment in the constantly aging male population throughout the world. The findings of the present study have shown that both BoNT-A and Lys treatment decreases the prostate weight in BPH. In terms of efficacy, there was no difference between both Groups.

Lys in BPH treatment has been first used in the present study. In rat BPH model, its prostate volume decreasing effect has been verified. As the

present study proposes a novel treatment modality in BPH it should be verified in large randomized human experimental studies.

ABBREVIATIONS

BPH = Benign prostate hyperplasia

BoNT-A = Botulinum toxin

EPV = Estimated prostate volume

LUTS = Lower urinary tract symptoms

Lys = Lysozyme

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Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. The study design was approved by the Süleyman Demirel University Animal Experiments Local Ethic Board, Turkey.

CONFLICT OF INTEREST

None declared.

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Prospective evaluation of vesicourethral anastomosis outcomes in robotic radical prostatectomy during early experience in a university hospital

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ABSTRACT

Purpose: Robotic assisted radical prostatectomy (RARP) presents challenges for the surgeon, especially during the initial learning curve. We aimed to evaluate early and mid-term functional outcomes and complications related to vesicourethral anastomosis (VUA), in patients who underwent RARP, during the initial experience in an academic hospital. We also assessed possible predictors of postoperative incontinence and compared these results with the literature.

Materials and Methods: We prospectively collected data from consecutive patients that underwent RARP. Patients with at least 6 months of follow-up were included in the analysis for the following outcomes: time to complete VUA, continence and complications related to anastomosis. Nerve-sparing status, age, BMI, EBL, pathological tumor staging, and prostate size were evaluated as possible factors predicting early and mid-term continence. Results were compared with current literature.

Results: Data from 60 patients was assessed. Mean time to complete VUA was 34 minutes, and console time was 247 minutes. Continence in 6 months was 90%. Incidence of urinary leakage was 3.3%, no patients developed bladder neck contracture or postoperative urinary retention. On multivariate analysis, age and pathological staging was associated to 3-month continence status.

Conclusion: Our data show that, during early experience with RARP in a public university hospital, it is possible to achieve good results regarding continence and other outcomes related to VUA. We also found that age and pathological staging was associated to early continence status.

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Minimally Invasive Surgical Procedures; Prostatectomy; Urinary Incontinence

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INTRODUCTION

Radical prostatectomy (RP) is the standard surgical therapy for localized prostate cancer, which is the second most common solid neoplasm in men worldwide and the fourth cause of cancer death (1). In Brazil, it occupies the first position regarding in-

cidence of cancer in men, and it is the second cause of mortality (2).

Robotic assisted radical prostatectomy (RARP) has become the most commonly performed surgical technique in several countries, and is increasingly being employed in Brazil (3). Advocated advantages of robotic surgery are decreased blood loss

and other complications, better early continence and sexual function, less positive surgical margins, and also diminished hospital stay and early return to regular activities (4-6).

Some technical features of the robotic system as the three-dimensional image with magnification, wristed instrumentation, and prevention of biological tremor may help on performing the challenging steps of RARP like bladder neck and neurovascular bundle dissection and vesicourethral anastomosis (VUA). Among those, VUA is one of the most technically demanding since it requires a watertight, tension-free suture, and minimal tissue damage, in order to obtain adequate healing. Even when performed with robotic assistance, an inadequate anastomosis may result in major complications like urinary leakage, prolonged urethral catheterization, increased length of stay, incontinence and bladder neck sclerosis (7). Overall complication rates with RARP may reach approximately 1.5% to 17.8%, even after the learning curve (8).

Although it is already widespread in the United States and Europe, RARP is still under implementation in several centers in Brazil and other countries, therefore we believed that it is crucial to evaluate results and complications during our early experience.

Considering the above mentioned, and since there is still few published data originated from robotic programs in our country, our objective was to assess early and mid-term functional outcomes and complications related to VUA, and to evaluate possible predictors of continence, in patients who underwent RARP during the initial experience in an academic hospital. We also compared these results with the literature.

MATERIALS AND METHODS

We conducted a prospective study from August 2013 to August 2015 in the urology department at Hospital de Clínicas de Porto Alegre (Porto Alegre, RS, Brazil).

Patients with clinically localized prostate cancer who were candidate to primary treatment were offered RARP. Data was collected prospectively from consecutive patients, and those with at least 6 months of follow-up were included in

the study. Operations were performed by two surgeons using the da Vinci SI robotic system with dual console. A total of 63 patients were submitted to RARP and VUA using monofilament barbed suture. The first 24 cases were mentored by an expert robotic surgeon.

Surgical Technique

Radical prostatectomy was performed transperitoneally in the following sequence: dissection of Retzius space, dorsal venous complex ligation, bladder neck incision, vas deferens ligation, seminal vesicles dissection, lateral prostatic pedicles and antegrade nerve bundle dissection, apical dissection and pelvic lymph node dissection (PLND), when indicated. We use a four-arm robotic approach for port placement, with the third working robotic arm positioned on the right and one assistant port on the left flank, as described by Chopra and colleagues (9). After completion of RP and PLND, posterior reconstruction with a modified Rocco Stitch (RS) and VUA were performed according to Van Velthoven's technique (10, 11). A 18F Foley catheter was left usually for 7 days.

RS and VUA were performed using V-LOC™ 90 3-0 CV-23 (17mm needle) or V-20 (26mm needle), depending on surgeon's preference. At the end of surgery a 15F Blake drain was placed in the pelvis through the right (3rd arm) robotic trocar.

Outcome Measures

Data was collected prospectively using standardized institutional protocol. Preoperative, demographic and postoperative data were recorded.

Surgical outcomes were time to perform both the RS and VUA individually. Also, intraoperative events related to suture material were recorded (suture breakage, shearing of urethra or bladder neck, loss of tension). Operative times were recorded by stopwatch during video playback of all cases. The VUA time was measured from the first bite on the bladder until confirmation of a watertight VUA, by filling of the bladder with 120mL of saline. Estimated blood loss (EBL) and transfusion rates were also recorded, since bleeding during or after surgery has been suggested to

negatively affect the quality of anastomosis and to be a predictor of urinary leakage (12).

Early postoperative complications were defined. Urinary leakage was considered as persistent drainage (more than 2 days) from drain or surgical incision, confirmed by elevation of creatinine in the fluid, or by contrast cystography. Cystography was not routinely used. Incidence of ileus was also presented and it was defined as requiring nasogastric tube placement as a result of an inability to resume a normal diet.

During follow-up, mid-term outcomes were evaluated prospectively during clinic visits. Urinary retention requiring catheterization, bladder neck sclerosis and continence were recorded.

Continence was assessed by phone calls or during visits to clinic on months 1, 3 and 6, by asking patients for need and number of pads used per day.

Possible predictors of continence were evaluated. Nerve-sparing status, age, BMI, EBL, pathological tumor staging, and prostate size were included as possible influencing factors and were compared to continence at 1, 3 and 6 months. Prostate size was derived from TRUS or MRI studies. Clinical and pathological stages were reported according to the 2009 TNM system and subcategorized into two groups: localized (pT2) or locally advanced disease (pT3 and pT4).

Nerve-sparing status was recorded prospectively according to surgeon's subjective assessment after surgery, or during video playback. Grading was defined for each neurovascular bundle as: non-nerve sparing, partial nerve-sparing or total nerve-sparing. For statistical analysis patients were subcategorized into two groups of preservation: at least 1 bundle totally spared or neither bundle spared.

Baseline characteristics and outcomes of all patients are presented as median (interquartile range) or mean (standard deviation) for continuous variables and frequencies and percentages for categorical variables. Logistic regression was used for univariate and multivariate analyses. A level of statistical significance of $p < 0.25$ was considered for including variables on a final multivariate model for predictors of incontinence. Reported p values were 2-sided and statistical significance was set at

$p < 0.05$ on multivariate logistic regression. All statistical tests were performed using SPSSv.18 (IBM Corp, Armonk, NY).

The procedure with its benefits and all possible complications was explained to the patients and all participants signed a written consent. The study was approved by the Local and National Ethics and Research Committee.

RESULTS

A total of 63 patients were consecutively submitted to RARP during the described period. Three patients were excluded from the analysis, two because they were operated by visiting surgeons from other institutions, and one because VUA was performed using monofilament non-barbed suture. Two patients had previous transurethral resection of the prostate. Sixty patients were included in the final analysis.

Median patient age was 64 (interquartile range 62–70). Median BMI value was 26.2 (23.8–29.2). Median baseline PSA value was 6.3ng/mL (5–8.2). The clinical stage of the primary tumor was cT1c in 28 (47%) patients, cT2a in 13 (22%) patients, cT2b in 4 (7%) patients, cT2c in 14 (23%) patients, and cT3 in 1 (2%) patient. Preoperative demographics of patients are summarized in Table-1. Table-2 shows postoperative pathological tumor staging.

The perioperative outcomes are summarized in Table-3. Mean time to complete VUA was 34 minutes, and to complete RS median time was 8 minutes. The mean total procedure duration and console time was 298, and 247 minutes respectively. Mean EBL was 95mL (± 158). No patients had blood transfusions during surgery, but two had postoperative bleeding requiring transfusion. Patients maintained Foley catheter for a median period of 7 days.

There were no relevant intraoperative events regarding suture material adequacy. We did not observe shearing of tissues, back slippage or suture breakage.

Regarding early and mid-term complications, two patients (3.3%) were diagnosed with urinary leakage, both of them treated conservatively without need for surgical reintervention. Ileus

Table 1 - Patients' demographics.

	n = 60	
Age, years	64 (62–70)	
BMI	26.2 (23.8–29.2)	
Preoperative PSA, ng/mL	6.3 (5–8.2)	
Clinical T Stage (n, %)		
	cT1c	28 (47%)
	cT2a	13 (22%)
	cT2b	4 (7%)
	cT2c	14 (23%)
	cT3a/b	1 (2%)
Gleason Score Biopsy (n, %)		
	6	27 (45%)
	7 (3+4)	21 (35%)
	7 (4+3)	7 (11.7%)
	8	4 (6.7%)
	9	1 (1.7%)
Prostate size at TRUS (median; range)	35.5 (28.1 – 46.1)	

median (interquartile range); **TRUS**: transrectal ultrasound

Table 2 - Postoperative pathological staging.

	n=60	
Pathological Staging; n (%)	pT2a	10 (16.7%)
	pT2b	7 (11.7%)
	pT2c	33 (55%)
	pT3a	4 (6.7%)
	pT3b	5 (8.3%)
	pT4	1 (1.7%)

Table 3 - Perioperative outcomes.

Variable	n = 60
Total procedure time (min)	298±73
Console time (min)	247±65
Rocco Stitch time (min)	8±6
VUA time (min)	34±16
Estimated blood loss (mL)	95.7±158
Transfusions (n, %)	2 (3.3%)
Length of stay (days)	3 (2-5)
Catheter duration (days)	7 (7-10)
J-Blake drain duration (days)	3 (2-3)

Mean±standard deviation; Median (interquartile range)

requiring nasogastric drainage occurred in 3 (5%) patients, and these were not the same patients that presented urinary leakage (Table-4). With an average follow-up of 18.6 (±8.2) months, no patients have developed bladder neck contracture.

Table-5 shows our continence results. Continence (defined as no pad use or only one safety pad) was 62.1%, 76.7% and 90% in 30 days, 3 months and 6 months respectively. When considering continent patients only those that reported using zero pads, our 6-month result was 78.3%.

We have assessed continence results at months 1, 3 and 6 regarding possible predictors. Incontinent patients (1 or more pads used per day) at 3 and 6 months of follow-up were

significantly older at univariate analysis. Table-6 shows these comparisons at 3 months of follow-up. For other variables, there was no statistically significant association.

Table-7 shows adjusted odds ratios estimated from a model of univariate logistic regression. The variables with p-value <0.25 were included on the multiple logistic regression model, where older age (p=0.02) and higher pathological staging (p=0.04) were associated to incontinence at 3 months. All the other clinical and pathological characteristics (nerve-sparing status, estimated blood loss, BMI and prostate size) were similar between groups at 3 months. There was

no association of factors with continence at first and sixth months post-operative. Nerve sparing technique did not affect continence status in this series of patients.

DISCUSSION

In the present study we analyzed the perioperative, short and mid-term outcomes and complications related to VUA during learning curve of RARP and compared them with the literature. To our knowledge this is the first study presenting early experience with RARP in a public university hospital in Brazil.

Previous reports showing results from learning curve have suggested an increased rate of complications, and that important outcomes as blood loss and positive margin status would improve after 100 or 250 cases, suggesting a steep learning curve (13). The number of surgical cases needed to achieve a low rate of complications related to the anastomosis is not clearly defined. In a single surgeon series, Ou et al. showed that the learning curve for significantly decreasing overall complications was 150 cases (14).

In Brazil, a few published studies have reported experiences with RARP, and none of them focused on VUA outcomes and complications. The first published paper discussing RARP in Brazil was in 2009 by Colombo Jr and from a private hospital. Results from these series are discussed here.

Several factors influence VUA quality and one of them is the type of suture material. Recently, two meta-analysis comparing barbed sutures (BS) to conventional monofilament sutures for VUA indicated shorter anastomosis time, operative time, and equivalent postoperative leakage rate, estimated blood loss, length of stay, and continence rates (15, 16). The authors deemed that it is easier doing the VUA with BS than with conventional sutures, and concluded that it is an important consideration especially for the novice surgeon (16).

Another factor that may influence continence and complications related to VUA is the type of reconstruction performed. Recent studies have suggested that a more complex posterior and anterior reconstruction of the peri-urethral struc-

Table 4 - Complications related to VUA.

Complications	n (%)
Urinary Leakage	2 (3.3%)
Ileus	3 (5%)
Urinary Retention	0
Bladder Neck Contracture	0

Table 5 - Funcional outcome.

Urinary continence over time (pads/day)	N, N {missing}
1 month	58 {2}
0	26 (44.8%)
1	10 (17.2%)
0-1	36 (62.1%)
2	10 (17.2%)
>2	12 (20.7%)
3 months	60 {0}
0	39 (65%)
1	7 (11.7%)
0-1	46 (76.7%)
2	8 (13.3%)
>2	6 (10%)
6 months	60 {0}
0	47 (78.3%)
1	7 (11.7%)
0-1	54 (90%)
2	4 (6.7%)
>2	2 (3.3%)

Table 6 - Comparison of factors according to continence status 3-months.

Factor	Univariate		
	Continent (n= 39)	Incontinent (n=21)	P
Age	61.93 (5.16)	65.39 (6.57)	0.028
Prostate size	42.48 (18.57)	37.67 (24.56)	0.491
IMC	26.43 (3.70)	26.41 (3.50)	0.991
EBL	104.1 (167.03)	80 (143.49)	0.578
Pathological staging	pT2	34 (87.2%)	14 (66.7%)
	pT3–pT4	5 (12.8%)	7 (33.3%)
NVB preservation	Total	22 (61.1%)	9 (47.4%)
	Non-total	14 (38.9%)	10 (52.6%)

BMI = body mass index; **EBL** = estimated blood loss; **NVB** = neurovascular bundle

Table 7 - Logistic regression analyses of 3-month urinary continence factors.

Factor	Univariate				Multivariate			
	P	OR	95% CI		P	OR	95% CI	
Age	0.03	1.12	1.01	1.25	0.02	1.13	1.02	1.28
Prostate size	0.48	0.98	0.95	1.02				
BMI	0.99	0.99	0.85	1.16				
EBL	0.57	0.99	0.99	1.00				
Pathological staging	0.06	3.4	0.93	13.29	0.04	4.58	1.12	21.67
Nerve sparing status	0.33	1.74	0.56	5.47				

BMI = body mass index; **EBL** = estimated blood loss

tures could improve early continence with a low incidence of complications (17, 18). The quality of nerve-sparing has also shown to influence postoperative continence (19).

During their early experience, Artibani et al. reported mean blood loss of 400mL, with 9.8% of the patients receiving blood transfusions (20). In the first reported series in Brazil, Colombo Jr et al. reported mean estimated bleeding of 480mL (100–1800) and transfusion necessary in two patients (2%). One study from Tobias-Machado and another from Lott found mean blood loss of 245.6mL and 212mL respectively (21, 22). In our series, mean EBL was 95mL (± 158), no patients had blood transfusions during surgery, but two (3.3%) required it during postoperative period.

Regarding operative times in initial experience series, surgical duration reported in previous

studies is extremely variable. One study that evaluated the first 100 cases in a secondary hospital, showed mean VUA time of 47.9 min. and mean console operative times of 225 min. (23). Reports from initial series in some high volume centers showed median surgical duration from 215 to 274 minutes (24, 25). Series from our country reported mean or median surgical times from 175 to 298 minutes (3, 21, 22, 26). In 2005 Patel et al. described an extremely short mean operative time of 141 min. in their initial 200 cases (27). In the present series, mean total console time, and time to perform anastomosis, were 298 and 34 minutes respectively.

Our study presents results from our initial experience with RARP in a laparoscopic naive center. During these procedures, lessening surgical time was not a main goal compared to achie-

ving good functional and oncological results. This reflected on our times of whole surgery and time to complete anastomosis being longer than current series of experienced surgeons, but allowed a very low incidence of complications related to the anastomosis, with no bladder neck sclerosis, no retention, and only 2 urinary leakage that did not require any intervention for its treatment. Another factor influencing total operative time is that most patients (65%) required pelvic lymph node dissection. We usually perform standard or extended PLND on patients with intermediate or high risk of progression, respectively.

Regarding these mid-term complications our results were also comparable to current literature. In 2008, Artibani and colleagues published their initial 41 cases and described one bladder-urethra anastomosis dehiscence, which required reintervention, and one bladder-urethra anastomosis stenosis using monofilament suture (20). Recently Jacobsen et al. reported their data from 236 consecutive patients, with an anastomotic leakage frequency of 2.9%, and anastomotic stricture of 4.9% (28). In another study that assessed complications during early experience of RARP, among 322 patients, urine leakage developed in 24 (7.5%) and anastomotic strictures requiring transurethral incision developed in 2 cases (0.3%) (29). One study that evaluated first 100 cases performed by 5 surgeons in a private hospital in Brazil, reported zero cases of leakage or bladder neck contraction (3).

Together with a low incidence of complications and apart from longer operative times than other series, our continence results were comparable to current literature. Publications from high-volume centers have found rates of continence (up to 1 pad/day) at 6 months of 71.7% to 93% on initial series (24, 30). One report from Brazil found 6 months continence rates of 93.3% (21). Lott et al. described continence rates of 88% in 6 months (22). Considering the criterion of continence up to 1 security pad, the present series showed 90% continence at 6 months.

Regarding factors predictive of return to continence, we found that patient age and pathological staging were significantly associated with 3-month continence status on multivariable analysis.

Patient age and Charlson comorbidity index have been found to be significantly associated with 12-month continence status after RARP in previous studies (31). Higher quality nerve-sparing was found to be associated positively with 1 year continence and to patients' perception of urinary recovery as measured by EPIC urinary outcome scores (19, 32).

Possible reasons for not finding a statistically significant association between the degree of preservation of neurovascular bundles and urinary incontinence are our sample size, lack of standardized score for the degree of preservation and lack of data on 12 months continence. Other factors that might affect continence outcomes were not included in our study such as bladder neck preservation, membranous urethral length, thickness of the *levator ani* muscle and urogenital diaphragm, higher pre-operative IPSS, type of urinary sphincter complex reconstruction, comorbidity score, among others, so that is a possible limitation of the model. Also, we did not use validated questionnaires for continence evaluation, although the number of pads/day is a broadly used variable, and the result of 78.3% of patients using no pads at 6 months represents a consistent finding considering an initial experience series.

Apart from these challenges and limitations, this was a prospective study with strict follow-up. Our main results are comparable to the literature, and reflect the experience and results starting from the very first case performed in a public training hospital. We believe that these results are relevant for other centers initiating their urologic robotic program. Our next goal is to compare these outcomes and other variables together with our open radical prostatectomy cases, and these data are being gathered for next publications.

CONCLUSIONS

Our data indicate that, during early experience with RARP in a public academic hospital, it is possible to achieve good continence results with low rates of bleeding and complications related to vesicourethral anastomosis. We also found that age and pathological staging was associated to early continence status.

ABBREVIATIONS

RP = radical prostatectomy
 RARP = robotic assisted radical prostatectomy
 VUA = vesicourethral anastomosis
 PLND = pelvic lymph node dissection
 RS = Rocco stitch
 EBL = estimated blood loss
 TRUS = transrectal ultrasound
 PSA = prostatic specific antigen
 BS = barbed sutures

Compliance with Ethical Standards:

Funding: The suture material (V-loc™) was donated by Covidien.

Ethical approval:

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:

Informed consent was obtained from all individual participants included in the study.

CONFLICT OF INTEREST

None declared.

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A case of retroperitoneal fibrosis responding to steroid therapy

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ABSTRACT

A 69-year-old man presented at the hospital with complaints of prolonged stomach pain extending from the week prior. Enhanced computed tomography (CT) revealed a low density area in the retroperitoneal space. A radiologist diagnosed the patient with retroperitoneal fibrosis. One week later, an enhanced CT revealed an exponential increase of the low density area and slight right hydronephrosis. Upon admission, prednisolone administration was initiated at a dose of 40mg/day. The size of the retroperitoneal soft tissue mass decreased gradually. Although the dose of prednisolone was tapered to 5mg, the patient is doing well without any sign of recurrence.

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INTRODUCTION

A 69-year old man presented to the hospital with complaints of prolonged stomach pain, lasting from the week prior. Enhanced computed tomography revealed a low-density area surrounding the aorta at the level of the inferior mesenteric artery and the right total iliac artery (Figure-1). With a suspected dissection of the inferior mesenteric artery, the patient was taken to our hospital by ambulance. A diagnostic radiologist at our hospital re-diagnosed the case as retroperitoneal fibrosis using a previous image obtained by enhanced computed tomography (CT). The serum biochemistry exhibited elevated levels of white blood cells (WBC) and C-reactive protein (CRP) (11300/ μ L and 5.37mg/dL, res-

pectively), indicating the possibility of an infection. In addition, the patient's serum creatinine level was within the normal limit (0.89mg/dL). For the purpose of observation and pain control, we administered NSAIDs and antibiotics upon admission. Since the pain was relieved by treatment with NSAIDs, the patient left the hospital momentarily the next day. However, when the patient came to the outpatient service for a follow-up study one week later, the enhanced CT revealed an exponential increase of the low-density area (Figure-2A) and slight right hydronephrosis (Figure-2B). Magnetic resonance imaging (MRI) demonstrated RPF masses to be of low to intermediate signal intensity in the T1 weighted images and low and high intensity (according to the level of inflammation) on the T2 wei-

Figure 1 - Enhanced computed tomography revealed a low density area surrounding the aorta at the level of the inferior mesenteric artery and the right total iliac artery.

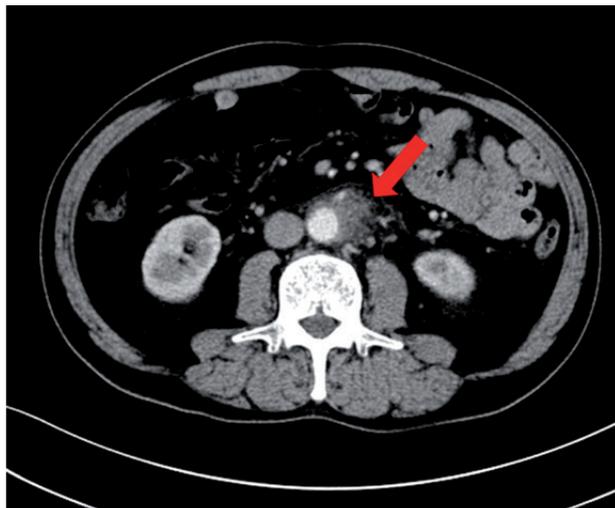
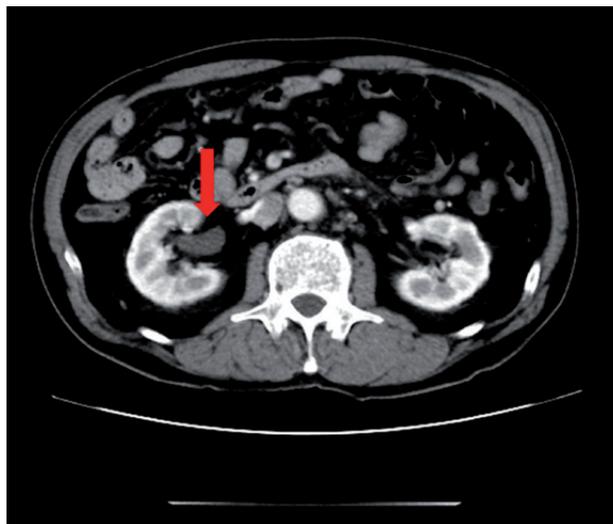


Figure 2 - B) Enhanced computed tomography also showed a slight right hydronephrosis.



ghted images (Figure-2C). Additionally, the patient complained once again of stomach pain and had a high fever. The patient's serum biochemistry revealed elevated levels of WBC 15000/ μ L, CRP 27.09mg/dL, and creatinine 1.11mg/dL; however, the serum IgG4 levels were within the normal limit (27.7mg/dL; normal range: 4.8-105). The patient was admitted once again, and prednisolone was initiated at a dose of 40mg/day under the diagnosis of retroperitoneal

Figure 2 - C) Magnetic resonance imaging demonstrated RPF masses as low and high intensity (according to inflammation) on the T2-weighted images.

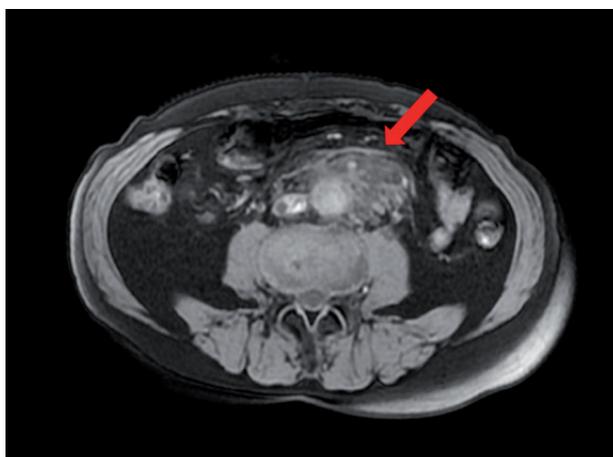
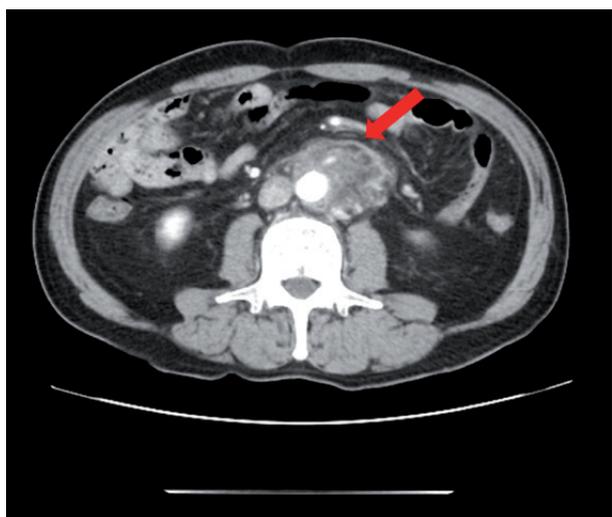


Figure 2 - A) Enhanced computed tomography revealed an exponential increase of the low density area.



fibrosis with RPF aggravation. The size of the retroperitoneal soft tissue mass gradually decreased after several days (Figure-3A). Right hydronephrosis disappeared completely and the serum creatinine levels normalized. The serum CRP level also normalized on day 15 post-admission. The dose of prednisolone was tapered by 10mg every five days (Figure-4). On day 15 post-admission, the dose of prednisolone was tapered to 10mg/day. The CT revealed a remarkable reduction in the size of the retroperitoneal mass. At

the time of admission, the serum levels of the soluble interleukin-2 receptor (sIL-2R) was 2990U/mL (normal range: 145-519); however, on day 19 following admission, the levels decreased to 1380U/mL. The patient was discharged on day 20. Since there was no sign of recurrence, the dose of prednisolone was tapered to 5mg/day one month after discharge. Although steroid therapy was continued for two months at a dose of 5mg/day, the CT revealed no sign of recurrence (Figure-3B).

Figure 3 - A) The size of the retroperitoneal soft tissue mass decreased on day 19 post-admission.

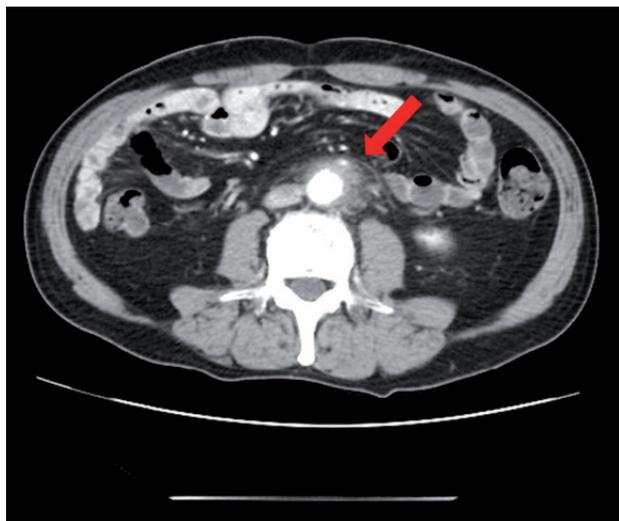
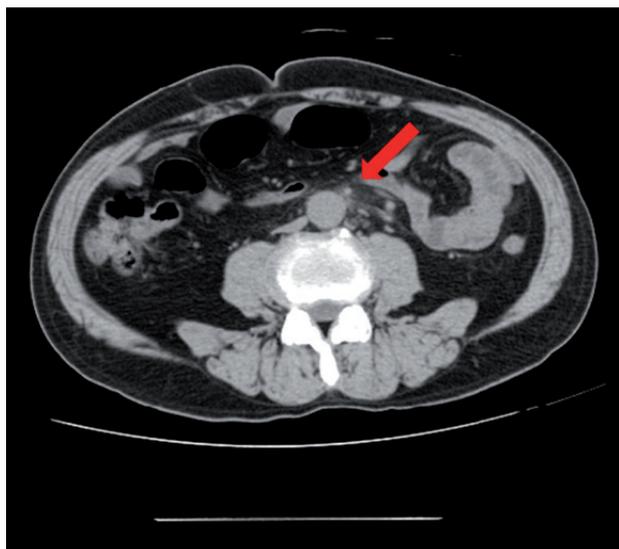


Figure3 - B) The size of the retroperitoneal soft tissue mass decreased two months after discharge.



The patient is currently doing well, without any sign of recurrence.

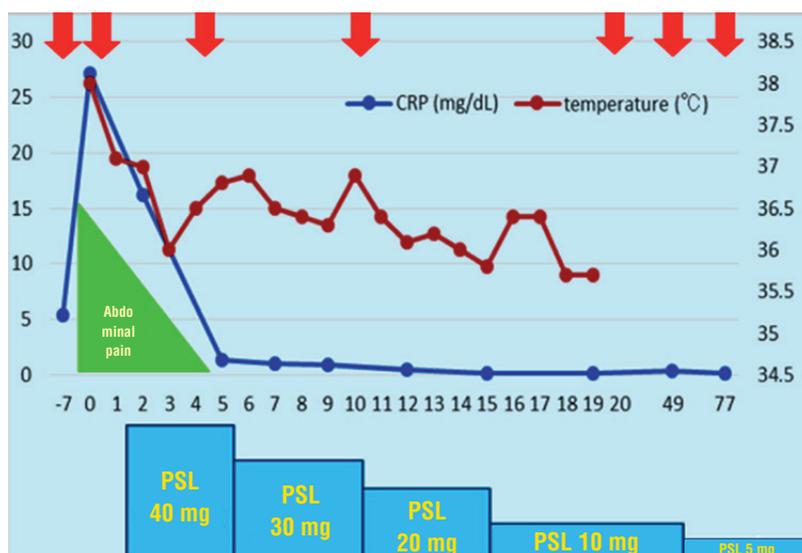
DISCUSSION

RPF was first reported by Ormond in 1948, and has since been widely documented (3). It is a rare condition characterized by the development of peritoneal inflammation and fibrosis, which often obstructs the ureters. RPF is further categorized as either idiopathic or secondary. Idiopathic RPF encompasses IgG4-related and non-IgG-related RPF (4).

The recently recommended concept of IgG4-related disease was derived from research on autoimmune pancreatitis (AIP). AIP is characterized by the abundant infiltration of IgG4-positive plasma cells and lymphocytosis, dense fibrosis, and the presence of obliterative phlebitis in the pancreas, a pattern termed lymphoplasmacytic sclerosing pancreatitis. This entity is associated with extra-pancreatic lesions exhibiting histological features similar to those of the pancreas, and is currently considered a pancreatic manifestation of IgG4-related systemic disease. RPF occasionally occurs as an extra-pancreatic lesion of AIP, and some forms of RPF can be classified as an IgG4-related disease (5).

Currently, a definitive diagnosis of IgG4-related RPF is based on the fulfillment of the following three criteria: 1) soft tissue masses surrounding the aorta and/or adjacent tissues on the CT and/or magnetic resonance imaging; 2) elevation of serum IgG4 levels ($\geq 135\text{mg/dL}$); and 3) the infiltration with IgG4-positive plasma cells (>10 IgG4-positive plasma cells per high power field and a ratio of IgG4-positive to IgG-positive cells of $>40:100$) (6). In the present case, we could not undertake a biopsy of the retroperitoneal tissue before initiating steroid therapy due to the rapid expansion of the fibrotic area and worsening of the patient's symptoms. Moreover, while the serum IgG4 levels were normal, approximately 30% patients exhibited normal serum IgG4 levels, despite having classical histopathological and immunochemical findings (2). Our case met only one of the necessary criteria for the diagnosis of IgG4-related RPF. Thus, we could not diagnose this case as IgG4-related RPF. In addition, we diagnosed this case as non-IgG4-related or idiopathic RPF.

Figure 4 - Clinical course (arrow: CT).



The serum CRP level was normalized on the fifteenth admission day. The dose of prednisolone was tapered by 10 mg every five days. On day 15 after admission, the dose of prednisolone was tapered to 10 mg/day then tapered to 5 mg/day one month after discharge.

The serum IgG4 levels have remained normal throughout the follow-up period.

Steroid therapy is recognized as the standard treatment for AIP. Therefore, steroid therapy is also strongly recommended for patients with IgG4-related RPF; however, for patients with non IgG4-related RPF, steroid therapy is also effective. Standard steroid treatment consists of an initial dose of 0.6mg/kg/day of oral prednisolone, which is reduced to a maintenance dose (5mg/day) over a period of three to six months. To prevent relapse, maintenance treatment for six months to three years is recommended (7). In the majority of cases, the pancreatic lesion or RPF improves following the initial course of treatment; however, a relapse can occur following steroid withdrawal in some cases. Our patient received standard steroid treatment resulting in almost complete remission three months after the initiation of medication. However, it is possible that we will need to increase the dose of steroids in the future due to the possibility of another recurrence. Additionally, adverse events associated with steroid therapy (e.g., gastrointestinal hemorrhage or impaired glucose tolerance) may prevent the continued use of steroid therapy. As a potential alternative to standard maintenance with steroid treatment, Fukuchi et.al. reported the efficacy and sa-

fety of Hochuekkito, a type of Kampo (i.e., traditional Japanese herbal remedy) medicine (8).

In the present case, we could not determine a definitive diagnosis prior to initiating steroid treatment. We have considered malignant lymphoma or the metastasis of malignant cancer as a differential diagnosis, but the images do not reveal any retroperitoneal nodular tumors following treatment in the area in which retroperitoneal fibrosis was present. Therefore, we therapeutically diagnosed this case as retroperitoneal fibrosis. If a case later proves to be malignant lymphoma or metastasis, we will treat for malignant lymphoma or cancer as appropriate. If we would undertake biopsy following steroid therapy, we will not be able to detect viable cells. Long term follow-up is essential for the detection of recurrence.

In the present case, the serum levels of sIL-2R were elevated, but did not provide clear evidence of malignant lymphoma because the serum levels of sIL-2R were also elevated due to increased inflammation. Moreover, the serum levels of sIL-2R decreased to 628U/mL two months after the patient was discharged.

Since we could rapidly initiate treatment with steroid therapy, we were able to avoid invasive treatment (e.g., ureter stent placement or nephrostomy)

for treating hydronephrosis or renal dysfunction, although we did not clearly diagnose the situation before commencing steroid therapy. Some cases have reported that stent placement was impossible owing to strong stenosis of the ureter. Therefore, the initiation of prompt therapy is important for the treatment of retroperitoneal fibrosis (9). It is possible that the origin of the fever in this case was attributed to right hydronephrosis and pyelonephritis. However, the CT demonstrated only moderate hydronephrosis and no sign of right pyelonephritis, thus, we considered the elevated inflammatory findings and presence of fever was due to the progression of RPF. Although we could have attempted stent placement prior to the initiation of steroid therapy, it would have been difficult due to the strong stenosis of the ureter. Therefore, we decided to initiate steroid therapy immediately and carefully follow-up hydronephrosis. If the RPF progressed and hydronephrosis worsened, we would attempt a stent placement or nephrostomy. Fortunately, the right hydronephrosis disappeared rapidly by day 3 after commencing the steroid therapy.

Disease progression can be monitored by a regular evaluation of CRP and creatinine levels, as well as radiological imaging via CT, MRI, or ultrasound (to monitor hydronephrosis). The prognosis is typically good, with a relapse rate of less than 10% to 30% after discontinuing treatment (10). However, there are currently no predictors of the response to treatment or probability of relapse; thus, long-term follow-up is essential.

CONCLUSIONS

In the present study, we report a case of retroperitoneal fibrosis responding to steroid therapy. Since we were able to rapidly begin treatment with steroid therapy, we were able to avoid invasive treatment, although we did not clearly diagnose the situation before commencing steroid therapy. Long-term follow-up by radiological imaging and blood tests are essential for detecting a recurrence.

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CONFLICT OF INTEREST

None declared.

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Gas surrounding the urinary bladder in emphysematous cystitis

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ABSTRACT

We report a rare case of emphysematous cystitis in a 66-year-old woman with a history of diabetes mellitus. The predisposition of diabetes mellitus and infection of gas-forming bacteria is considered to precede the manifestation of emphysematous cystitis. The present recommended diagnosis test is computed tomography, which have definite value in the evaluation of gas accumulation in bladder wall, or an air-fluid level in bladder.

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Cystitis; Tomography, X-Ray Computed; Diabetes Mellitus

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INTRODUCTION

A 66-year-old woman with a 10-year history of diabetes mellitus presented to the emergency department for painful urination and gross hematuria. Similar episodes had occurred several times in the 6 months preceding presentation, along with episodes of acute urinary retention and bladder catheterization. The physical examination was unremarkable. Laboratory investigations revealed mild anemia (Hb:9.2g/dL) and elevated blood glucose (BG:171mg/dL). Urinalysis findings indicated urinary tract infection. The culture of voided midstream urine showed evidence of *Escherichia coli*. Urinary system ultrasonography

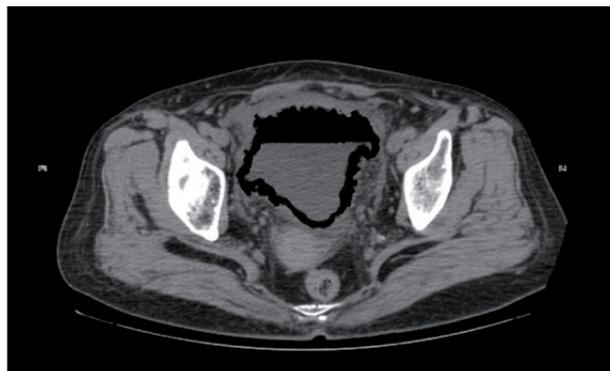
revealed an irregular thickened bladder wall with post-void residual volume of 140mL. Computed tomography (CT) of the abdomen and pelvis without administration of contrast material revealed diffuse gas within the bladder wall (Figure-1) and a prominent air-fluid level (Figure-2). This pattern of gas surrounding the urinary bladder on computed tomography is a typical manifestation of emphysematous cystitis (EC) in which natural fermentation of glucose for gas-forming bacteria infected mostly in diabetic women (1, 2).

EC is a rare, but severe infection of the bladder characterized by gas accumulation surrounding the bladder wall. It occurs predominantly in females over 60 years old, with 60-70% of cases

Figure 1 - Gas surround the bladder wall on computed tomography.



Figure 2 - A prominent air-fluid level within the bladder on computed tomography.



being diabetic patients (1). Diabetes mellitus and female gender are the highest risks for developing EC. The typical presentation spectrum of EC includes lower abdominal pain, bacteremia, and dysuria. Urinalysis often indicates bacteriuria, pyuria and hematuria. CT is the most sensitive diagnostic protocol for EC (3). Current concepts about the pathogenesis of gas formation in the bladder is postulated that bacteria such as *Escherichia coli* ferment the glucose in the urine of diabetic patients but in non-diabetic patients remains still

unknown. EC is often successfully managed with drainage and appropriate antibiotics. About 10% of cases require surgery and estimated mortality rate is 7% (4, 5). Our patient was treated with levofloxacin 500mg for 5 days and was discharged in stable condition.

CONFLICT OF INTEREST

None declared.

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Robotic Assisted Radical Cystoprostatectomy and Intracorporeal Ileal Conduit Urinary Diversion for a Kidney Transplant Recipient

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ABSTRACT

Introduction and Objectives: Robotic assisted radical cystectomy (RARC) is an alternative to open radical cystectomy. As experience is gained with the RARC approach the technique is being applied to more complex surgical cases. We describe here our technique for RARC with intracorporeal ileal conduit urinary diversion for a renal transplant recipient.

Materials and Methods: The patient is a 60-year old man with high-grade muscle invasive bladder cancer. He has a history of renal failure due to polycystic kidney disease and received a deceased donor renal transplant in 2008. His hospital course at time of transplant was complicated by low-level BK virus viremia. Interestingly his trans-urethral bladder tumor resection specimen at time of bladder cancer diagnosis stained positive for SV40. His native kidneys were anuric so bilateral laparoscopic nephrectomy was performed in a staged fashion 2 weeks prior to RARC. Our surgical technique utilizes 6 trocars, of note a 12-mm assistant trocar is placed 1 cm superior to the pubic symphysis, and this trocar is solely used to pass a laparoscopic stapler to facilitate the excision of the ileal segment and the stapled enteric anastomosis. Surgical steps include: identification of native ureters bilaterally (removed *en bloc* with the bladder specimen); identification of the transplanted ureter at the right bladder dome; posterior bladder and prostate dissection along Denonvilliers' fascia; development of the space of Retzius; ligation and transection of the bladder and prostate vascular bundles; apical prostate dissection and transection of urethra; left pelvic lymphadenectomy; ilium resection for creation of the ileal conduit; stapled enteric anastomosis; ureteroileal anastomosis; maturation of the ileal conduit stoma.

Results: The surgery had no intraoperative complications. Operative time was 443 minutes (7.4 hours). Estimated blood loss was 250 cc. Length of hospital stay was 5 days. The patient did not experience any postoperative complications. The patient maintained good renal graft function with no decline in eGFR to date.

Conclusions: As surgeon comfort and experience with robotic assisted surgery grows, robotic surgery can successfully be applied to less frequently performed procedures. Here we successfully performed a robotic assisted radical cystoprostatectomy with intracorporeal ileal conduit urinary diversion for a renal transplant recipient.

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Full-thickness skin mesh graft vaginoplasty: a skin sparing technique

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ABSTRACT

Introduction: The ideal vaginoplasty method should promote good cosmetic and functional results with low morbidity. We describe a new technique for congenital vaginal agenesis using a full-thickness perforated skin graft.

Materials and Methods: We report an 18 year old patient with vaginal agenesis (Morris syndrome) that undergone a modified version of McIndoe vaginoplasty.

Patient is set in a low lithotomy position and lateral traction sutures are placed in labia and a 16Fr urethral catheter inserted. An inverted "V"-shaped incision is made in the mucosal plaque below the urethra. Blunt dissection in a cephalic posterior direction forms a space between the rectum and urethra. Special care is taken to avoid rectal tear during this maneuver. A full-thickness skin graft is removed from the lower abdomen measuring 12.0x6.0cm as an aesthetic abdominoplasty. The fat tissue is removed, remaining epidermis and dermis and the graft is perforated, allowing a great surface increase. After suturing over a mold, the graft is fixed in the created space. The donor site is closed with intradermal transversal suture.

Results: From January 2009 to August 2015, seven patients diagnosed with vaginal agenesis underwent this technique. There were no major complications or need for blood transfusions. At the six-month follow-up, all patients reported satisfactory sexual intercourse. There were no significant complications at donor site or neovagina that needed surgical intervention.

Conclusion: Vaginal reconstruction using the perforated graft is viable with excellent functional results. Applying this modification, we yielded the good results of a classic McIndoe technique with lower donor site morbidity.

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Surgical management of female paraurethral cyst with concomitant stress urinary incontinence

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ABSTRACT

Paraurethral cysts are usually asymptomatic and frequently detected incidentally during routine pelvic examination, however, patients can present with complaints of a palpable cyst or with lower urinary tract symptoms (LUTS) and also dyspareunia. In most cases, diagnosis can be made on physical examination but for more detailed evaluation and to differentiate from malign lesions ultrasonography (US), voiding cystourethrogram (VCUG), computerized tomography (CT), or magnetic resonance imaging (MRI) can also be used. Management of symptomatic paraurethral cyst is surgical excision.

In this video our objective is to show the surgical management of female paraurethral cyst with concomitant stress urinary incontinence (SUI).

A 37 year-old woman presented with an 8-year history of progressive urinary symptoms, consisting of dysuria, urinary frequency, urgency urinary incontinence, SUI and dyspareunia. Physical examination in the lithotomy position revealed a cystic lesion located in the left anterolateral vaginal wall. Also cough stress test for SUI was positive. Her preoperative ICI-Q, UDI-6, IIQ-7 and SEAPI scores were 16, 8, 9 and 18 respectively. Vaginal US revealed a solitary 2 cm paraurethral cyst, localized in the distal urethra. Pelvic MRI also revealed a benign cystic lesion in the distal urethra. The patient underwent surgical excision of the cyst and anterior colporrhaphy for SUI. At third month visit the patient was very satisfied. The ICI-Q, UDI-6, IIQ-7 and SEAPI scores were 0.

Sometimes the LUTS concurring with the paraurethral cyst can be dominant. Herein we want to show that extra surgical procedures can be necessary with paraurethral cyst excision for full patient satisfaction.

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Alternative approach of a Fibroepithelial polyp

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ABSTRACT

A 41-year-old male presented at Emergency Department (ED) with right flank pain associated with hematuria for 3 days. Patient had a previous history of nephrolithiasis. The physical examination and blood tests were normal. Urine analyses showed haematuria > 1.000.000/ μ L. After clinical evaluation, a computer tomography (CT) showed right ureteral dilatation caused by a 5 mm proximal stone and a distal intraluminal mass of 8 cm in length. In this setting, an ureteroscopic biopsy was performed and revealed a large polypoid lesion histologically suggestive of fibroepithelial polyp. Due to technical difficulties (intraluminal mass length and technical issue for the passage of guidewire) and after discussing all available minimally invasive options, we opted for a laparoscopic approach. Instead of ureterectomy of the affected segment of the ureter, as classically performed, we proceeded with an ureterotomy, blunt dissection of the tumor and ureterolithotomy, with complete removal of the mass. This approach did not require ureteral anastomosis and the ureteral dilatation facilitated its primary closure. No complications occurred, even after 3 years of follow-up.

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Re: Unusual intravesical foreign body in a young female migrated from the vagina due to autoerotism

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To the editor,

We read with interest the recent case of an unusual intravesical foreign body reported by Bansal et al. (1). A case is presented of an 18 year old female who presented with lower tract symptoms and was found to have a supratrigonal fistula following self insertion of a plastic pen per vagina for sexual gratification 6 months earlier. The operative management is described and high quality radiological and cystoscopic images are provided.

The authors allude to the array of intravesical bodies that have been reported and mention the psychological reasons for self insertion (1). It should be acknowledged that in certain patient cohorts, urethrovesical foreign body insertion is a form of manipulative behaviour as it requires mandatory transfer to an acute hospital (2) and that the practice is frequently mimicked by other institutionalised patients(3). Specific to the incarcerated population higher rates of emergency surgical intervention have been reported following urethral foreign body insertion (4).

The important role of radiology in determining the lucency, location and size of foreign bodies is discussed and the preference for endoscopic management is mentioned (1). The increasing role of the interventional radiologist in imaged guided retrieval of self inserted foreign bodies, should not be underestimated as illustrated by Young et al. (5).

The authors conclude by discussing urogenital fistulae as a consequence of foreign body insertion. Recent reports have highlighted the additional acute complication of urethral avulsion following polyembolokoilamania necessitating emergency urethroplasty (6).

Finally, it should be acknowledged that not all cases of self embedding behaviour require intervention as some patients deliberately request no intervention (7) and reports exist of cases that have been managed conservatively (8).

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