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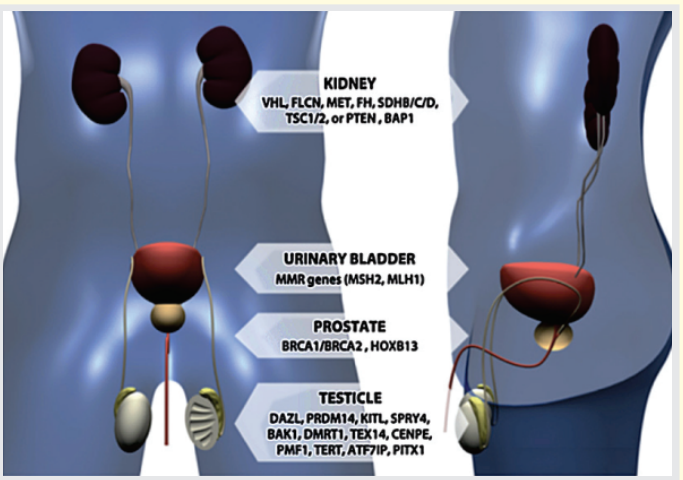

Figure 1 - Main genes related to urological malignances linked to genetic syndromes. (Page 193)

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Reviewers: the soul of a scientific journal

Peer review system is like democracy: it is absolutely imperfect, but the best available. It allows criticism to articles, suggestions for improvement under different perspectives and allows the editor to make a fairer decision about the future of an article.

The reviewer is the soul of a medical journal like International Brazilian Journal of Urology. He/she dictates the quality of publishing, the velocity by which each article reaches the reader, being a voluntary job that takes time. A good reviewer is rapidly identified by different magazines and is overloaded by many requests.

A good reviewer is not necessarily famous in his/hers specialty, but a committed person that spends precious time without payment in order to evaluate a paper and to decide if its valuable enough to be published or if it needs improvements and guidance to authors.

International Brazilian Journal of Urology has received 641 new submissions in 2016, added to other 275 that were revised and resubmitted. 456 reviewers participated in that task and it is important to nominate, as we have been doing in the past years, the most efficient and more collaborative.

We created a formula by which this choice is not subjective and only based on the opinion of the Associated Editors:

$$\textit{Reviewer score} = \frac{\textit{(completed revisions/invitations)x R-score}}{\textit{Median time consumed for revision}}$$

The result is multiplied by 100.

R-score: given note that each AE gives to each revision (1= bad revision to 3-excelent review)

Based on that formula we have elected the 5 most efficient reviewers of 2016 and we specially thanks them for their dedication:

John Denstedt, University of Western Ontario London, ON, Canada

Fabio Vicentini, Departamento de Urologia, Hospital das Clínicas, Faculdade de Medicina da Universidade de São Paulo, SP, Brasil

Renato Pedro, UNICAMP, Campinas, SP, Brasil and Hospital Israelita Albert Einstein, São Paulo, SP, Brasil

Marco Arap, Hospital Sírio Libanês, São Paulo, SP, Brasil

Lisias Castilho, Instituto de Ensino e Pesquisa do Hospital Sírio Libanês, São Paulo, SP, Brasil

EDITORIAL
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Al of them evaluated at least 10 papers in 2016 and took 2 to 6 days to perform the revision. This effort and the agility of the Associated Editors allowed a median time of 54.2 days from submission to final decision for publishing, with an acceptance rate of 27.1%.

With the update of the journal website (www.intbrazjurol.com.br) and the policy of fast publication on line (Ahead of Print) of the accepted papers, today it is possible to have a paper published at International Brazilian Journal of Urology and consequently at Medline in a median time of 90 days after submission.

Another novelty of the website (very important to Brazilian authors and urologists) is the possibility to access on line all editions in full of the old Jornal Brasileiro de Urologia, since number 1, 1974.

The International Brazilian Journal of Urology is totally costed and supported by Brazilian Society of Urology and all publishing production is made by regularly employed officials at the Society headquarters. The joint effort of Ricardo de Morais, Technical Editor, Bruno Nogueira, Production Editor and Patricia Gomes, Secretary, has made our Journal more interactive and more competitive over the years.

Sidney Glina, MD, PhD

Professor Titular Disciplina de Urologia da
Faculdade de Medicina do ABC, Santo André, SP, Brasil

EDITORIAL

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Selection of best videos of the year for 2016

Dear esteemed colleagues and friends,

As we begin a new year, I would like to take this opportunity to thank each of you for your commitment in supporting our journal and expanding its readership. This past year has been truly exceptional in the quality of submissions we have received within the video section of the *International Brazilian Journal of Urology*. We are committed in publishing the highest quality videos detailing novel surgical techniques and approaches by leading surgical teams from across the world. Similarly, we encourage groups pushing the envelope in terms of how we can continually refine the art of surgery and ultimately improve the outcomes of our patients. In this regard, I am pleased to share with you this year's selection for best videos of the year for 2016. Many individual criteria were taken into account in making this selection including novelty, superior quality in terms of video depiction and narration, and lastly vides that best depict re-defining surgical approaches to urological diseases. On that note, here are the selections:

First Prize:

Robotic ureteroureterostomy for treatment of a proximal ureteral stricture by Andrade HS et al. from the Glickman Urological and Kidney Institute, Cleveland Clinic (Cleveland, Ohio) published in volume 42(5):1041-1042, September-October 2016 (available at: http://www.intbrazjurol.com.br/video-section/andrade_1041_1042) (1). This video is truly a masterful depiction on how minimally invasive robotic surgery can be used to tackle benign ureteral stricture disease. Excellent perioperative surgical outcomes are reported and the authors provide sufficient follow-up of 27 months to insure these favorable outcomes are maintained with adequate follow-up. There is no question this surgical approach and specifically this video can be used by surgeons skilled and interested in conducting such surgical procedures.

Second Prize:

Robotic repair of vesicovaginal fistula- initial experience by Jairath A et al. from the Department of Urology, Muljbhai Patel Urological Hospital (Nadiad, India) published in volume 42(1):168-169, January-February 2016 (available at: http://www.intbrazjurol.com.br/video-section/video-library/jairath_168_169) (2). The authors presents a surgical series of 8 vesicovaginal fistulas approached using a

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robotic assisted laparoscopic transabdominal extravesical approach. The authors report excellent outcomes with no recurrences at short-term follow-up of 3 months.

Third Prize:

Retzus-sparing robotic-assisted laparoscopic radical prostatectomy: A step-by-step technique description of this first Brazilian experience by Tobias-Machado M et al. from the Departmento de Urologia, Faculdade de Medicina do ABC, Santo Andre, SP, Brazil and other centers of excellence in Sao Paulo, SP, Brazil published in volume 42(6):1250, November-December 2016 (available at: http://www.intbrazjurol.com.br/video-section/tobias-machado_1250_1250) (3). This surgical video is quite elegant in its demonstration that Retzus sparing RRP is not only feasible and reproducible but can enhance continence recovery following initial catheter removal. This is furthermore an excellent depiction of the wonderful surgical collaborations taking place in Brazil.

I would like to conclude this editorial by once again thanking each of you for the support of the **International Brazilian Journal of Urology**. We are committed in publishing the very best original articles and videos from across the world and similarly will continue to do so in a very timely manner, with a rigorous peer review process by the very best subject leaders. Lastly, I send my very best wishes to you and your families for 2017.

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DIFFERENCE OF OPINION

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Radical Cystectomy is the best choice for most patients with muscle–invasive bladder cancer? *Opinion: Yes*

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Keywords: Urinary Bladder Neoplasms; Cystectomy; Chemoradiotherapy, Adjuvant; Neoadjuvant Therapy

Around the World, radical cystectomy (RC) with bilateral pelvic lymphadenectomy (PLND) and perioperative chemotherapy is regarded as the standard treatment for patients with muscle-invasive bladder cancer (MIBC). This management approach is supported by numerous renowned organizations and guidelines, such as the National Comprehensive Cancer Network (NCCN), as well as by the European Association of Urology (EAU) guidelines. In fact, the latter has assigned RC a grade A recommendation for treating MIBC (T2-T4aN0M0) and high-risk non-muscle invasive bladder cancer.

Furthermore, several studies demonstrate that quality surgery with RC and PLND offers excellent locoregional control for patients with clinically localized or locally advanced bladder cancer, even at extended long-term follow-up (1, 2). Therefore, RC accomplishes a paramount target in bladder cancer. It achieves oncologic control with acceptable morbidity. However, in the modern World, oncologic control is not the only goal when physicians decide to treat cancer patients, as quality of life is very important as well. In the absence of prospective randomized studies, it is not possible to compare RC versus other forms of therapy. There would be a strong bias because patients are subjected prior to therapy for selection of a specific treatment approach and hence patients are prepared for the disadvantages and advantages associated with the different treatment approaches. As such, there is a real risk that patient-led preferences would be inappropriately labeled against RC.

Even though there can be considerable complication rates, we believe that RC remains the preferred choice for most patients with MIBC. To support this reasoning, we ask ourselves one simple question: Is there some other treatment with equivalent or better oncological outcomes than RC?

Organ-preserving therapies have been developed as an alternative to radical treatments for organ-localized cancer disease. The purpose of organ-preserving therapies is to improve patients' quality of life and to reduce the associated morbidity of radical treatments, while maintaining similar oncologic outcomes. For instance, head and neck, breast, anal, and prostate cancers have well-established organ-preserving therapies. In patients with MIBC, there are also several bladder preservation options, such as single

modality treatments that include transurethral resection of a bladder tumor (TURBT), radiation therapy (RT), or chemotherapy alone, as well as multimodality treatments. Tri-modality therapy (TMT) is currently the most accepted form of multimodality bladder preservation therapy.

Large retrospective non-selected series from single institutions have shown that the recurrence--free survival (RFS) rates following RC ranges from 62% to 68% and 50% to 66% at 5 years and 10 years, respectively (3-5). Moreover, Hautmann et al. published similarly long-term oncologic outcomes among 1,100 patients who underwent RC; they exhibited an overall 10-year cancer-specific survival (CSS) rate of 67% (6).

TMT for MIBC is the most studied and most effective bladder-sparing treatment. TMT consists of maximal TURBT followed by radiotherapy and concomitant radio-sensitizing chemotherapy. It also includes prompt salvage RC in patients with incomplete response or among those who developed invasive recurrence.

In a systematic review, contemporary bladder-preservation modalities were assessed, while focusing specifically on TMT in MIBC (7). This review included 83 studies in its synthesis, which consisted of 5 prospective TMT Phase III trials, and 2 Phase III randomized controlled trials (RTCs). The remaining articles consisted of large retrospective series and Phase II trials that were conducted with small cohorts. Overall, the mean response rate following TMT was 73%. The types of cancers that were optimal and eligible for bladder preservation were those with low-volume T-2 disease without hydronephrosis or extensive carcinoma *in situ* who underwent resection of all visible disease prior to therapy. Following TMT, the rate of recurrent bladder tumors ranged from 24%-43%, and the salvage cystectomy rate was 25-30%. Although feasible, salvage cystectomy after TMT is associated with a slightly higher risk of complications, and almost all patients end up receiving ileal conduit diversion instead of orthotopic neobladder reconstruction. In the same review, bladder preservation approaches with TMT were not found to be free of complications; the acute grade 3-4 toxicity rates ranged from 10%-36%. Most studies with TMT had small cohorts (<50 patients) or limited follow-up data; they also featured heterogeneous treatment protocols that provided few data on long-term oncologic control or late toxicity.

In a recent multicenter randomized trial, oncological outcomes in patients with BC that underwent RT alone or in combination with chemotherapy were assessed. Overall, 83% of patients had clinically organ-confined disease (<cT2), 33% of patients received neoadjuvant chemotherapy and 13% developed late grade 3-4 toxicity. As patients on clinical trials are generally biased towards better outcomes, it is somewhat unexpected, that the 5-year overall survival (OS) rates were only 48% (95% CI: 40-55) in the TMT group and 35% (95% CI: 28-43) in the radiotherapy group (8,9).

Recently, researchers at the Massachusetts General Hospital published the largest single-institution experience of TMT for MIBC, where 475 patients were treated between 1986 and 2013 (10). The patients enrolled in this study presented with the following characteristics: 67% were in the cT2 stage, 88% were without hydronephrosis, and 76% showed an absence of tumor-associated CIS. However, patient selection criteria became more stringent after 2005 where 97% of patients had cT2 disease, 100% were without hydronephrosis, 81% showed absence of CIS. This retrospective analysis where 73% of patients received adjuvant chemotherapy following TMT yielded actuarial 5-year and 10-year CSS rates of 66% and 59% respectively after a median follow-up of 6.9 years among survivors. Moreover, the risk of salvage cystectomy at 5 years was 29%. This study demonstrates that in well-selected patients, TMT offers comparable outcomes. Our center has had a long-standing interest in TMT. We strongly believe that patient selection remains critical to good outcomes. We have reported on our earlier experience when patients were not selected appropriately (e.g. only 10% had complete TURBT), the 5-year CSS was 38% (11). However, our more recent experience with more stringent criteria and properly selected patients yielded significantly improved outcomes with 3-year CSS of 71% (12).

Patient selection improves outcomes even in patients treated with radical cystectomy. Many papers attempt to compare TMT with non-selected surgical series where outcomes can appear similar. However, if one uses similar selection criteria as within the TMT studies, the outcomes of surgical series are excellent. A recent study that clearly illustrates this point was from the University of Texas MD Anderson

Cancer Center and the University of Southern California, who treated patients with MIBC (cT2) who are considered low risk (i.e., absence of hydronephrosis, palpable mass, >cT3b disease, or lymphovascular invasion on TURBT), showed an excellent 5-year CSS rate of 83% (13).

Lymphadenectomy has been associated with improved survival rates in multiple surgical studies (14), and the number of lymph nodes removed is an independent predictors of survival (15, 16). This therapeutic benefit is not fully addressed in TMT studies. There are also some concerns to consider complete response on transurethral biopsy after TMT as an excellent marker of good response because there are contrasting studies showing presence of residual tumor after RC in patients labeled complete responders. For example, the Memorial Sloan Kettering Cancer Center experience demonstrated that after methotrexate, vinblastine, doxorubicin and cisplatin (M-VAC), chemotherapy alone, 30% of patients had residual MIBC on cystectomy that was not detected on the preoperative TUR (17).

While one can use arguments to support both therapeutic sides, a comparison between different bladder-preservation modalities and RC remains difficult. Most published studies are largely heterogeneous with regards to the patient sample, tumor staging (clinical versus pathological), treatment modalities, and data reporting and analysis methods. As such, it is difficult to obtain good evidence to settle the debate on whether TMT has equivalent oncological outcomes to RC. A direct comparison between RC and TMT has been confounded by selection biases and discordance between clinical versus pathologic staging. There are no prospective randomized trials to date that have compared TMT with RC and pelvic lymphadenectomy following neoadjuvant chemotherapy. Since the "Selective Bladder Preservation Against Radical Excision" trial failed to recruit patients (18), it is likely that RCTs in this domain will never be completed. Overall, cumulative data have shown that TMT leads to acceptable outcomes and may be considered a reliable alternative option in well-selected patients. However, based on a large body of evidence, RC remains the optimal choice for most patients with MIBC.

While we are presenting the 'Pro' side for RC in this manuscript, it is important to highlight that the two approaches (RC or TMT) should be used in a complementary fashion to ensure most patients with MIBC are actually receiving definitive therapy. Recent studies raise concern that 50% of patients with MIBC are not receiving any form of definitive therapy (19, 20). Most patients who are not surgically fit to undergo radical cystectomy will be able to tolerate TMT. Furthermore, even in patients that are not ideal candidates for bladder preservation therapy and who are not surgical candidates, they should still be offered TMT as cure can still be achieved.

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DIFFERENCE OF OPINION

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Radical Cystectomy is the best choice for most patients with muscle-invasive bladder cancer? *Opinion: No*

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Progress is a hallmark of human civilization. Over the past centuries, it became easier, faster and safer to travel from point A to point B, and the choices of transportation are now numerous, making it at times challenging for travelers to make their selection and forcing them to buy guidebooks, research travel websites and sometimes even contact the travel agents. Perhaps sailing was once the only way of crossing the ocean, but with advent of aviation it is no longer the case. Similarly, upfront radical surgeries were once patients' only hope for cure - radical mastectomy for patients with breast cancer, amputation for patients with extremity sarcoma, laryngectomy for patients with laryngeal cancer, radical prostatectomy for patients with prostate cancer, abdominoperineal resection for patients with anal carcinoma. However, over the past 40 years the field of oncology has embraced organ preservation, often, but not always, through randomized clinical trials showing equivalent outcomes with smaller surgeries and adjuvant radiation therapy, or replacement of upfront surgery with definitive chemoradiation therapy, reserving organ extirpation for salvage in case of local recurrences. The management of muscle-invasive bladder cancer (MIBC) is no different (1).

Stating that one treatment – such as radical cystectomy – is the best choice for most patients with a particular disease – such as muscle-invasive bladder cancer – is no different from advertising a particular mode of transportation. One treatment never fits all, and the best choice of treatment modality for an individual patient starts with a multi-disciplinary evaluation and inclusion of patients and their caregivers in the decision process. An open discussion of all issues related to radical cystectomy (RC) and reconstruction options on one hand, and tri-modality bladder preservation therapy with chemoradiation (TMT) on the other hand allows for the ultimate informed consent and highest patient satisfaction. Most discussions of bladder preservation for MIBC are centered on two themes – efficacy and toxicity.

Survival of patients who undergo cystectomy or tri-modality bladder preservation

A UK randomized trial "SPARE" for patients with MIBC closed in 2007 due to poor accrual, in part because patients did not want to be randomized to the surgical arm.

Based on 45 randomized patients, the trial showed no difference in overall survival, a trend towards decreased post-treatment grade 3 and 4 toxicity in the bladder preservation arm, and a salvage cystectomy rate of 18% for patients with local recurrence following TMT (2). An Egyptian randomized trial of 160 patients with MIBC, which included a high percentage of patients with squamous cell carcinoma of the bladder – a histology more common in Egypt than other countries -- showed no difference in overall survival between upfront radical cystectomy vs TMT (3). A high rate of salvage cystectomies (30%) due to local recurrences were due to low radiation dose of 46 Gy, a dose that is not used in current European and North American TMT practices. Similar 5- and 10-year survival rates from large published cystectomy and bladder preservation treatment series (4) made the National Comprehensive Cancer Network (NCCN) Bladder Cancer Guidelines committee in 2013 accept tri-modality bladder preservation therapy as an alternative treatment option for patients fit to undergo radical cystectomy.

In 2015 Italian physicians have conducted a meta-analysis and compared the overall survival outcomes in over 3,000 patients treated with TMT on 29 published studies to outcomes in over 10,000 patients treated with RC on 30 clinical series. The worse 5-year overall survival rates were associated with undergoing RC (5). What could explain the observed inferiority of RC? Radical cystectomy has a significant risk of post-operative mortality, which increases as patients age. A large SEER database analysis of over 10,000 patients treated in the US with RC between 1984 and 2004 showed that at 90 days mortality is 1% for patients younger than 60 years of age, 6% between ages 69 and 83 and 14% for patients over age 89 (6). If post-operative mortality could be reduced by limiting cystectomy to young and healthy patients, one could hypothesize that cystectomy outcomes would be in line, or superior, to outcomes with tri-modality bladder preservation treatment.

It is important to consider TMT as an attempt to preserve the native bladder without jeopardizing the ultimate outcomes for patients with MIBC - survival. The vast experience in Europe and North America indicates that over 70% of carefully selected patients with MIBC achieve durable local control and avoid the need for salvage cystectomy. Patients who have undergone bladder preservation therapy must be followed closely by their urologists with frequent cystoscopic evaluations, so that an early local recurrence in 30% of these patients could be effectively treated with salvage cystectomy. The reconstructive options may be limited for these patients, due to presence of irradiated bowel, which may be unacceptable for a continent reservoir or a neobladder creation. However, peri-operative morbidity and mortality rates in almost 100 patients who underwent salvage cystectomy at Massachusetts General Hospital (7) were remarkably similar to rates for immediate cystectomies without radiation as reported by other centers specializing in treatment of bladder cancer.

Quality of life of patients who undergo cystectomy or tri-modality bladder preservation

The most common myth pertains to the overwhelming radiation-induced side-effects and poor quality of life for patients who opt to keep their bladders. Among 157 patients who underwent TMT for MIBC on national clinical protocols with a median follow-up of 5.4 years, only 7% experienced late grade 3 genitourinary or gastrointestinal toxicities (8). There were no late grade 4 toxicities and no treatment-related deaths. No cystectomies were performed due to treatment-related toxicity in any of these patients. 200 patients were followed with a median follow-up of 6 years and were asked to undergo urodynamics study (9) and fill out quality of life questionnaires (10). Seventy-five percent of patients had normal functioning bladders by UDS. Reduced bladder compliance was seen in 22% of patients, however only a third of these patients experienced distressing bladder symptoms. Bowel symptoms occurred in 22% of patients and caused distress in 14% of patients. Thirty-six percent of men reported normal erections and another 18% less firm erections, but sufficient for intercourse. Of note, the median age of male patients in this study at the time of the questionnaire was 68 years. The rate of erectile dysfunction after RC, on the contrary, is almost 100%.

A recent retrospective study of 173 patients who underwent TMT at Massachusetts General Hospital [64], or RP at University of North Carolina [109] on multivariate analysis revealed association be-

tween TMT treatment and better general quality of life, better bowel function, and better sexual function, while urinary symptoms were similar between the two group of patients (11).

Clinical situations when RC may be desirable for patients with MIBC

A multi-disciplinary evaluation of patients and discussion of treatment options may reveal that a particular patient may be a suboptimal candidate for TMT. History of prior pelvic irradiation for other malignancies may prevent additional dose of radiation that would be adequate for bladder tumor eradication. Poor baseline bladder function due to tumor and/or previous intravesical therapies, such as BCG or mitomycin, may indicate that upfront RC would be expected to lead to better quality of life in these particular clinical scenario. For large or multi-focal tumors that cannot be grossly removed by maximal transurethral resection of bladder tumor (TURBT), or in the setting of hydronephrosis, the local control with chemoradiation therapy may be lower than 70%. Finally, patients who adamantly desire neobladder may decide to pursue upfront RC, in order to avoid a situation when neobladder may not be possible in the setting of local recurrence after TMT.

CONCLUSIONS

Multi-disciplinary evaluation of patients with MIBC and discussion of all treatment options is key to best outcomes and patient satisfaction (12), similarly to other malignancies (13). Ultimately, overall survival for patients with MIBC – showed to be in 40-50% range at 5 years in large published series of RC and TMT (4) - is dictated not so much by the choice of local therapy – radical cystectomy or TMT with chemoradiation therapy – but by the high risk of systemic disease progression and comorbid medical conditions that are so prevalent in patients afflicted with bladder cancer. Based on growing evidence in the literature, TMT is associated with similar or better outcomes to cystectomy for patients with MIBC. Quality of life in these patients is excellent. Similarly to other solid malignancies, local recurrence rate is significant, necessitating a thorough and frequent cystoscopic follow-up for patients after completion of tri-modality bladder preservation. Thirty percent of patients will require salvage cystectomy for local disease recurrence, usually identified within the first three years, and the morbidity and mortality from salvage cystectomy is no different than from upfront radical cystectomy.

Is sailing from France to England the best option for most travelers? A “Yes” answer is not substantiated by safety and satisfaction results from studies of randomly assigning travelers to various transportation modes. A “Yes” answer may not fit the needs of particular travelers exploring their options. A “Yes” answer is clearly biased towards financial support of the ferryboat industry.

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REVIEW ARTICLE

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Urological cancer related to familial syndromes

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ABSTRACT

Cancer related to hereditary syndromes corresponds to approximately 5-10% of all tumors. Among those from the genitourinary system, many tumors had been identified to be related to genetic syndromes in the last years with the advent of new molecular genetic tests. New entities were described or better characterized, especially in kidney cancer such as hereditary leiomyomatosis renal cell carcinoma (HLRCC), succinate dehydrogenase kidney cancer (SDH-RCC), and more recently BAP1 germline mutation related RCC. Among tumors from the bladder or renal pelvis, some studies had reinforced the role of germline mutations in mismatch repair (MMR) genes, especially in young patients. In prostate adenocarcinoma, besides mutations in BRCA1 and BRCA2 genes that are known to increase the incidence of high-risk cancer in young patients, new studies have shown mutation in other gene such as HOXB13 and also polymorphisms in MYC, MSMB, KLK2 and KLK3 that can be related to hereditary prostate cancer. Finally, tumors from testis that showed an increased in 8 - 10-fold in siblings and 4 - 6-fold in sons of germ cell tumors (TGCT) patients, have been related to alteration in X chromosome. Also genome wide association studies GWAS pointed new genes that can also be related to increase of this susceptibility.

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INTRODUCTION

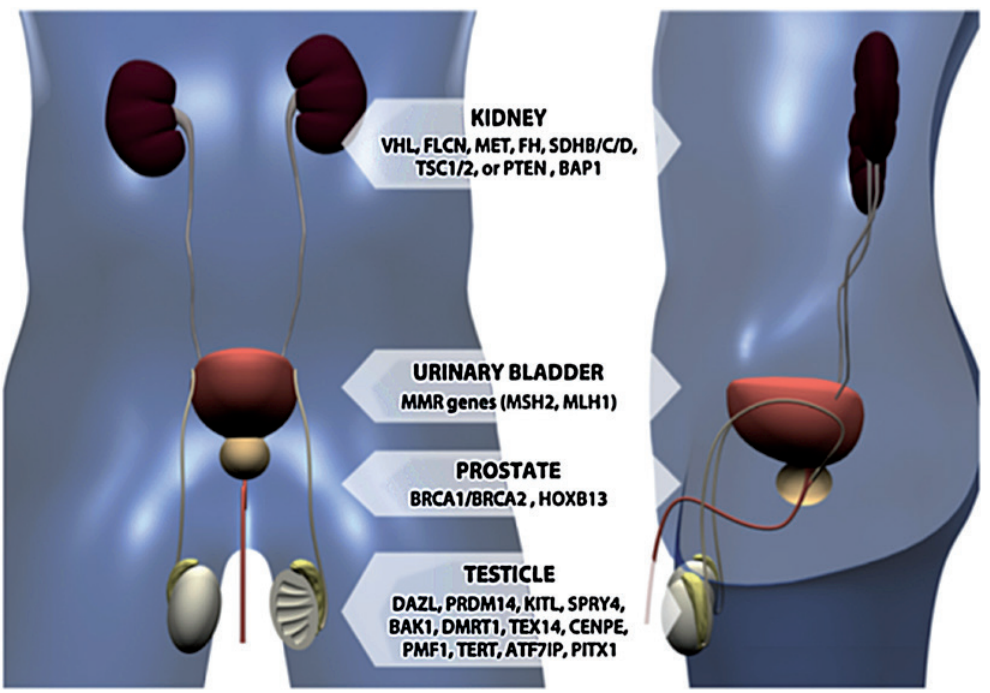
Hereditary cancer syndromes account for 5 to 10% of all cancers and are characterized by a high predisposition to develop tumors. Most are autosomal dominant diseases characterized by a familial clustering of early onset cancer. Over the last two decades, clinical molecular genetic testing for diagnosing the underlying molecular alteration responsible for one of the known cancer predisposition syndromes has increasingly become available. This has enabled the implementation of effective screening guidelines as well as the detection of asymptomatic carriers among family members. Currently, there are approximately 100 genes known to predispose to one or more forms of cancer in carriers of germline pathogenic mutations.

Tumors of urinary tract can be a manifestation of several different genetic syndromes (Figure-1). In this review we focused on organ based tumors and highlight the principal subtypes and clinical morphological parameters that should be recognized in other to further investigations.

MATERIALS AND METHODS

We comprehensively searched the MEDLINE and Cochrane databases on 4 July, 2015. Search terms included the USA National Library of Medicine's Medical Subject Headings (MeSH): familial prostate cancer, hereditary prostate cancer, familial kidney cancer, hereditary kidney cancer,

Figure 1 - Main genes related to urological malignances linked to genetic syndromes.



kidney cancer syndromes, familial testis cancer, hereditary testis cancer, Lynch Syndrome, hereditary urothelial carcinoma, familial urothelial carcinoma, P53 mutation, TP53 mutation, Li-Fraumeni Syndrome. Both free text and MeSH search for key words were used. Data from a period of 22 years, from 1993 to 2015, were included in the search.

RESULTS

Kidney

Renal cancer accounts for 2 - 3% of all malignant disease in adults. The incidence of renal cancer is rising at a rate of approximately 2.5% per year in the USA. Hereditary renal cell carcinoma (RCC) represents 5% to 8% of kidney neoplasms. Family history, clinical manifestations common in familial syndromes, bilateral or multifocal tumors and relatively young patients age (up to 46 years old) are findings that should suggest hereditary RCC.

Several hereditary RCC syndromes have been described, including von Hippel-Lindau (VHL), Birt-Hogg-Dube´ (BHD), hereditary papillary renal cell carcinoma (HPRC), hereditary leiomyomatosis RCC (HLRCC), succinate dehydrogenase kidney cancer (SDH-RCC), tuberous sclerosis (TS), and Cowden syndrome (CS). These syndromes have been shown to be associated with germline mutations in VHL, FLCN, MET, FH, SDHB/C/D, TSC1/2, or PTEN genes, respectively. Recently, germline mutations of BAP1 gene have been described as a possible finding that leads to hereditary predisposition to RCC.

VHL disease is the first described and the most common hereditary renal cancer syndrome. It represents an autosomal dominant inherited disorder caused by germline mutations in the VHL gene (1). Affected individuals are at risk for the development of tumors in several organs, including multifocal clear cell RCC, pancreatic cysts, neuroendocrine tumors, pheochromocytoma, retinal angiomas and central nervous system (CNS) hemangioblastomas. It affects approximately 1 in 36.000 live births worldwide. The VHL gene is a

tumor suppressor gene located on the short-arm of chromosome 3 (3p25). Loss of heterozygosity (LOH) of VHL is commonly found in clear cell RCC (ccRCC) in patients with VHL syndrome as well as those in the sporadic setting (2). The syndrome specific lesions differ in mean age of onset, frequency and in the underlying type of germline mutation (3). In fact, the disease is classified into types 1 and 2 based on the presence of pheochromocytoma. The second group is further subdivided into 2A, 2B and 2C depending on the presence of RCC (Table-1).

Though less penetrants, renal tumors usually present aggressive behavior (7). Affected individuals harbor a germline heterozygous loss-of-function mutation of the Krebs cycle enzyme, fumarate hydratase (FH) gene, which acts as tumor suppressor. Patients with HLRCC inherit a germline mutation of the FH gene as well as a wild-type copy. LOH at 1q43 is observed in up to 80% of patients, which suggests a biallelic inactivation of the FH gene, in a "two-hit" manner; one allele inactivated by the germline mutation and the other by LOH. The exact mechanism of tumorigenesis in

Table 1 - Type and characteristics of VHL genetic syndrome.

Type 1	VHL loss or mutation that affects the protein folding	Haemangioblastoma Renal Cell Carcinoma Low risk of phaeocromocytoma
Type 2A	VHL missence mutation	Haemangioblastoma phaeocromocytoma Low risk of Renal Cell Carcinoma
Type 2B	VHL missence mutation	Haemangioblastoma Renal Cell Carcinoma Phaeocromocytoma
Type 2C	VHL missence mutation	Phaeocromocytoma only

Approximately two thirds of patients present multiple renal cysts and RCC patients with VHL disease can develop up to several hundred cysts and tumors. RCC occurs with an age dependent frequency ranging from 25% up to 70%. The lifetime risk for developing RCC is 25% to 45%, and when renal cysts are included, the risk rises to 60% (4). Much controversy exists regarding the biological characteristic of VHL - associated tumors. Some groups have reported that the growth rate of VHL associated tumors is slower than that of sporadic tumors whereas others could not confirm such findings (5, 6). Life expectancy of patients is around 50 years old and RCC is the leading cause of death (3).

HLRCC is a hereditary syndrome characterized by the presence of cutaneous and uterine leiomyoma. Renal tumors have been identified in approximately one-third of HLRCC families. HLRCC is unknown, but evidences suggest a pseudo - hypoxic pathway, similarly to the molecular mechanism in VHL - deficient kidney cancer.

HPRC is an autosomal dominant hereditary syndrome in which patients develop multifocal and bilateral papillary type 1 renal tumors. It presents a variable degree of penetrance and so far, the development of renal tumors is the only described clinical manifestation of the syndrome (8). Interestingly, patients typically present late onset tumors (sixth and seventh decades) although some reports show patients with early onset renal tumors, between the second and third decades. HPRC has been associated to an activating mutation in the c-Met oncogene on chromosome 7 (7q31). MET gene encodes the cell surface receptor for hepatocyte growth factor (HGF). MET mutation causes aberrant activity of the intracellular tyrosine kinase domain of

the membrane-bound c-Met receptor, producing the cascade of activation of HGF (9).

BHD is a rare, autosomal dominant genodermatosis caused by mutations of the folliculin - codifying gene (FLCN) located on the 17p11.2. Folliculin apparently regulates the m-TOR pathway through folliculin - interacting protein (FNIP1) and 50-AMP - activated protein kinase (AMPK). Besides cutaneous lesions, affected individuals are at risk for the development of pulmonary cysts and spontaneous pneumothorax; and bilateral, multifocal kidney cancer (10). Renal tumors may present with various histologic types, and there are reports of hybrid tumors. Such histological heterogeneity is often observed within the same kidney. Mean age at diagnosis in Pavlovich et al. cohort was 51 years (11). As in VHL and HPRC patients, these tumors may be observed safely up to a size of 3cm before intervention.

Initially described in 2004 by Vanharanta et al. SDH-RCC is an inherited kidney cancer characterized by germline mutations in Krebs cycle succinate dehydrogenase B (SDHB) in patients with hereditary paraganglioma (PGL) (12). Further reports described the presence of RCC in patients with SDHB mutations either with or without a personal or family history of PGL. Later studies have also reported an association of mutations in SDH subunits C (SDHC) and D (SDHD) and inherited RCC (13, 14). The metabolic basis of HLRCC and SDH - RCC are similar and result in an early age onset, aggressive form of RCC with high metastatic potential. The enzymatic loss of function of FH might lead to a metabolic similarity with the impairment of Krebs cycle function, reliance on glycolysis, and a metabolic shift to aerobic glycolysis (14). Patients should be managed with prompt surgical intervention regardless of the tumor size. Importantly, such tumors have the potential for the development of multifocal and metachronous RCC; and nephron - sparing surgery should be considered when feasible.

TS is an autosomal dominant disorder and it affects 1 person in 6000. It is characterized by the presence of multifocal renal tumors, mental retardation, seizures and development of hamartomas in multiple organs (15). In a series of 167 TS patients, Rakowski et al. described the presence of renal lesions in 58% of patients. Of such patients, 85% had angiomyolipomas, 45% had renal cysts, and 4% had ccRCC (16). Germline mutations in TSC1 (9q34) and TSC2 (16p13.3) characterize the syndrome. Differences in the phenotype have been associated to mutations in either TSC1 or TSC2 with evidences suggesting more severe manifestations, including mental retardation and renal lesions, highly associated with mutations in TSC2 (17). TSC2 loss has been shown to result in accumulation of HIF1α and increased expression of HIF - related genes, including vascular endothelial growth factor (VEGF) (18).

CS is inherited in an autosomal dominant manner with an estimated incidence of 1 in 200.000 individuals (19). About 70% of patients with CS have germline mutations in PTEN. PTEN acts as a tumor suppressor gene through the action of its phosphatase protein product that results in inhibition of the AKT signaling pathway. Clinical manifestations include macrocephaly, multiple hamartomas, dermatologic disorders such as acral keratosis and facial tricholemmomas and increased risk for breast, endometrial and thyroid cancers. The first description associating CS and kidney cancer was made by Mester et al. who estimated that these patients had a thirtyfold increased risk of developing renal tumors of variable histology, including clear cell, papillary and chromophobe types (20). In a recent series of 24 CS patients, Shuch et al. described a 16.7% penetrance of CS - related renal tumors (21).

A recent study evaluating 82 unrelated probands with unexplained familial RCC, first described a germline mutation of BAP1 gene (BRCA1 - associated protein-1) (17). BAP1 functions as a classic two-hit tumor suppressor gene and is somatically mutated in ccRCC, uveal melanoma (22). In Farley et al. study, only one from 82 patients presented BAP1 germline mutation, however previous data described an overall germline frequency of approximately 3.8% (range of 1.9% in a group of individuals with uveal melanoma to 8.0% in a subset of apparent sporadic mesotheliomas) (17). BAP1 encodes a nuclear ubiquitin carboxyterminal hydrolase, which was initially described as binding to the BRCA1 RING finger and enhancing BRCA1 - mediated cell growth suppression (23). A later mul-

ticenter study from France evaluating families that included individuals identified as carrying germline deleterious BAP1 mutations demonstrated a significantly increased risk for RCC, which suggests that BAP1 is an RCC - predisposition gene (24).

The diagnosis of hereditary RCC syndromes can be difficult for a number of reasons such as: features with incomplete penetrance, de novo mutations, sex-specific manifestations (such as uterine leiomyomas in HLRCC), and others, such as HPRC that do not present extra-renal manifestations. Additionally, there is the need for prolonged follow-up periods for the identification of patients with bilateral or multifocal disease. The current recommendation is that all patients with bilateral or multifocal disease and patients age 46 years or younger with RCC should be referred for genetic counseling, which should be performed by an experienced team that is able to advise patients on the current clinical recommendations (Table-2).

Bladder and Renal Pelvis (Urothelial carcinoma)

Urothelial carcinomas (UC) is the 4th tumor of the urinary tract that affect specially the bladder but it can occur from renal pelvis to urethra. Among familial cancers, UC has been mainly associated to Lynch syndrome.

Hereditary non-polyposis colorectal cancer (HNPCC), also known as Lynch syndrome (LS) is an autosomal dominant familial syndrome characterized by germline mutations in mismatch repair (MMR) genes such as mutS homolog 2, colon cancer, non-polyposis type 1 (MSH2) and mutL homolog 1, colon cancer, non-polyposis type 2 (MLH1) (25). Such events lead to genetic instability and result in tumors with a high level of microsatellite instability (MSI), which is detected as alterations in short and repetitive sequences of DNA called micro-satellite regions (26). The autosomal dominant mutations are inherited with high penetrance and result in familial clustering of colorectal cancer. The extra-colonic tumor spectrum of LS includes endometrial, ovarian, urothelial, gastric, small bowel, pancreatic, hepatobiliary, brain, and sebaceous tumors. In fact, urological neoplasms represent the third most frequent Lynch-associated tumors (5%) after colonic (63%) and endometrial (9%) cancers (27).

Upper urinary tract urothelial carcinoma (UUT) is relatively rare in the normal population but patients with LS are at increased lifetime risk with an incidence as high as 6%. Ureteral carcinoma in patients with LS carries a 22% increased relative risk when compared to the risk in the general population (28). Actually, UC is considered part of the classical Lynch-syndrome tumor spectrum. Previous studies showed that the male predominance is less than that in the general population and UUT cancer is up to 7-fold more common in MSH2 than in MLH1 mutated family members (29, 30). Crockett et al. describe 39 patients with LS and UC with a predominance of ureteral tumors compared to tumors of the renal pelvis but no particular difference in pathologic parameters such as stage or histologic grade (29).

With respect to bladder cancer (BC) and LS, a number of studies have shown conflicting results. Sijmons et al. study reported a 14-fold RR (95% CI, 6.7-29.5) for UUT cancer, but the risk for developing UC of the bladder was not increased (31). While patients with MSH2 mutations have been shown to be at increased risk for UUT UC, the risk of BC in these subjects has been less investigated. Goecke et al. described a higher incidence of BC in MSH2 carriers compared with MLH1 carriers, suggesting that BC is indeed part of LS (32). Geary et al. also found a 3.6 relative risk of BC (p=0.001) in MSH2-positive compared with MSH2-negative families (30). In a recent multicenter study, Skelton et al. described a 6.21% (11/177) prevalence of BC in patients with MSH2 mutations compared with 3 of 129 patients with MLH1 mutations (2.32%) (33).

In summary, patients with LS and their relatives should be screened for UC. However, most familial UC still remains unexplained. UC seems to be a polygenic disorder, although rare familial single-gene disorders may exist.

Prostate

Prostate cancer (PC) is the second most common tumor in men, with approximately 500.000 new cases annually in the U.S. and Europe. Despite being well studied, the etiology and pathogenesis of the disease remains poorly understood. Age, race and family history are among the known risk factors of

Table 2 - Familial syndromes related to development of renal cell neoplasia.

Syndrome	Incidence	Genes Envolved	Molecular Pathway affected	Renal Type	Others characteristics
VHL	1:36,000	VHL	Hypoxic pathway(through HIF)	Clear cell RCC	pancreatic cysts and neuroendocrine tumors, pheochromocytoma, retina l angiomas, hemangioblastomas
Birt-Hogg-Dubé	rare(unknown)	FLCN	m-TOR	Variable subtypes	cutaneous lesions, pulmonary cvsts and spontaneous pneumothorax
HPRC	rare (less then 1:1,500.00	MET	C-MET	Type 1 papillary RCC	not specific
HLRCC	Rare (unknown)	FH	Krebs cycle	HLRC related RCC	Multiples cutaneous and uterine leyomiomas
SDH-RCC	rare(unknown)	SDHB/SDHC/ SDHD	Krebs cycle	SDH related RCC	Paragangliomas/ Pheocromocytoma GIST
T5	1:6,000	T5C1/T5C2	m-TOR	Angiomyolipomas Clear Cell RCC Renal Cvsts	mental retardation, seizures and development of hamartomas in multiple organs
Cowden	1:200,000	PTEN	AKT signaling pathway	Various histologyc subtypes	macrocephalv, multiple hamartomas, dermatologic disorders such as acral keratosis and facial trichilemmomas and increased risk for breast, endometrial and thyroid cancers.

PC. It is estimated that up to 42% of cases of PC can be assigned to familial and hereditary factors (34). First-degree relatives of men with PC present twice the risk of developing the disease. An extensive meta-analysis comprising 33 studies described that risk was greater for those men with affected brothers (relative risk [RR] 3.4; 95% CI 3.0-3.8) than for men with affected fathers (RR 2.2; 95% CI 1.9-2.5). Furthermore, the presence of two or more first degree relatives affected by PC results in higher relative risk of disease diagnosis. It is estimated that 15% of men with the disease have a first-degree relative with PC compared to 8% of the general population (35).

Unlike other types of tumors such as breast and kidney, it is suggested that the model of PC susceptibility is considerably more complex than initially thought. In fact, it is believed that multiple genes may be involved, thereby configuring what is called polygenic inheritance (36). Rare genetic events are involved with high fami-

lial risk, however due to its rarity they account for less than 5% of the total cases. Thus, in most cases there is involvement of multiple loci that confer moderate or low risk (37).

Despite extensive study, few high penetrance genes are associated with risk of developing PC. Men with mutations in BRCA1 and BRCA2 genes are known to be high-risk patients. Comparatively, the impact of BRCA1 mutation was shown to be considerably less important than BRCA2. BRCA2 mutation involves a lifetime risk of developing PC ranging from 30-40%, while BRCA1 mutation carriers present a 3–8% lifetime risk with a modestly elevated incidence of early onset prostate cancer but no increased risk in older men (38). Besides being characterized by diagnosis at earlier ages, men with BRCA1/BRCA2 mutations have high-grade tumors and more advanced stage when compared to the general population (39). Thus, this subset of patients should be managed in a specific way and strategies for prevention and treatment should be personalized.

A recent meta-analysis describes G84E germline mutation in the HOXB13 gene to be associated with a significantly increased risk of familial PC (40). HOXB13 is a homeobox transcription factor gene, which is important in prostate development. Furthermore, HOXB3 mRNA and protein are over-expressed in primary PC tissues compared to the adjacent normal prostate tissues, which suggests a potential role of HOX on prostate carcinogenesis (41). In Huang et al. study, men with PC were more likely to carry G84E allele (carrier frequency, median=1.40%; range from 0.1 to 4.9%) comparing with control subjects (carrier frequency, median=0.08%; range from 0 to 1.4%). Men with the HOXB13 G84E variant had a 4.51-fold higher relative risk of PC compared with non-carriers (95% CI 3.28–6.20). This risk effect was more pronounced in younger patients and high-grade tumors.

With the advent of new genomic tools, genome wide association studies (GWAS) have been developed, which enabled researchers to simultaneously assay up to millions of common variations in single base - pairs called single-nucleotide polymorphisms (SNPs). SNPs with a minor allele frequency >5% are called common variant alleles. GWAS have identified 76 prostate cancer risk SNPs (36). The SNPs identified are mainly in regions that had not previously been known to be associated with prostate cancer risk that might be clinically relevant, such as MYC, MSMB, KLK2 and KLK3 genes. Such SNPs explain an estimated 20% of inherited prostate cancer risk, which is highly significant when compared with breast and colon cancer (42). Recent studies have attempted to associate the presence of specific SNPs not only to the risk of developing PC but also to predict clinical outcomes and therapeutic response.

PC seems to be a polygenic disorder. The identification of patients at high risk for PC diagnosis has the potential to be a useful tool in selecting patients for screening. It is an extremely controversial issue, especially after the negative recommendation from the US Preventive Services Task Force in 2012. Incorporation of risk associated SNPs might address some of the weaknesses of PSA based screening by enabling risk stratification in screening protocols, particularly in patients who are predisposed to aggressive disease, such as BRCA2 mutation carriers. Explanation of PC inheritance will likely require an improved understanding of the interaction between these distinct genomic regions. Overall, there is limited information about benefits and harms of screening men at higher risk of PC. In addition, there is little evidence to support specific screening approaches in PC families at high risk. It is recommended that high-risk men should engage in shared decision-making with their health care providers in order to develop individualized plans for PC screening based on their risk factors.

Testis cancer

The incidence of testicular germ cell tumors (TGCT) has steadily increased 3% to 6% annually for the last 40 years (43). Approximately, 2% of TGCT patients report an affected first degree relative. It is estimated that the relative risk of TGCT is increased 8-10-fold in siblings and 4-6-fold in sons of TGCT patients. The latter supports a role for genetic susceptibility in TGCT. The higher familial risk among brothers than father-son pairs suggest the involvement of a recessive mode of inheritance or an X-linked susceptibility locus (44).

The identification of predisposing genes has been hindered by the rarity of the disease and the wide histologic variation in TGCT presentation. GWAS of TGCT have identified eight associated SNPs at six loci, which together account for >11% of the genetic risk of TGCT (45-47). Initially, two independent genome wide association studies (GWAS) identified allele variation within KITLG on 12q22 as the strongest genetic risk factor for TGCT, with a per allele odds ratio (OR) greater than 3 (45, 48). In a recent study by Ruark et al., nine new loci for TGCT were identified, which brings the total number of TGCT--associated loci to fifteen. These fifteen loci have provided considerable new insights into TGC tumorigenesis, implicating genes involved in germ cell differentiation (DAZL, PRDM14) including the KIT-KITL signaling pathway (KITL, SPRY4, BAK1) and genes involved in sex-determination (DMRT1), microtubule assembly (TEX14, CENPE, PMF1) and telomerase regulation (TERT, ATF7IP, PITX1). The authors suggest that the nine new susceptibility alleles would account for 4% of the excess familial risk to brothers and 6% to sons of men with TGCT, which brings the cumulative totals to 15% and 22%, respectively (49).

There is strong evidence supporting a hereditary component to TGCT. However, collaborative efforts may be required to overcome the challenge posed by the rarity of this tumor.

CONCLUSIONS

The incidence of cancer is increasing worldwide. Although population aging is in large part responsible, a more comprehensive familial risk history and advances in genomics have uncovered an increasing proportion of tumors related to cancer-syndromes. Among urinary tumors, the recognition of new entities such as SDH and HLRCC renal cell carcinomas were possible after better characterization of those syndromes.

Familiarity with the various familial phenotypes and suggestive clinical features such as early age of presentation and multicentricity should point to the possibility of a cancer related syndromes.

CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Diagnostic relevance of metastatic renal cell carcinoma in the head and neck: An evaluation of 22 cases in 671 patients

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ABSTRACT

Purpose: Renal cell carcinoma (RCC) is a malignant tumor that metastasizes early, and patients often present with metastatic disease at the time of diagnosis. The aim of our evaluation was to assess the diagnostic and differential diagnostic relevance of metastatic renal cell carcinoma (RCC) with particular emphasis on head and neck manifestations in a large patient series.

Patients and methods: We retrospectively evaluated 671 consecutive patients with RCC who were treated in our urology practice between 2000 and 2013.

Results: Twenty-four months after diagnosis, 200/671 (30%) of RCC had metastasized. Distant metastases were found in 172 cases, with 22 metastases (3.3%) in the head and neck. Cervical and cranial metastases were located in the lymph nodes (n=13) and in the parotid and the thyroid gland, tongue, the forehead skin, skull, and paranasal sinuses (n=9). All head and neck metastases were treated by surgical excision, with 14 patients receiving adjuvant radiotherapy and 9 patients receiving chemotherapy or targeted therapy at some point during the course of the disease. Five patients (23%) survived. The mean time of survival from diagnosis of a head and neck metastasis was 38 months, the shortest period of observation being 12 months and the longest 83 months.

Discussion and conclusion: Our findings show that while RCC metastases are rarely found in the neck, their proportion among distantly metastasized RCC amounts to 13%. Therefore, the neck should be included in staging investigations for RCC with distant metastases, and surgical management of neck disease considered in case of resectable metastatic disease. Similarly, in patients presenting with a neck mass with no corresponding tumor of the head and neck, a primary tumor below the clavicle should be considered and the appropriate staging investigations initiated.

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INTRODUCTION

Renal cell carcinoma (RCC) is a malignant tumour of the kidney that metastasizes early. Most commonly, metastases occur in lung, bone or liver and often in multiple sites (1). Head and neck metastases are rare but there is little evidence in the literature as to their pattern and management.

Numerous single case reports and small series of metastasis of RCC into the head and neck region are available in the literature. These case reports focus mainly on particular, unusual, and especially extranodal location of the metastases as well as unusual clinical courses (2-8). The aim of this study was to assess the differential diagnostic and also the therapeutic relevance of metastatic RCC in a large series of RCC and to evaluate if a systematic examination of the head and neck is appropriate in the context of staging RCC. We present an analysis on RCC metastasizing into the head and neck region based on a large group of 671 consecutive patients with an RCC treated in our unit.

PATIENTS AND METHODS

Medical records of 671 consecutive patients who were diagnosed with an RCC in the Department of Urology of Ruppiner Kliniken, a large District General Hospital, between 2000 and 2013 were evaluated. All patients were followed-up until the time of their death; surviving patients were followed-up for at least 24 months from the date of diagnosis. All data were collected from case notes, anonymized and maintained in an Apache OpenOffice4 database and analyzed using a statistical software package (Apache OpenOffice4 Calc with R4Calc R extension). As this study was a retrospective case notes study, formal ethical approval was not required. Written consent was obtained from all patients prior to undertaking any procedures but for this retrospective case note audit, formal written consent was not required. All investigations and treatments were carried out according to accepted clinical practice and were compliant with the medical principles of the Declaration of Helsinki.

RESULTS

Of 671 consecutive patients diagnosed with RCC, 200 (30%) had distant or regional lymph node metastases either at the time of diagnosis or within 24 months of diagnosis. The overall metastatic rate, including locoregional metastases, was 17% (111/671) at the time of diagnosis of RCC, with an additional 13% (89/671) diagnosed following treatment.

Distant metastases were found in 172 patients (26%), and regional lymph node metastases in 22 patients (3%). In 92 patients (14%), metastases were identified at the time of diagnosis of the primary tumour, and in the remaining 80 patients (12%) metastasis occurred over the course of the following 24 months despite curative intent treatment (Table-1).

Metastases of RCC in the head and neck were found in 22 patients (3%). Sixteen patients were male and six were female. The mean age of these patients at the time of diagnosis was 66 years (32-81 years). In 10 patients (45%), head and neck metastases appeared simultaneously to the primary tumour, or the metastasis was the first manifestation of the RCC. In 12 patients (55%), metastases were detected at follow-up following curative intent treatment after 24 months on average. The longest period between treatment of the primary RCC and the detection of metastases in the Head and Neck was 87 months.

The histological type of renal cell carcinoma was clear cell renal carcinoma in 14 cases, poorly differentiated or undifferentiated carcinoma in six cases, nephroblastoma in one case and small cell renal carcinoma in one case. Tumour Grade was G2 in 8 cases, G3 in 9 cases, and undetermined in 5 cases. Initial TNM stages ranged from T1N0M0 to T3N2M1 at the time of diagnosis.

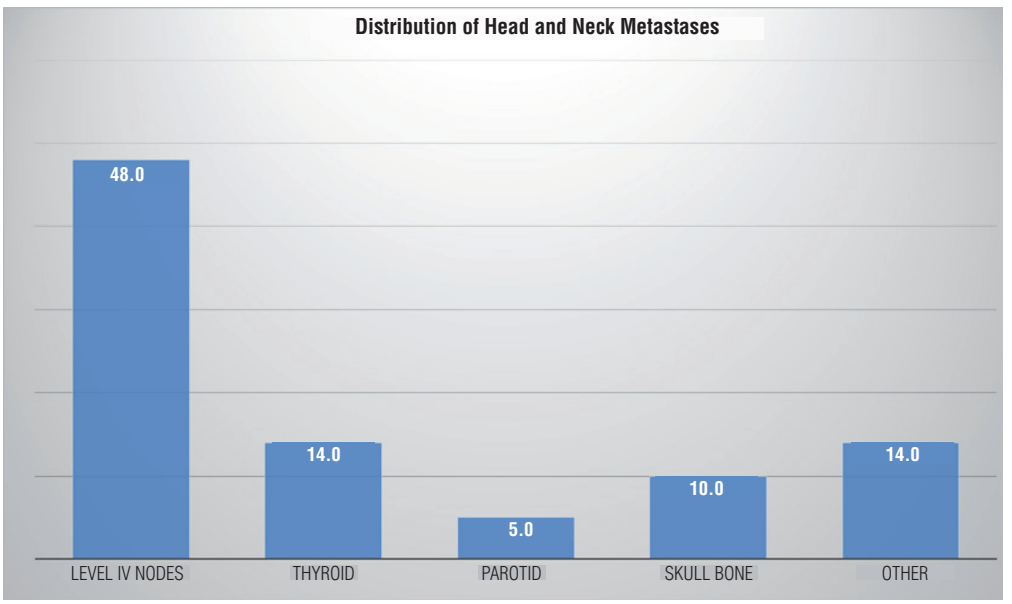
Metastases to cervical lymph nodes were found in 12 out of 22 cases. Organ metastases were found in the parotid (n=1) and thyroid gland (n=3) and skull bone (n=2). Other locations (n=3) included the tongue, facial skin and frontal sinus (Figure 1). Recurrence in context of a metachronous cervical metastasis was seen in one case. In 19 out of 22 patients, synchronous disseminated metastases were detected in other organs at some stage during the course of the illness. In 10 patients, this occurred simultaneously with the head and neck metastases. The most important metastatic target organs in these 19 cases were the lung (n=12) and the skeletal system (n=9). Other less frequent locations were the liver, the brain, and the peritoneum. In 3 out of 22 patients, metastasis occurred solely in the head and neck.

Table 1 - Patient and tumour characteristics.

No.	Age *	Histology	Grade	TNM at diagnosis	primary management	Time to Metastasis **	Location Metastasis	Other metastases	Management of metastatic disease	Survival after Metastasis (H&N) in months
1	70	clear cell	2	pT2bpNxM1	nephrectomy	0	Level IV Lymph node	Lung, bones	Excision	2
2	58	clear cell	2	pT3apN0M1	nephrectomy	5	Thyroid Gland	Retroperitoneum, pancreas	Excision (Thyroidectomy), Sunitimib	24 (patient alive)
3	74	clear cell	***	unknown	nephrectomy	78	Parotid Gland	none	Excision (Parotidectomy),	56 (patient alive)
4	52	clear cell/poly	3	pT3pN2 M1	nephrectomy	5	Level IV Lymph node	Lung, bones	Excision, radiotherapy, chemotherapy	14
5	78	clear cell	2	pT2NxM1	nephrectomy	65	Frontal Sinus	Brain, lung, bones, liver	Radiotherapy	2
6	69	undifferentiated	3	cT3cN1cM1	chemotherapy	0	Level IV Lymph node	Bone, liver, peritoneum	Excision, radiotherapy, chemotherapy	14
7	73	clear cell	2	pT1bNxMx	nephrectomy	94	Level IV Lymph node	Lung, bones	Excision, radiotherapy, chemotherapy, Sorafenib	7
8	69	clear cell	3	pT1cN0cM0	nephrectomy	14	Level IV Lymph node	Peritoneum	Excision, radiotherapy	4
9	70	undifferentiated	***	TxN1M1	chemotherapy	0	Level IV Lymph node	Lung, mediastinum	Chemotherapy, Sunitimib	28
10	69	undifferentiated	***	pT3cN0cM1	nephrectomy	24	Level IV Lymph node	Lung, bones, liver, adrenal glands	Excision, chemotherapy, Sunitimib	12
11	32	Nephroblastoma	3	pT3bpN0cM1	nephrectomy	0	Level IV Lymph node	Lung, liver	Chemotherapy, radiotherapy (other centre)	24
12	56	clear cell	3	pT3acN1cM1	nephrectomy	5	Tongue	Lung, bones, mediastinal nodes, soft tissue finger	Excision (glossectomy), radiotherapy	3
13	66	polymorph	3	pT1bNxMx	nephrectomy	75	Frontal Skull bone	Lung, bones, soft tissue back	Sunitimib, chemotherapy radiotherapy	13
14	81	undifferentiated	***	unknown	declined treatment	0	Level IV Lymph node	none	Declined treatment	0
15	69	clear cell	2	pT1acNxM0	nephrectomy	69	Level IV Lymph node	Retroperitoneal lymph nodes (paraaortal)	Excision, chemotherapy	19
16	68	clear cell	2	pT1bpN0cM0	nephrectomy	36	Facial Skin (forehead)	Lung, adrenal glands, jejunum	Excision	19 (patient alive)
17	48	clear cell	2	pT1bN0M0	nephrectomy adrenalectomy	87	Thyroid Gland	Lung, bones, mediastinum	Excision, laminectomy, chemotherapy, Sunitimib	27 (patient alive)
18	72	small cell	***	cT4cN1M1	resection metastasis	0	Frontal Skull bone and mandible	Lung, brain, mediastinal lymph nodes	Excision, radiotherapy	13
19	73	clear cell	2	pT1a cN0 M1	nephrectomy	40	Thyroid Gland	Lung, bones, mediastinum	Excision, radiotherapy	86 (patient alive)
20	78	clear cell	3	pT2bNxM0	nephrectomy	6	Level IV Lymph node	Lung, retroperitoneum	Excision, Sunitimib	7
21	63	undifferentiated	***	unknown	nephrectomy	0	Level IV Lymph node	Lung	Excision, none to lung	2
22	65	undifferentiated	***	unknown	unknown	unknown	unknown	unknown	unknown	Lost to follow up

*Patient age at the time of head and neck metastasis
**Time from first diagnosis of RCC to head and neck metastasis in months
***Tumour Grade undetermined

Figure 1 - Distribution of head and neck metastases by location (in % of n=22 patients).



All 22 patients received curative intent treatment at the time of diagnosis, except for one patient, who declined treatment. Eighteen patients, all of whom had the primary tumour diagnosed first or synchronous with the head and neck metastasis, received a nephrectomy.

In the 18 cases where metastases in the head and neck were found after diagnosis of the primary tumour or at staging of the primary tumour, patients received curative intent treatment at the time of initial diagnosis and were then followed-up by either a hospital or community urology tumour surveillance programme.

Nephrectomy was performed in 17 patients, total nephrectomy in 13 patients and partial nephrectomy in 4 patients. When metastases of the head and neck occurred, they were treated by surgical resection and adjuvant radiotherapy. In the 10 patients where diagnosis of the RCC head and neck metastasis preceded (4 patients) or coincided (6 patients) with diagnosis of the primary tumour, patients received surgical treatment of the head and neck metastasis first followed by surgery to the primary tumour followed by adjuvant radiotherapy.

Radiotherapy was performed in 14 cases. Radiotherapy after primary tumour resection was performed in one patient, following resection of the head and neck metastasis in 4 cases, and following detection of other metastases in 9 cases. The dose of radiotherapy was 40 Gray except in four patients who requested palliative treatment; 25 Gray were administered in such cases.

Chemotherapy was performed in 9 patients, usually following the diagnosis of disseminated metastatic disease. Due to the long observation period, chemotherapy regimens changed over time and included both standard chemotherapy, chemoimmunotherapy and targeted therapy. In particular, targeted therapy with either Sunitinibe or Sorafenibe was given to 6 patients.

Sixteen patients (73%) with head and neck metastases died from RCC. The time of death was on average 25 months after an RCC was first diagnosed, and 13 months after diagnosis of a head and neck metastasis. The median survival time after a RCC was first diagnosed was 28 months, meaning that 11 patients (50%) were still alive at 28 months after their RCC was diagnosed. The median survival time after diagnosis of a head and neck metastasis was 13 months. Patients died from either disseminated disease or local recurrence with the exception of one case, who died from an acute event.

Five patients (23%) survived and one patient was lost to follow-up. The mean time of survival from diagnosis of a head and neck metastasis was 38 months, the shortest period of observation being 12 months and the longest 83 months (standard deviation 30 months) (Figure-2).

DISCUSSION

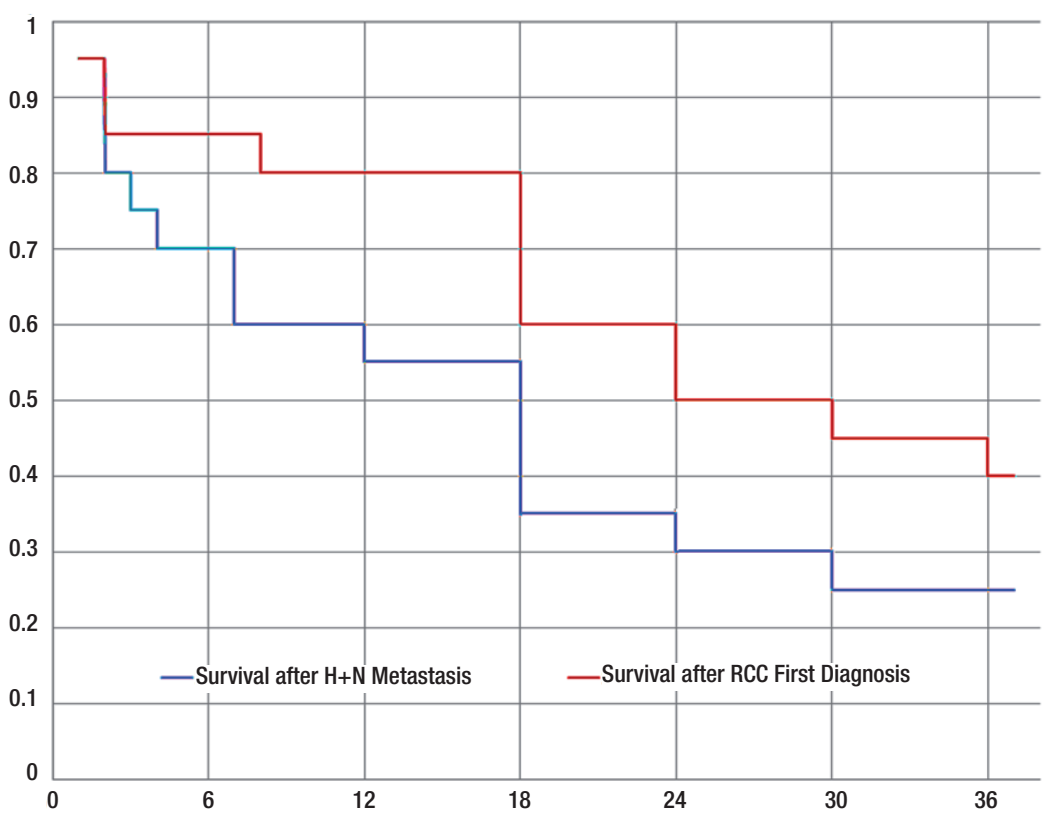
RCC are slowly growing, capsule-forming tumours and most frequently metastasize into lung and the lymph nodes, followed by the skeletal system and the liver – in the majority of the cases, several organ systems are affected simultaneously (1). The metastatic rate of 17% (111/671) in our patient group at the time of diagnosis, and an additional 13% (89/671) in the further course of the disease, is lower than described in other studies (2). This could be explained by the fact that 66% of our cases had been diagnosed in an early stage (61% in stage I) and had been well differentiated (Grade 1 + Grade 2: 68%).

RCC are considered to be the third most frequent infraclavicular tumour metastasizing to the head and neck. Supraclavicular metastases were found in 3% (22/671) of all our patients with RCC. In the literature, there are reports of metastatic rates of up to 15% (3-6). Whether these results can be compared to those presented here, remains to be discussed with regards to size of study, stage of disease, duration of follow-up and whether all patients were staged specifically for metastases of the head and neck.

While the proportion of RCC metastasizing to the head and neck was low at 3%, we observed head and neck metastases in 11% (22/200) of all metastasized RCC and in 13% (22/172) of all distantly metastasized RCC.

According to our results, RCC metastasizing into the head and neck area primarily metastasize into the cervical lymph nodes. In the literature,

Figure 2 - Survival after diagnosis of a head and neck metastasis, shown in months with standard deviation.



there are several reports about unusual manifestations of RCC in different organs of the head and neck. Single case observations refer to the parotid gland, the skull, the skin, the oral cavity, and the paranasal sinuses (3, 5-10), which were also seen in our patient group. Two large multicentre studies also reported metastatic spread of RCC into the thyroid gland, a phenomenon that was also observed in two of our patients (10, 11).

Our observations also show the variable pattern of cervical metastasis of RCC. In some cases, a cervical metastasis may represent the first manifestation of an RCC, in other cases, cervical metastases may occur months or years after curative intent treatment of an RCC (5, 6, 12). In 3 out of 22 patients, a solitary cervical lymph node metastasis was the first manifestation of a previously unknown RCC. At the other end of the spectrum, a solitary metastasis appeared in the parotid gland 6 years after diagnosis of the primary tumour. In the other 19 patients, the metastatic spread of the RCC into the head and neck occurred at the same time as metastasis into other organ systems.

Lymph node metastases and metastases of the parotid gland generally occur as painless, relatively slowly growing tumours (7, 9, 13). Facial nerve palsies in conjunction with parotid metastasis of a RCC are rare (3). Metastases within the upper aerodigestive tract such as the oral cavity and the pharynx are often painful. They are usually diagnosed when patients present with sore throats or oral pain, and grow nearly always submucosally, show signs of increasing vascularization and are often distinguished from mucosa by their red discolouration. Such lesions will bleed profusely when biopsied or haemorrhage spontaneously, and life-threatening haemorrhage has been reported. Metastases in the supraglottic larynx may cause narrowing of the upper airway, stridor and dyspnoea. Manifestations of the nasal cavity or the paranasal sinuses lead to nasal obstruction, sinusitis-like complaints, or significant haemorrhage (14).

According to our observations, the head and neck were involved in 13% of distantly metastasized RCC. This must be considered in patients who are due to undergo extensive surgery of either the primary tumour or metastases in other locations. Appropriate staging procedures would include imaging of the neck by either computed tomography (CT) or magnetic resonance imaging (MRI) with contrast and, if upper aerodigestive tract symptoms are present, a laryngo-pharyngoscopy.

Surgery as therapeutic option of metastasized RCC has an great significance. Good oncologic clearance is achieved in particular if metastases occur more than two years after treatment of the primary tumour, and where there is good surgical access. This applies to large case series on treatment outcomes of lung and liver metastases of RCC (15-17), and international guidelines recommend surgical therapy of metastases despite improvements of chemotherapy outcomes (2). Larger series of surgical therapy of supraclavicular metastases have only been published for patients with thyroid gland metastases. The five-year survival rate of those patients amounted to 51% (10, 11). Only case reports only exist about the surgical therapy of RCC metastases in other supraclavicular locations. Curative therapeutic options exist in cases of single metastasis into the head and neck (7, 8), but surgical management of head and neck metastases can also be appropriate for symptom control in cases of airway obstruction, haemorrhage, or pain (13). We observed survival of 23% of patients with a head and neck metastasis following treatment, and would therefore have no hesitation in recommending curative intent management of head and neck metastases in all patients fit for surgery.

CONCLUSIONS

Our results show that 3% (n=22) of all patients with an RCC (n=671) treated in our unit developed metastatic disease into the head and neck. This accounts for 9% of metastasized RCC. It remains open for discussion whether inclusion of the head and neck into the staging procedure should be recommended – it should, however, be considered in all cases of metastasized RCC. It is also of note that head and neck metastases of RCC may occur at any time during the course of the illness and any patient reports of head and neck-related symptoms such as neck swelling,

sore throat, dysphagia or foreign body sensation should prompt an otolaryngologist examination and an ultrasound examination of the neck and thyroid at the very least, bearing in mind that while most metastases occur in supraclavicular lymph nodes, they may also present in an unusual location. Surgical management of such metastases should be considered in all patients fit for surgery for both curative intent and palliative treatment.

CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Open partial nephrectomy for entirely intraparenchymal tumors: a matched case-control study of oncologic outcome and complication rate

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ABSTRACT

Purpose: To compare the oncologic and clinical outcomes for open partial nephrectomy (OPN) performed in patients with entirely intraparenchymal tumors versus case-matched controls, with exophytic lesions.

Material and methods: Patients having undergone OPN between 2007 and 2012 were investigated. Exclusion criteria included patients with a benign tumor, advanced malignancy, malignancies other than renal cell carcinoma, end-stage renal failure, or 3 or more co-existing chronic diseases. Individuals with tumors that were invisible at the renal surface were identified, and then matched with 2 controls chosen for tumor size, pathology, age, follow-up period, and presence of a solitary kidney. Oncological status, perioperative, and postoperative data were collected and compared between groups.

Results: 17 individuals with entirely endophytic RCC tumors and available oncologic status were identified. For five patients, only one suitable control could be identified, bringing the control group number to 29. All tumors were clear cell carcinomas staged at pT1a. Median tumor size was 25mm for endophytic lesions, and 27mm for exophytic masses (P=0.32). The operative period was extended by 20 minutes for intrarenal tumors (P=0.03), with one case of a positive surgical margin in each group (P=0.7). There were no significant differences in perioperative or postoperative complications. Median follow-up was 47 and 43 months for patients with endophytic and exophytic tumors respectively. Disease recurrence was recorded in one patient after endophytic tumor resection, and in four controls (P=0.4).

Conclusions: OPN shows equivalent safety and efficacy for both intrarenal RCC tumors and exophytic tumors of the same size and type.

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INTRODUCTION

In 2012, renal cell carcinoma (RCC) represented the nineth most common malignancy worldwide (1). Radical nephrectomy (RN) was considered the gold standard for RCC treatment but stage migration, advances in surgical technique, and an increased appreciation of the morbidity associated with renal insufficiency, have resulted in an expansion of the indications for nephron-sparing techniques. Partial nephrectomy (PN) for localized T1 RCC has an oncologic outcome similar to that of radical surgery, and incurs in a minimal risk of postoperative renal insufficiency (2-5). According to the guidelines of both the European Association of Urology and American Urological Association, PN is strongly recommended for patients with T1a tumors.

Despite these clear recommendations, PN utilization is variable and depends on a number of factors including the physician's preference, surgical skills, and tumor characteristics. It has been emphasized by many authors that PN of hilar and endophytic tumors is associated with a higher complication risk. The feasibility of performing a partial nephrectomy, in the case of an entirely endophytic tumor that does not extend to the renal surface, is of particular concern. These cases of tumor localization pose several challenges to surgeon, including the intraoperative identification of the tumor and its extent. The aim of this study was to compare the oncologic and clinical outcomes of PN for completely intraparenchymal tumors, with case-matched controls operated for exophytic lesions. To the best of our knowledge, this study is the first to compare results of nephron-sparing techniques for tumors not extending to the renal surface, with a matched control group of exophytic masses.

MATERIALS AND METHODS

Patient Selection and Outcome Measurements

Patients with a renal mass who underwent PN from 2007 to 2012, in a single department, were reviewed. Those with benign tumors, advanced malignancies other than RCC, a contralateral tumor, end-stage renal failure, or three or more co-existing chronic diseases, were excluded from the study. Within the patient group termed "ENDO", with entirely endophytic RCC tumors, tumors were defined as renal masses localized exclusively in the parenchyma of the kidney, and macroscopically invisible at the kidney's surface during surgery. Each of these patients was then matched with two others, with tumors visible at the renal surface (termed group EXO). The investigated group (ENDO) was matched with controls for age (±15 years), pathological subtype, clinical stage, tumor size (+/-1.5cm), time of surgery (same year), and type of indication for PN (elective vs. absolute).

Patient data were gathered for specified demographic and clinical variables, tumor characteristics, perioperative, and oncologic outcomes. Pathologic data comprised histological subtype, stage (assigned according to the 2009 TNM classification system) (6), grade (7), and surgical margins (8). Perioperative outcomes included clamping time, length of stay (LOS), transfusion, and surgical complications graded according to the Clavien - Dindo classification (9). A trifecta defined as warm ischemia time <25 minutes, negative surgical margins, and no perioperative complications was also calculated. Postoperative follow-up included ultrasound or CT imaging, performed every six months. Oncologic status for this study was assessed using CT performed at least 2 years after surgery. If any suspicion of disease progression arose, a complete restaging for further treatment was performed.

Surgical Technique

PN was performed using a lateral retroperitoneal approach in all patients. In the endophytic group, the renal parenchyma was incised over the tumor after identification of the mass by intraoperative US or palpation. The renal pedicle was always visualized and prepared for clamping. If necessary, warm ischemia was applied by clamping the main artery or its specific branch. Wedge resection, sometimes combined with enucleation, was utilized. Vessels were unclamped as soon as the major source of bleeding was found and controlled. Otherwise a standard procedure was applied (10-14).

Statistical analyses

Continuous variables were presented as medians accompanied by ranges or interquartile ranges (IQR). Differences between groups were evaluated using the U Mann-Whitney test for continuous variables, and by the chi-square test for categorical variables. As follow-up was inconsistent, with a six-month time span for control imaging, the precise time until recurrence could not always be reliably ascertained. For this reason, recurrence-free survival (RFS) was estimated using the modified Kaplan-Meier method for interval--censored data. Difference in survival between the endophytic group and the controls was assessed

using the log-rank test. For all statistical analyses, a 2-sided P value <0.05 was considered statistically significant. Statistical analyses were performed using STATISTICA 12 (StatSoft, USA).

RESULTS

During the reported period, 313 patients underwent PN in the department, with seventy--eight individuals excluded according to the criteria detailed earlier. Among the remaining 235 patients, we identified 24 individuals with entirely endophytic RCC tumors. For 7 of these patients, their oncological status was unavailable at the time of the study, leading to their exclusion. The final ENDO group therefore comprised 17 patients, with a median follow-up of 47 months (IQR 34). For five of these patients, we could identify only one control that matched all of our criteria. As a result, the control group comprised 29 patients, with a median follow-up of 43 months (IQR 23) (P=0.51).

All tumors in both groups were clear cell RCC, stage pT1a. Five patients, two with endophytic and three with exophytic tumors, had solitary kidneys. Other absolute indications for PN were not identified in the studied groups. Median tumor size and distribution showed no significant differences (Table-1). Six endophytic lesions (35.3%) and 4 exophytic tumors (13.8%) were 11-20mm, 3 endophytic tumors (17.6%) and 14 exophytic tumors (48.3%) were 21-30mm, while 6 (35.3%) endophytic tumors and 11 (37.9%) exophytic tumors were 31-40mm. Tumor localization was comparable for both groups: nine (52.9%) endophytic tumors and 13 (44.8%) exophytic tumors were entirely polar (P=0.6). A hilar location was found in 2 (11.8%) endophytic tumors, and 7 (31.8%) exophytic tumors (P=0.31). Also, there were no significant differences between the groups in terms of age, indication, and health status (Table-1).

Surgery in the ENDO group took approximately 20 minutes longer (P=0.03) and was performed more frequently under ischemia, than in the EXO group (P< 0.01; Table-2). If the artery was clamped, ischemia time never exceeded 20 minu-

Table 1 - Demographic and clinical characteristics of the patients who underwent open partial nephrectomy for entirely endophytic or exophytic renal tumors.

Variable	ENDO	%	EXO	%	P
n	17		29		
Age	61 (11)		63 (9)		0.95
Sex (Male/Female)	10/7		19/10		0.65
ASA score:					0.4
1 and 2	13	76.5	25	86.2	
3	4	23.5	4	13.8	
Hypertension	10	58.8	9	31	0.06
Diabetes	2	11.8	4	13.8	0.8
Preoperative serum creatinine (mg/dl)	1 (0.3)		1 (0.3)		0.99
Tumor size (mm)	25 (14)		27 (11)		0.3
Laterality (right)	9	52.9	11	37.9	0.3
Solitary kidney	2	11.8	3	10.3	0.9

ENDO = endophytic tumors; **EXO =** exophytic tumors; **ASA =** American Society of Anesthesiologists
Qualitative data are presented as median (interquartile range)

Table 2 - Perioperative and postoperative outcomes for patients having undergone open partial nephrectomy for entirely endophytic or exophytic renal tumors.

Variable	ENDO	%	EXO	%	P
n	17		29		
Operative time (minutes)	120 (34)		101 (35)		0.03
Renal artery clamped	9	52.9	4	13.8	<0.01
Warm ischemia time (minutes)	12 (3)		12.5 (7.5)		0.9
Intraoperative US use	7	41	0	0	<0.0002
PSM	1	6	1	3.4	0.7
LOS (days)	9 (34)		9 (23)		0.4
Trifecta	15	88.2	25	86.2	0.8
Follow-up (months)	47 (34)		43 (23)		0.5
Disease recurrence	1	5.9	4	13.8	0.4
Serum creatinine in follow-up (mg/dL)	0.9 (0.2)		1 (0.4)		0.96

ENDO = endophytic tumors; **EXO =** exophytic tumors; **US =** ultrasound; **PSM** = positive surgical margin; **LOS =** length of stay
Qualitative data are presented as median (interquartile range)

tes, with median values for both groups of 12 min. For localization of the endophytic tumors, in 7/17 cases intraoperative ultrasound was used, with the aid of a radiologist. Oncologic status was determined between January 2014 and April 2015. In both groups, all patients were alive at the time of the study. There were also no significant differences in perioperative and postoperative complications (P=0.8 and P=0.6 respectively). The renal collecting system was opened and repaired in 1 patient with an endophytic tumor and in 3 patients from the control group. Only one (6%) postoperative complication was observed in the ENDO group (wound infection and ileus, Clavien 2), with 3 events (10%) in the EXO group (hypokalemia and hypertension, Clavien 1; transient ischemic attack, Clavien 2; reoperation due to bleeding from intercostal artery, Clavien 3). No perioperative, cardiovascular life-threatening incidents occurred. Blood transfusions were carried out for 2 patients in the ENDO group, and 3 patients in the control group; each transfusion required 2 units of packed red blood cells. Positive surgical margins were found for 1 (6%) endophytic tumor, and 1 (3.4%) exophytic tumor (P=0.7). Disease recurrence was detected in one patient from the ENDO group (local relapse), and in four patients from the EXO group: two patients suffered a local relapse, one patient developed a multifocal recurrence in the ipsilateral kidney, and one presented with metastasis to the adrenal gland. The difference in recurrence-free survival was insignificant (P=0.3). In two patients from the EXO group (both with a solitary kidney), and in one patient from the ENDO group, creatinine levels exceeded normal values throughout the follow-up period. However, the median values for serum creatinine were unchanged for both groups during follow-up (Tables 1 and 2).

DISCUSSION

Despite explicit guideline recommendations, radical nephrectomy remains the most widely used treatment for T1 RCC tumors (15). Moreover, high tumor complexity diminishes the use of PN, even at high volume academic centers, from frequencies of 75-100% for cases with low nephrometry scores, to 0-45% for those with high scores (16). Frequent use of radical nephrectomy seems to be the result of concern about possible complications and poor surgical benefits. When considering the usefulness of nephrometry as a predictive tool, we believe that it is essential not to overestimate an endophytic location when predic-

ting PN outcome. Despite the additional challenge presented by these tumors, entirely intrarenal T1a tumors should always be considered as candidates for the nephron-sparing approach. The extent of adhesion to the renal collecting system is of more importance than tumor visibility alone. Therefore, when considering operative options, and candidacy for PN, we consider not only the absolute indications and staging, but principally the relationship of the tumor to the hilar structures. Hilar T1 tumors that dislocate vessels and/or the renal collecting system are therefore usually disqualified from PN.

Demographic and pathologic data did not differ significantly between cases and controls, which confirmed that our exclusion criteria and the matching process were adequate. There were no statistically significant differences in terms of positive surgical margins or recurrence. The length of stay was also similar for cases and controls. Although renal vessels were clamped significantly more often during endophytic PN, the warm ischemia times were comparable, and within safe limits.

The longer operative time for PN could be attributed both to vessel dissection and to the use of ultrasound. In cases where small exophytic tumors were localized far from the hilum, we could perform PN with neither artery clamping, nor artery dissection. This meant that the whole procedure, from skin incision to closure, could take as little as 60 minutes. In almost half of the cases of intraparenchymal lesions, intraoperative ultrasound was used to localize the tumor site, while in other cases, parenchymal bulging, especially when examined in ischemia, was the guiding parameter. Calling on the assistance of the radiologist prolongs surgery, with additional time needed to examine the kidney intraoperatively, along with logistic issues. Additionally, requesting the assistance of the radiologist when the surgeon does not have adequate ultrasound experience can cause delays, which can be avoided should the urologist be familiar with the apparatus. Although not proven statistically, our experience was that the smaller the intraparenchymal tumor, the more challenging it was to find. Additionally, appropriate patient selection and technical skills could adequately prevent major complications such as fistula formation. Transfusion was required infrequently in both groups. Only minor complications occurred, and no significant difference in total complication event number was noted.

We are, however, aware of some limitations of the study. Our study cohort was small, which impacts statistical power. Moreover, even with data-censoring, inconstant follow-up remains a significant limitation. Additionally, its retrospective nature renders our analyses prone to selection bias. Contrary to some reports (17, 18), the intrarenal tumors in our material were localized predominantly to the poles. This could be explained by selection bias or by the fact that in the majority of previous studies, endophytic lesions were defined as all those extending intrarenally by >50% of their diameter. Indeed the lack of any significant differences in relation to polar line and entirely endophytic versus exophytic masses has already been described (19).

Initial studies of the use of an open nephron-sparing treatment for endophytic lesions suggested that the resection of intrarenal RCC tumors was associated with longer ischemic times and higher peak creatinine levels in the immediate postoperative period (20, 21). On the other hand, initial laparoscopic procedures led to the concern that endophytic tumors were associated with a higher complication rate (22), and that corticomedullar growth patterns were the most significant predictor of postoperative complications (23). Contemporaneous with the development of minimally invasive PN, several reports have now confirmed similar outcomes for open (24, 25) versus robotic PN of complex tumors (18, 25-27). Authors have emphasized the low rate of major complications (19, 25, 26), oncological safety (19, 25, 26), short time of warm ischemia (19, 26), and superior preservation of renal function (24); all of which are in line with our data. The introduction of particular management measures and tools, such as intraoperative US (28), which is now strongly advised for complex tumors, may also result in improved outcomes. In terms of contemporary series on endophytic renal tumors and robot assisted partial nephrectomy (19), the surgical management of such lesions is technically extremely challenging. Therefore, open surgery remains the best option

for countries and regions where surgical robots are unavailable. Under such circumstances, the low morbidity and favorable oncologic data presented in our study, would lead us to advocate the use of partial nephrectomy over radical nephrectomy.

CONCLUSIONS

Our results confirm that an open approach for PN of endophytic T1a tumors is both safe and efficient, and should also be considered for patients with lesions located entirely interstitially. This localization, for small renal masses, carried no increased risk of complication or recurrence, and should not be incorporated in nephrometry scores.

LIST OF ABBREVIATIONS

RCC=Renal cell carcinoma
RN=Radical nephrectomy
PN=Partial nephrectomy
LOS=Length of stay
CT=Computed tomography
IQR=Interquartile ranges
RFS=Recurrence-free survival
US=Ultrasound

CONFLICT OF INTEREST

None declared.

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Comparative analysis of short – term functional outcomes and quality of life in a prospective series of brachytherapy and Da Vinci robotic prostatectomy

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ABSTRACT

Introduction: There is a growing interest in achieving higher survival rates with the lowest morbidity in localized prostate cancer (PC) treatment. Consequently, minimally invasive techniques such as low-dose rate brachytherapy (BT) and robotic-assisted prostatectomy (RALP) have been developed and improved.
Comparative analysis of functional outcomes and quality of life in a prospective series of 51BT and 42Da Vinci prostatectomies DV
Materials and Methods: Comparative analysis of functional outcomes and quality of life in a prospective series of 93 patients with low-risk localized PC diagnosed in 2011. 51patients underwent low-dose rate BT and the other 42 patients RALP. IIEF to assess erectile function, ICIQ to evaluate continence and SF36 test to quality of life wee employed.
Results: ICIQ at the first revision shows significant differences which favour the BT group, 79% present with continence or mild incontinence, whereas in the DV group 45% show these positive results. Differences disappear after 6 months, with 45 patients (89%) presenting with continence or mild incontinence in the BT group vs. 30 (71%) in the DV group.
65% of patients are potent in the first revision following BT and 39% following DV. Such differences are not significant and cannot be observed after 6 months.
No significant differences were found in the comparative analysis of quality of life.
Conclusions: ICIQ after surgery shows significant differences in favour of BT, which disappear after 6 months.
Both procedures have a serious impact on erectile function, being even greater in the DV group. Differences between groups disappear after 6 months.

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INTRODUCTION

Prostate cancer (PC) is the most common non-cutaneous cancer detected in males in the Western world (1). Retropubic radical prostatectomy has been the treatment of choice for localized PC in patients with a life expectancy ≥10yrs. Nowadays, the growing interest in achieving higher survival rates with lower morbidity has led to the development and rise of minimally invasive techniques, such as low-dose rate BT and robotic--assisted prostatectomy (RALP) (2).

A variety of therapies can be used to treat low-risk PC, according to D'Amico classification

(3); BT and RALP are two of them. Nevertheless, the use of one technique or another depends on the consensus between physician and patient. Current systematic studies on the management of localized PC conclude that all the treatments affect functional outcomes and quality of life with varying degrees, severity and duration. But, so far, there is not enough evidence to support one clinical procedure over the other.

The objective of the present study is to compare functional outcomes and quality of life in a prospective series of 51BT and 42Da Vinci robotic prostatectomies (DV) performed in our institution, being to this date the only report comparing both techniques, currently at their peak.

MATERIALS AND METHODS

From January through December 2011, 93 males diagnosed with low-risk localized PC in our institution chose BT or Da Vinci prostatectomy treatment. The choice was a personal decision once patients had been orally informed about the different therapies and after they had filled up a Validated Tool for Decision-making (4), which is a simple document explaining the different therapies for PC and side effects. Once patients had read the document and solved any doubts, 51 chose low-dose rate BT and 42DV prostatectomy.

Low-dose rate BT consists in the permanent implantation of Rapid Strand Iodine-125 seeds at a dose of 145Gy. Transperineal implantation of the seeds is performed in lithotomy position guided by transrectal echography, performing planimetry and previous dosimetry in the same procedure (real-time scheduling).

Robotic prostatectomy was carried out through laparoscopy using 3 ports in an inverted--U configuration of the robot arms (left ilioinguinal access port, left and right pararectal ports), a supraumbilical port for the optical trocar, a right secondary ilioinguinal port (12mm) and an optional right pararectal port (5mm). We performed antegrade dissection with neurovascular bundle preservation.

Both procedures were carried out by the same team of 4 urologists with wide experience.

The inclusion criteria for both techniques were strictly observed: clinical staging T1-T2a, Gleason score <7, PSA level <10, Body max index <35, prostate volume <50cc.

In our prospective series, we compared functional outcomes and quality of life before and after surgery during the first follow-up year. At months 3, 6, 9 and 12 patients filled up ICIQ (International Consultation on Incontinence Questionnaire) (5), IIFE (International Index of Erectile Function) (6) and the short-form SF36 test (physical and mental) (7) validated for Spanish. Through the ICIQ we evaluated urinary incontinence as mild (1-7), moderate (8-12) and severe (13-21) and with the IIEF score, erectile dysfunction was rated as severe (<15), moderate (15-20) or mild (21-25).

For the descriptive analysis, qualitative variables were expressed as absolute and relative frequencies and quantitative variables as median and interquartile range, when the distribution was normal or mean and standard deviation, when the distribution was not normal (Kolmogorov-Smirnov test). Chi square test was used to compare qualitative variables and Student T test or Mann Whitney U test for quantitative variables. A value of $p < 0.05$ was considered as statistically significant. SPSS v20 was used to perform the statistical study.

RESULTS

Mean age of patients in the BT group was 64yrs vs. 60yrs in the DV group, being this difference significant ($p < 0.05$). The groups under study were homogeneous and no statistically significant differences were observed in regard to tumour staging, PSA level at diagnosis, Gleason score or preoperative IPSS. We found differences in the surgical removed piece volume, preoperative maximum flow and SF36 (physical) (Table-1).

Most prostatectomy specimens were pT2c (51%), 27% of pT3 were understaged (24% pT3a and 3% pT3b) and 9% had positive surgical margins (predominantly unifocal). No death occurred in the mean follow-up period of 8 months (4-10).

The comparison of pre-and postoperative data revealed differences in IPSS and maximum flow which worsen significantly in the BT group (Table-2).

Table 1 - Pre-treatment description of age, PSA level, Gleason score, IPSS, maximum flow and SF36 in the two groups of patients.

Pre- Intervention	BT	DV	p
Age (mean)	64	60	*
PSA	5.8	6.3	NS
Gleason	6	6	NS
IPSS	6	6	NS
Maximum flow	22	15	*
SF 36 (physical)	50	53	*
Prostate volume	31	39	*

NS = No significant; * = <0.05

Table 2 - Comparison of pre and post-treatment outcomes in the BT and DV groups.

	BT	p	DV	p
IPSS (pre/post)	6 / 14	*	6 / 7	NS
Maximum flow (pre/post)	22 / 16	*	15 / 16	NS
Hemoglobin (pre/post)	15 / 14	*	15 / 13	*
ICIQ < 7 (pre/post)	83%/79%	NS	79%/45%	*
IIEF > 21 (pre/post)	60%/24	*	45%/10%	*
SF 36 (Pre/post)	105/ 105	NS	103/ 101	NS

Pre/Post = before intervention / 1 month after intervention; **NS =** No significant; * = <0.05

ICIQ at the first revision shows significant differences in favour of the BT group, as 40 patients (79%) were continent or present with mild incontinence, whereas only 19 patients in the DV group showed such positive results. Differences disappear after 6 months, when we found the same percentage of continent patients in both groups, 89% (45 patients) in BT and 71% (30 patients) in DV group (Table-2 and Figure-1).

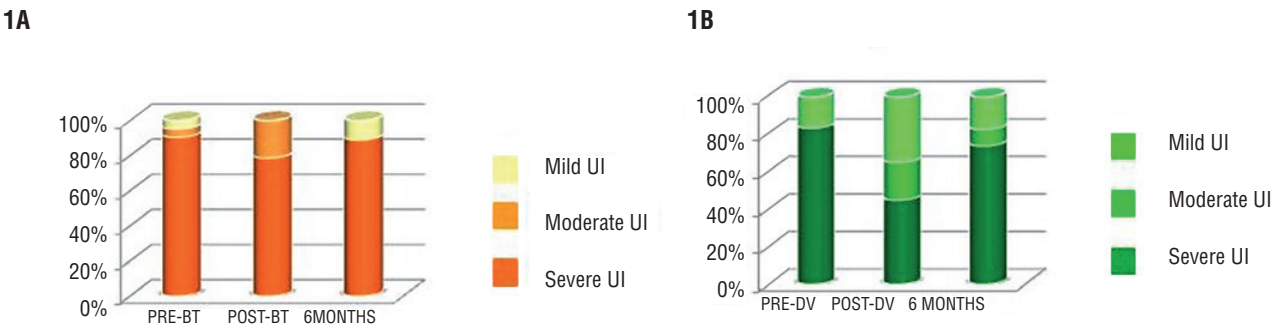
Both techniques have a serious impact on erectile function, with significant worsening of postoperative IIEF, being even more significant in the DV group (Table-2). Differences disappear after 6 months (65% with IIEF >15 in the BT group vs. 39% in the DV group) (Figure-2).

No significant differences were observed when we compared quality of life before surgery and in the different revisions (at months 3, 6 and 9) (Table-2).

In regard to further variables that could affect quality of life, hospital stay was significantly longer in the DV group (3 vs. 1 days) as well as postoperative pain, assessed by the Visual Analog Scale (VAS) (2 vs. 1) and mean indwelling catheter time (16 vs. 1 days).

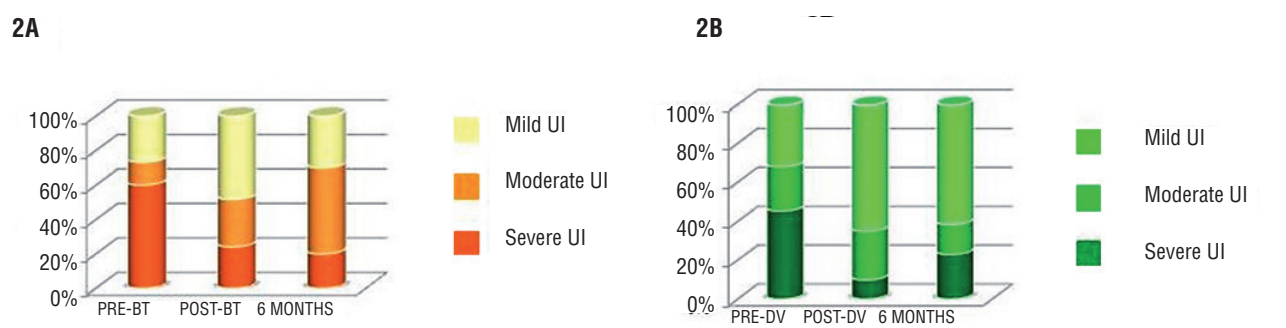
We observed a significant decrease of pre--and postoperative hemoglobin levels in both series, being greater in the DV group (Table-2). Although no differences are found in the number of patients showing complications (4 in each group)

Figure 1 - Percentage of mild, moderate or severe incontinence pre-treatment, at 3 months after treatment and from month 6 onwards.



1A) BT Group, 1B) BV Group

Figure 2 - Percentage of mild, moderate or severe erectile dysfunction pre-treatment, 3-6 months after treatment and from month 6 onwards.



2A) BT Group, 2B) BV Group

Table-3, the severity of complications, according to Clavien-Dindo classification, varies, with 2 patients in the DV group (48%) requiring conversion to open surgery.

DISCUSSION

Prostate cancer (PC) is the most common non-cutaneous cancer affecting males in the Western world. Since the prostate-specific antigen (PSA) screening became widely used for the early detection of PC in the early 90s, cancer-specific mortality has changed drastically (8). As an increasing number of tumours detected are localized, now the main objective of physicians is to improve morbidity (incontinence and erectile dysfunction) while maintaining the control of the disease (9). Thus, minimally invasive techniques have emerged in recent years to treat low-risk PC (BT, cryotherapy, HIFU, robotic prostatectomy). All of them try to provide positive oncological outcomes and improve the functional outcomes of radical prostatectomy. Recently, different series of patients undergoing low-dose rate BT or robotic-assisted prostatectomy have been reported with promising outcomes (10-13).

Both low-dose rate BT and robotic-assisted prostatectomy are equally recommended to treat PC in low-risk patients (according to D'Amico classification) with PSA levels <10ng/mL, Gleason score ≤6 and tumour staging T1-T2a. Nowadays, the final decision to undergo one procedure or another depends on the consensus between physician and patient. In our series, 27% of the radical prostatectomies were pT3, similar to other series (11).

Table 3 - Post-treatment complications.

Procedure	Complication	Clavien-Dindo	Approach
BT	AUR	II	Conservative
	AUR	II	Conservative
	UI	I	Medical
	UI	I	Medical
DV	Bleeding	III	Reconversion
	Difficult dissection	III	Reconversion
	Bleeding	II	Transfusion
	Urinary leak	II	Conservative

AUR = Acute Urinary Retention; **UI =** Urinary Infection.

Among the advantages of BT we must mention that it is a short, minimally invasive procedure (45-90 minutes) which does not require prolonged hospital stay. Also, BT delivers radiation in the prostate gland minimizing the radiation dose to surrounding healthy tissues. This would diminish radiation-related side effects such as erectile dysfunction or urinary incontinence, thus improving patients' quality of life. The improvement of functional outcomes with the use of robotic-assisted prostatectomy is due to the greater precision and technical skill achieved with the use of articulated instruments, ergonomic manipulation and 3D vision with 10x magnification.

A systematic review of reports comparing prostatectomy and BT to treat organ-confined PC concluded that BT shows similar outcomes to other therapies used for this type of tumours, at least in low-risk patients (14).

Nevertheless, none of the two procedures lack shortcomings or complications. Although the learning curve of robotic prostatectomy seems to be faster and this procedure provides ergonomic advantages for surgeons in comparison to conventional laparoscopy, the time required to prepare the Da Vinci system is twice the time necessary in conventional laparoscopy and the main drawback it presents is the loss of tactile sensation (15). On the other hand, according to some reports, BT shows a high incidence of local recurrence and complications (16).

Despite their limitations both procedures are two minimally invasive techniques which have proven useful to treat PC. However, there is no clear evidence to decide which of the two is the most adequate treatment for low-risk localized PC.

In our study, both the patients undergoing BT and those undergoing DV had similar PSA levels, Gleason score and tumour staging. The preoperative characteristics of the patients included in each group can be compared with those of the series so far published (9-13, 17-18). In our series, we found differences regarding mean age which can be explained by a tendency of ageing patients to choose BT to avoid the anesthetic complications of surgery. Age (64 vs. 60) is statistically different but we think it is not clinically relevant. SF36 and maximum flow could be clinically relevant, and because there is no randomization those differences can only be explained because people who understand that they are physically impaired prefer braquitherapy. Nevertheless, after treatment there is not differences in SF-36. Flowmetry and IPSS worsen significantly after BT (IPSS 6 and maximum flow 22 before intervention vs. IPSS 14 and maximum flow 16 after intervention). This is one of the possible side effects of BT, mainly due to a syndrome of mixed urinary incontinence (irritative symptoms provoked by acute cystitis and obstructive symptoms resulting from the local inflammation following radioactive seeds implantation) which can be partially controlled with the administration of alpha-blockers and anti-inflammatories.

Although DaVinci surgery is said to reduce hospital stay in comparison to open surgery, in the group of patients undergoing DaVinci prostatectomy included in our study, we

observed an increase in hospital stay, surgical times and surgeon's fatigue when compared with patients undergoing BT. Likewise, DV patients showed worse outcomes in VAS and had to use an indwelling catheter for a longer period of time.

The comparison of functional outcomes between the two groups is the main basis of our report. The ability to attain a firm enough erection for sexual intercourse depends on a variety of factors such as age, comorbidity (diabetes and peripheral vascular disease), psychological factors, habits and social factors. Also, we must consider the effect of the surgical technique or procedure employed. All this together with the multiple definitions of the terms sexual potency and erectile dysfunction and the use of different validated questionnaires makes it difficult to evaluate sexual function and to compare outcomes (9, 22). The same is true when we analyse urinary incontinence.

The percentage of potent patients following DV prostatectomy reaches 7-86% (23-32), which varies depending on the series, age of patients and unilateral or bilateral neurovascular bundle preservation. In the BT group, the percentage of potent patients ranges between 11 and 98% depending on previous sexual function, age, race and BMI (33).

Sexual function is seriously affected in both groups. Although no statistically significant differences are observed, impairment of sexual function is greater in the DV group (IIEF >15 in 65% of patients in BT group vs. 39% in DV group). Differences disappear after the six month.

A high percentage of DV patients present with early recovery of urinary continence (91-96%) (12, 34, 35). Our BT group showed a higher continence in the revisions at months 3 and 6 (79% patients without incontinence or with mild incontinence in the BT group vs. 45% in the DV group). Differences disappear after 6 months (71% patients without incontinence or mild incontinence in the DV group vs. 89% in BT group).

Bradley et al. in a report of 2004 compared radical prostatectomy, BT and BT combined with external radiotherapy and found no differences regarding quality of life. They observed worse initial functional outcomes in the radical prostatectomy group, which disappeared with time (36).

CONCLUSIONS

In our study, both patients undergoing BT and robotic-assisted prostatectomy experience a significant impairment of urinary continence and erectile function, especially the latter. Although initial functional outcomes are slightly better in the BT group, after six months patients undergoing robotic-assisted prostatectomy attain similar results to the BT group. No differences are observed in relation to quality of life when we compare both therapies.

To this date, there is no clear evidence to favour one technique over the other in relation to functional outcomes. Further studies comparing both techniques are necessary as well as the use of standardized validated questionnaires (IIEF, ICIQ, SF-36) before and after treatment and the objective quantification of urinary leaks (pad test) instead of the use of subjective quantification parameters.

CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Overexpression of UHRF1 gene correlates with the major clinicopathological parameters in urinary bladder cancer

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ABSTRACT

Introduction: Recently, expression of the UHRF1 gene was found to be up-regulated in numerous neoplasms, including the urinary bladder transitional cell carcinoma (TCC).
Objective: The aim of our study was to determine if the expression levels of UHRF1 gene correlates with the major pathological characteristics of the tumor and patients' clinical outcome.
Materials and Methods: In our study, we have analyzed the tissue samples derived from group of 70 patients with histologically confirmed TCC of the urinary bladder, while normal urinary bladder mucosa obtained from 40 patients with nonmalignant diseases was used as a negative control group. Expression of UHRF1 gene in each patient sample was determined using reverse transcriptase-polymerase chain reaction.
Results: UHRF1 gene expression was found to be app. 2.5 times higher in samples from patients with TCC in comparison with normal epithelium derived from control group patients. Analysis show that gene expression correlates with the malignancy of the tumor. A highly significant differences were found between the expression values of samples from low and high grade TCC, as well as between the high grade and control group. UHRF1 expression was higher in patients with non-muscle invasive disease than in those with muscle invasive disease.
Conclusions: The result of this study indicates that UHRF1 gene expression levels correlates with the major pathological characteristics of TCC samples and with the clinical outcome of those patients. Determination of UHRF1 gene expression could have a potential to be used as a sensitive molecular marker in patients with urinary bladder cancer.

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INTRODUCTION

Transitional cell carcinoma (TCC) of the urinary bladder cancer is a common malignancy in industrialized countries associated with high mortality thus having significant impact on public health (1-4). At the time of diagnosis, the majority of patients present with superficial neoplasm (restricted to the epithelium or the subepithelial connective tissue), while approximately 50% to 70% of patients develop disease recurrence with 10–40% of cases ultimately progressing to muscle invasive stage of advanced or metastatic disease over 5 years (5). Thus far, no validated and clinically useful molecular markers are established for urinary bladder TCC, as opposite to other urological cancers (6).

The Ubiquitin-like containing PHD Ring Finger 1 (UHRF1) protein, also called ICBP90, is

a multidomain protein encoded by the UHRF1 gene that seems to have a complex transcription pattern. UHRF1 binds to specific DNA sequences, creates a complex with histone deacetylase 1 and DNA methyltransferase 1 and thus regulates gene expression. As a target of E2F transcription factor, UHRF1 facilitates the cell-cycle G1/S transition (7). In addition, UHRF1 downregulates the expression of several tumor suppressor genes including p16INK4A, hMLH1, BRCA1 and RB1 and functions in the p53-dependent DNA damage checkpoint during cell-cycle regulation. Some authors consider UHRF1 as a caretaker that has a critical role in the maintenance of the genome integrity (8).

Latter, it was described that UHRF1 gene expression is only detectable in proliferating cells, not in quiescent cells and that this gene is overexpressed in numerous malignant neoplasms including lung, breast, prostate, pancreatic and cervical cancer (9, 10). Some authors consider UHRF1 gene as an oncogene which overexpression leads to hypomethylation phenomenon in cancer genomes (11).

Recently, overexpression of UHRF1 gene was described in urinary bladder TCC, but conflicting results were obtained regarding the correlation and clinical significance of this molecular abnormality (12-14). Furthermore, it was demonstrated that the upregulated UHRF1 promotes bladder cancer cell invasion by epigenetic silencing of KiSS1 metastasis suppressor gene coding for kisspeptin (15).

The purpose of our study was to determine if the expression levels of UHRF1 gene in patients with TCC correlates with the major pathological characteristics of the tumor and patient's clinical outcome.

MATERIALS AND METHODS

Patients and samples

'In this study we analyzed tissue samples from 70 patients with histopathologically confirmed transitional cell carcinoma of the urinary bladder, collected by transurethral resection of bladder tumor at the University Clinic of Urology in Skopje between October 2009 and March 2011. Sixty-one male and 9 female patients with median age of 64.29±9.51 years (range 38-79) were included in the study. Clinicopathological parameters that were evaluated included: histopathological grade according to WHO 2004 classification system (LG and HG), pathological stage (non--muscle invasive: pTa, pTis or pT1, and muscle invasive: pT2 or higher), as well as clinical history (incidence of local recurrence, distant metastases and cancer-related death in the 2-year follow-up period).

Histological tumor grading and staging were considered at the time when the tissue sample was first obtained from the patient.

As a negative control patient group, tissue samples of histologically normal urinary bladder mucosa obtained by open retropubic prostatectomy for benign prostate hyperplasia (34 patients) or hysterectomy for nonmalignant purposes (6 patients) were used. The patients from the negative control group had age mean 64.24±12.58 years (range 34-81) and very similar gender distribution as those from the UBC group.

All patients signed written information consent and the study was approved by the Ethics Board of the Urology Clinic in Skopje (No 03-1165 from December 28, 2009).

RNA isolation

Total cellular RNA was isolated from frozen tissue samples using TRI-reagent according to the manufacturer's instructions. Residual DNA was digested by RNase-free DNase I at 37°C for 30 min. After thermal inactivation of the enzyme at 95°C for 5 min, the RNA was reprecipitated with isopropanol and rehydrated with RNase-free water containing 20 units of ribonuclease inhibitor. All above mentioned reagents were purchased from Sigma-Aldrich, UK.

RT-PCR analysis

UHRF1 gene expression was measured by semiquantitative RT-PCR relative to housekeeping β-actin gene using reverse transcription system Enhanced Avian HS RT-PCR (Sigma-Aldrich, UK) following the manufacturer's protocol. Oligo-dT primer was used for first-strand cDNA synthesis, while amplification of UHRF1 gene-specific mRNA transcripts was performed by primer pairs:

forward, 5'-CCA GCA GAG CAG CCT CAT C-3' and reverse, 5'-TCC TTG AGT GAC GCC AGG A-3' with the following PCR program: initial denaturation at 94oC for 3 min, followed by 33 cycles of 94oC for 1 min, 60oC for 1 min and 68oC for 1 min, 45 secs, as described previously (16). Terminal extension at 68oC for 10 min was applied. Primer pair used for β-actin transcript was: 5'-GCT CGT CGT CGA CAA CGG CTC-3' and 5'-CAA ACA TGA TCT GGG TCA TCT TCT C-3'. All oligonucleotide primers were ordered from Sigma-Genosys, UK). Each RT-PCR reaction was run in triplicates on GeneAmp System 2400 thermocycler (Perkin Elmer, USA) with control reaction with no RNA template included. Amplification products sizes were 74bp for UHRF1 and 311bp for β-actin gene transcript. After agarose gel electrophoresis and digital imaging, fluorescence intensity of the electrophoretic bands was quantified using the gel analysis function of ImageJ 1.48 software (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997-2014). To quantify the relative levels expression of the target UHRF1, the value of the internal standard (β-actin) in each test tube was used as the background measurement (1.00) of gene expression in sample. The relative value of UHRF1 expression was calculated from ratio of the arbitrary units of the target gene to that of β-actin in the same PCR.

To determine the threshold value for overexpressed UHRF1 gene in our TCC patient's group, the expression values form control mucosa samples were analyzed. As those values were consistently between 0.0803 and 1.3850 (mean 0.5826) ±standard deviation (1.866), values of 2.4484 (mean+10 standard deviations) or more were considered to show overexpression of the UHRF1 gene (17).

Statistical analysis

Normal distribution of the gene expression data was evaluated by Shapiro-Wilk test. Correlations between the UHRF1 relative expression values from different patient's groups were analyzed using non-parametric unpaired, two-tailed Mann--Whitney test comparing independent samples with unequal variance. P-values less than 0.05 and 0.01 were considered to be statistically significant and highly significant, respectively. Frequency of overexpressed UHRF1 gene was compared between patient's subgroups by two-tailed Fisher exact test with calculations of Odds and Risk ratios at 95% confidence intervals. Statistical analyses were performed using Real Statistics Resource Pack software v. 4.3 on Microsoft Excel 2016.

RESULTS

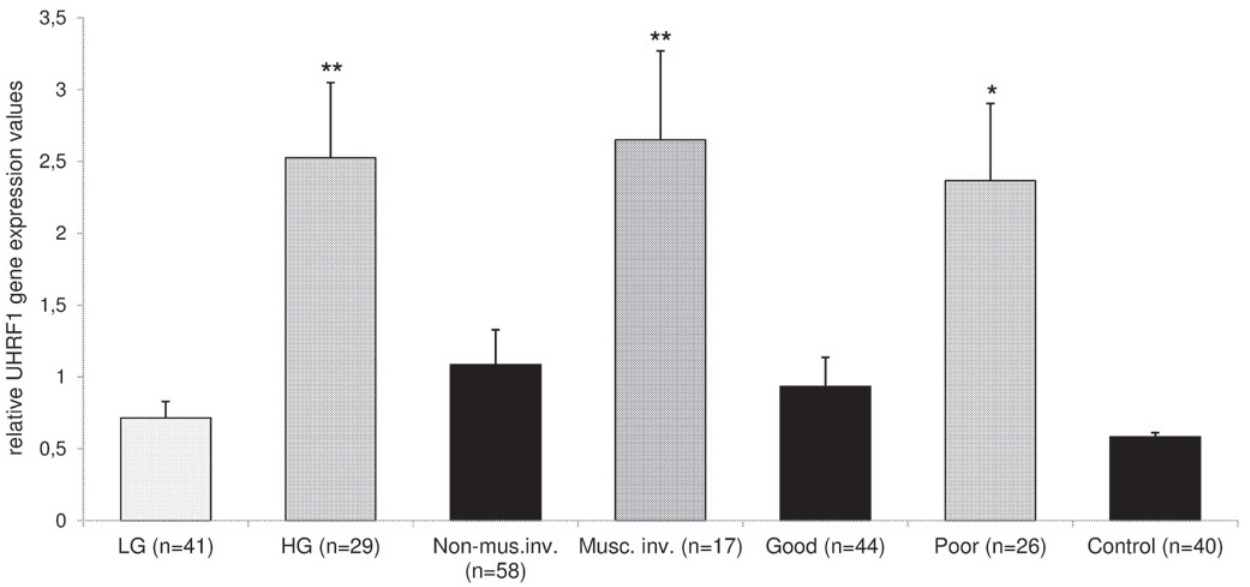
In this study, RT-PCR analysis revealed that the mean UHRF1 gene mRNA expression values in bladder cancer patients group were approximately 2.5 times higher than in the normal urothelium control group. Interestingly, the patients with solitary TCC has a significantly higher UHRF1 expression than the patients with multiple tumors ($p<0.05$).

We found that the obtained gene expression data correlates with the malignancy of the tumor (Figure-1). Namely, UHRF1 gene expression levels are lowest in control mucosa samples and in low grade TCC samples, but is significantly increased in the high grade samples ($p<0.01$, the exact p-values are given in Table-1). Statistical analysis revealed highly significant differences between the expression values from low and high grade samples, as well as between the high grade and the control group ($p<0.01$). On the opposite, the expression of UHRF1 gene was not significantly different between patients with TCC low grade and the control group with normal urinary bladder mucosa ($p>0.05$).

Considering the histopathological staging categories, the expression value means were significantly lower in the non-muscle invasive than in the muscle invasive stage ($p<0.05$). There was a statistically highly significant difference between the muscle invasive stage TCC patient's subgroup and control patients ($p<0.01$). However, the UHRF1 expression was not significantly higher in the non-muscle invasive stage than in the normal mucosa control samples ($p>0.05$).

Of the 70 analyzed patients, 26 had local tumor recurrences requiring multiple interventions, had a distant metastasis or died from this

Figure 1 - UHRF1 expression correlation with the T-classification grades, stages and the clinical outcome during 2-year follow-up of urinary bladder TCC patients.



LG and HG = low and high grades, respectively; **Non-musc. inv. =** non-muscle invasive stage; **Musc. inv. =** muscle invasive (infiltrative) stage; **Good** outcome, no clinical signs of recurrence, metastasis or cancer-related death within the 2-year evaluation period; **Poor** clinical outcome indicates recurrence, metastasis or cancer-related death in the same period. **Columns**, means; bars, standard errors; *and**, $p<0.05$ and $p<0.01$, respectively, versus control.

cancer within 2 years of obtaining the tissue sample. The mean expression values of UHRF1 in this poor outcome patient's subgroup was significantly higher than in the control group ($p<0.05$). For the purpose of prognosis prediction, the frequency of patients with UHRF1 overexpressed above arbitrary estimated threshold value was analyzed instead of semiquantitative data. The UHRF1 gene was overexpressed in 10 patients (38.46%) out of the TCC subgroup with poor outcome during the 2-years follow-up in comparison with the 3 patients (6.82%) in the subgroup with good outcome ($p=0.0016$). The calculated Odds Ratio is 8.54 (at 95% confidence interval from 2.08 to 35.12) and Risk Ratio is 1.51 (at 95% confidence interval from 1.11 to 2.07).

No significant differences of UHRF1 expression values were found regarding the other demographic and clinicopathological parameters: gender, smoking history and tumor size.

DISCUSSION

The overexpression of UHRF1 gene was recently described in many cancers, including urinary bladder TCC. However, although the expression levels were significantly higher in tumor samples than in the histologically normal urinary bladder, some authors did not identify any correlation between those values and the major pathological characteristics, the incidence of disease recurrence or patient's survival (18). On the contrary, another study showed an association between UHRF1 gene expression and tumor recurrence in superficial bladder cancer of Chinese cases (13). Considering the relatively small number of studies published thus far, it is rather difficult to conclude the exact nature of those results discrepancies.

We examined the correlations between UHRF1 expression in urinary bladder TCC patients and selected demographic and clinicopathological parameters. The expression levels of UHRF1 correlated significantly with the WHO 2004 histological grading category HG, as well as with both stages (non-muscle invasive and muscle invasive), but no difference was found between low grade TCC and control samples, as well as between the non-muscle invasive stage and controls, suggesting that the UHRF1 gene expression correlated

Table 1 - UHRF1 gene expression values regarding the demographic and selected clinicopathological parameters in urinary bladder TCC versus control patient's samples and differences between the urinary bladder TCC subgroups.

Patients groups	Demographic and clinicopathological patient subgroups		Patients number n (%)	UHRF1 expression (mean±SE)	Difference vs. control group (p-value)	Difference between the two subgroups (p-value)
TCC	**Total**		**70 (100)**	**1.47±0.25**	**0.1826**	/
	Gender	Male	61 (87.14)	1.53±0.28	0.3090	0.4719
		Female	9 (12.86)	1.02±0.34	0.0814	
	Smoking history	No	23 (32.86)	1.56±0.48	0.2332	0.7546
		Yes	47 (67.14)	1.42±0.29	0.2664	
	Tumor number	Solitary	41 (58.57)	1.75±0.36	0.0348*	0.0927
		Multiple	29 (41.43)	1.06±0.30	0.9177	
	Tumor size (cm)	≤3	38 (54.29)	1.09±0.25	0.4268	0.4330
		>3	32 (45.71)	1.91±0.45	0.1275	
	Grades	Low	41 (58.57)	0.72±0.11	0.9060	0.0090**
		High	29 (41.43)	2.53±0.52	0.0057**	
	Stages	Non-muscle invasive	53 (75.71)	1.09±0.24	0.6222	0.0305*
		Muscle invasive	17 (24.29)	2.65±0.62	0.0087**	
	Outcome	Good	44 (62.86)	0.93±0.20	0.4654	0.1127
		Poor	26 (37.14)	2.37±0.54	0.0410*	
Control	**Total**		**40 (100)**	**0.58±0.03**	/	/
	Gender	Male	34 (85.00)	1.58±0.03	/	0.6359
		Female	6 (15.00)	1.60±0.05	/	

*and**, $p<0.05$ and $p<0.01$, respectively, versus control; **SE =** standard error.

with the malignancy. Considering the mean expression levels were very similar in controls and in the low grade TCC samples, we hypothesized that UHRF1 overexpression initiates relatively late during the carcinogenesis and that this could be a consequence of a p53 dysfunction. Namely, some authors suggest that UHRF1 overexpression in cancer cells is partially due to p53 protein inactivation, since the last one is involved in UHRF1 gene regulation (13, 16). Previously, it was demonstrated that tumor-suppressor p53 protein indirectly downregulates UHRF1 expression by increasing the p21/WAF1 level and inactivation of E2F1 (19).

As experimentally induced UHRF1 gene knockdown in cancer cell lines leads to suppressed growth, the UHRF1 protein is essential for malignant cell's proliferation and accordingly could be an attractive drug target (20). Furthermore, considering the estimated Risk Ratio (1.51), UHRF1 gene overexpression in TCC patients increases the risk of poor outcome for 51% in the 2-year evaluation period, compared to TCC subgroup with normal gene expression levels. Our results are in accordance with some of the previous studies about potential usefulness of UHRF1 overexpression as a prognostic prediction factor in urinary bladder cancer (12-14).

Moreover, our study confirms the importance of UHRF1 gene overexpression in urinary bladder epithelial carcinogenesis and further suggests that it could be used as a clinically useful molecular marker. Using of this molecular marker in combination with another one or additional parameters could potentially provide even greater sensitivity or patient's outcome prediction.

The result of this study indicates that UHRF1 gene expression levels correlates with the high grade urinary bladder cancer, as well as with the muscle invasive stage. Further, results indicate that overexpression of this gene may predict the patient's outcome in 2-years postoperative period. Determination of expression of UHRF1 gene could have a potential to be used as a very sensitive molecular marker with a prospective value in clinicopathological evaluation and prognosis of patients with urinary bladder cancer. More studies with larger patient's groups and method validation are needed to confirm our results and establish stronger clinical usefulness of this molecular marker.

CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Prostate specific antigen and acinar density: a new dimension, the "Prostatocrit"

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ABSTRACT

Background: Prostate-specific antigen densities have limited success in diagnosing prostate cancer. We emphasise the importance of the peripheral zone when considered with its cellular constituents, the "prostatocrit".
Objective: Using zonal volumes and asymmetry of glandular acini, we generate a peripheral zone acinar volume and density. With the ratio to the whole gland, we can better predict high grade and all grade cancer. We can model the gland into its acinar and stromal elements. This new "prostatocrit" model could offer more accurate nomograms for biopsy.
Materials and Methods: 674 patients underwent TRUS and biopsy. Whole gland and zonal volumes were recorded. We compared ratio and acinar volumes when added to a "clinic" model using traditional PSA density. Univariate logistic regression was used to find significant predictors for all and high grade cancer. Backwards multiple logistic regression was used to generate ROC curves comparing the new model to conventional density and PSA alone.
Outcome and results: Prediction of all grades of prostate cancer: significant variables revealed four significant "prostatocrit" parameters: log peripheral zone acinar density; peripheral zone acinar volume/whole gland acinar volume; peripheral zone acinar density/whole gland volume; peripheral zone acinar density. Acinar model (AUC 0.774), clinic model (AUC 0.745) (P=0.0105).
Prediction of high grade prostate cancer: peripheral zone acinar density ("prostatocrit") was the only significant density predictor. Acinar model (AUC 0.811), clinic model (AUC 0.769) (P=0.0005).
Conclusion: There is renewed use for ratio and "prostatocrit" density of the peripheral zone in predicting cancer. This outperforms all traditional density measurements.

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INTRODUCTION

PSA and derived densities, whole gland density, PSAD (1, 2) and the transition zone density PSATD (3), have a limited role in diagnosing cancer despite initial optimism. This is partly due to age related changes (4).We propose a new way of using the zones of the prostate taking into account their absolute volumes and the asymmetry in the amount of glandular acini within each (5-7). Hence the relative contribution of each zone, in terms of both epithelial acinar cells and their PSA production, to the entire gland. This highlights the contribution of the peripheral zone acinar volume (PZav) and its acinar density (PZad). We divide the serum

PSA into the differing amounts of acini within zones of differing volumes.

The peripheral zone is an intrinsically more stable entity (in terms of volumes and relative amounts of stroma and acini). This allows an intuitive approach to use volumes with appropriate densities (not the arbitrary divising of entire PSA into the transition zone). It is these acinal cells that produce PSA, a marker of epithelial activity, and which undergo mitotic events causing cancer. Regardless of the nature of the nodule, there is always an increase in stroma relative to epithelium (8) and a diluting effect on glandular components. We have taken the overall glandular quantity in the entire gland to be 70%, most of which is concentrated in the peripheral zone (6, 8-10). This varies with age. The peripheral zone is far less variable in acini and stroma, and we extrapolate our other values from this constant entity.

We can better predict cancer of all grades, and more importantly, better predict high grade cancer than conventional densities. Finally, we propose that we will be able to model both the benign gland as it ages, in terms of each zone, its divisions into acinar and stromal elements, and further, that we can contrast this with the relative growth dynamics of the malignant gland.

MATERIALS AND METHODS

Our study population included 672 patients admitted to a district general hospital, for transrectal ultrasound and biopsy, from 2007 to 2012, because of elevated PSA, anxiety or abnormal rectal exam. This was performed by one physician. The inner gland and the outer, peripheral zone were measured. 409 were benign and 263 malignant. We measured the whole gland volume (WGv) using the ellipsoid formula, then the inner gland (transition zone and central gland combined). We subtracted this from the WGv to yield the peripheral zone volume (PZv). We documented those with a positive family history, a first degree relative affected by prostate cancer. Also whether they had had a previous negative biopsy and whether the prostate felt suspicious of cancer or was clearly malignant (Table-1).

We have accepted the established glandular, acinar component of the prostate of 70%. The WGv x 0.7 yields the whole gland acinar volume (WGav). By definition, 1–WGav yields the whole gland stromal volume (WGsv). The peripheral zone was attributed a percentage of 80%. 0.8 x PZv yields the peripheral zone acinar volume (PZav).

1-PZav yields the peripheral zone stromal volume (PZsv). The WGav – PZav yields the transition zone acinar volume (TZav).

1-TZav yields the transition zone stromal volume (TZsv) (Figure-1). Figure-2 demonstrates how the zones vary with a growing gland.

Densities

The serum PSA is divided into WGav to yield the whole gland acinar density. The peripheral zone acinar density is calculated by multiplying the WGad times the ratio of the PZav/WGav.

The transition zone acinar density is WGad–PZad.

Formula:

0.7WGv = WGav
PSA/Wgav = WGad
0.8PZv = PZav
Wgav - Pzav = TZav
WGad x PZav/Wgav = PZad
WGad - Pzad = TZad
WGv - Wgav = WGsv
TZv - Tzav = TZsv
PZv - Pzav = PZsv

Statistics

Univariate logistic regression was initially used to find significant predictors for all grades of prostate cancer and then for high grade (Gleason 7 and above). Backwards multiple logistic regression was used to generate ROC curves using the new model and comparing it to whole gland density, PSA density and to PSA alone using Medcalc statistical software.

Table 1 - Patient characteristics.

		BPH	PC
Age years	Mean	63.0	68.1
	Median	63.0	68.0
	Interquartile range	58-68	63-75
PSA ng/mL	Mean	8.1	16.4
	Median	6.6	8.5
	Interquartile range	4.6-9.1	5.8-13.3
Whole gland volume cc	Mean	57.6	48.0
	Median	51.0	40
	Interquartile range	35-71	29-59
Transition zone volume cc	Mean	29.8	22.4
	Median	24.0	16.0
	Interquartile range	14-39.5	9-29
Peripheral zone volume cc	Mean	27.7	25.7
	Median	24.0	24.0
	Interquartile range	18-34	17-31
Previous negative biopsy		93 (23%)	73 (28%)
Family history		48 (12%)	37 (14%)
DRE	Suspicious	102 (25%)	83 (32%)
	Cancer likely	4 (1%)	11 (4%)

RESULTS

See Table 1
See Table 2
See Table 3

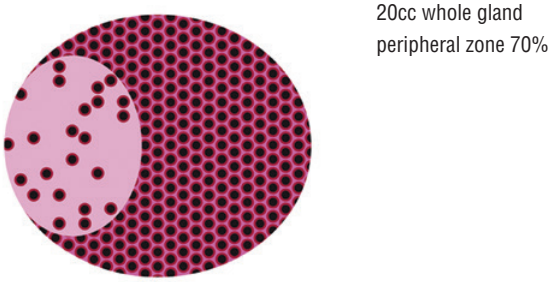
See Figure 3
See Figure 4

DISCUSSION

Developing the model

To tackle the problem of PSA and its variable zonal densities, we need to take into account the distribution of acini within the gland in the normal man and the age related changes of hyperplasia within nodules. The periphery was noted, in autopsies, to account for most of the glandular material (6) and the inner gland to account for most of the ducts and stroma. The asymmetry of gland distribution was further enforced when normal (autopsy) and enlarged (suprapubic prostatectomy) glands were compared (8). In the glands with benign prostatic hyperplasia, the glandular component was only 12% and this was mostly due to "dilution" by stromal growth. Growth rates were elucidated showing two phases, a rapid increase in growth from ten years old to thirty years then a slow gradual increase from thirty onwards. The normal stroma constituting about 50% of the mass and epithelium and acinal lumen the other 50%. Hyperplasia is associated with growth of both components to varying degrees (11). The ratio of stroma to epithelium, within adenomas, varies with symptoms (4.6) and without symptoms (2.6) (12). The stroma forms 62% of the symptomatic gland and epithelium 38%. The conclusion is that BPH is primarily a stromal process. Using enzymatic staining it was found the stroma to constitute 76% (12, 13) and that it is the ratio increase leading to symptoms.

Figure 1 - Normal gland 20cc schematic diagram. 70% PZ by volume 30% TZ by volume. PZ 80% acini 20% stroma (cells red, lumen black, stroma pink).

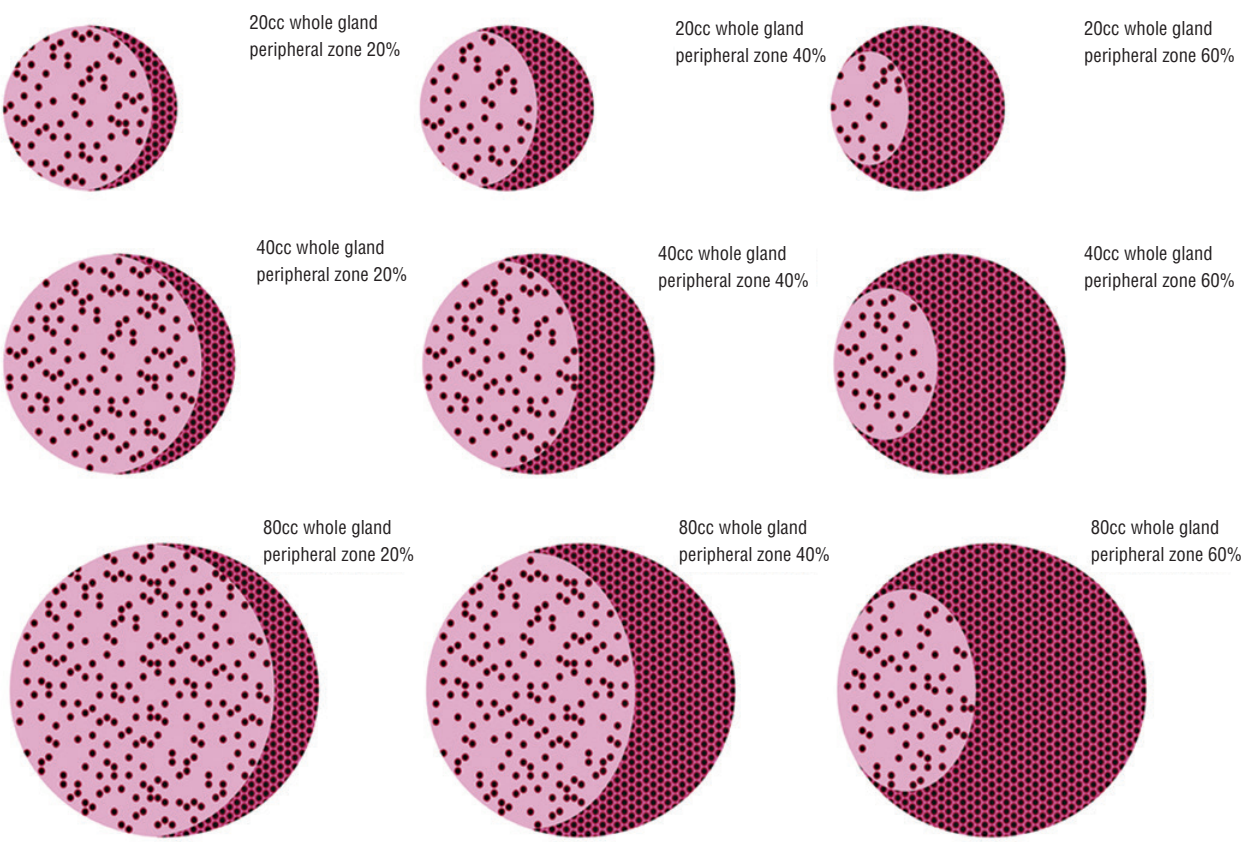


That PSA is related to prostate size, the amount of epithelium and testosterone was well established. But size is not a good predictor of PSA because of the great variation in acini and whole gland volume with a range of stromal component (14). It was proposed that epithelium itself is not a good guide to the amount of PSA because the architecture of acini can be disrupted by disease processes both benign and malignant. Further, PSA secretion is androgen independent and this wanes with advancing years.

The predominance of epithelium within the PZ compared to TZ, yet the lack of correlation of PSA with the PZ and the greater correlation of PSA with TZ has been confirmed (7). Further, the variability of the TZ and the relative constancy of the PZ is documented. The confounding problem of PSA is that it appears to be highly dependent on the TZ rather than the PZ. The rate of BPH epithelium growth is x 9 the normal gland rate and stromal growth within BPH x 37 the normal rate (15). In regard to ratio, the converse is true for cancers.

The androgen receptor is present in both stroma and epithelium, but 5 alpha reductase is only present in stromal cells and that they have an inductive influence on the epithelium (16). The

Figure 2 - Prostate gland with variation in whole gland size and peripheral zone as 20, 40, 60% of whole gland volume.



Whole gland volume 20cc top row; Whole gland volume 40cc middle row; Whole gland volume 80cc bottom row

Table 2 - All grade and high grade models.

All grade model	Variable	Coefficient	Standard error	P	Odds ratio	Confidence interval
	Age	0.089	0.01	<0.0001	1.09	1.067-1.11
	DRE = cancer	2.14	1.07	0.0466	8.5	1.03-70.0
	Family history positive	0.57	0.27	0.0382	1.7	1.03-3.08
	LnPZAd	0.58	0.16	0.0003	1.8	1.30-2.48
	Previous negative biopsy	-0.55	0.24	0.0218	0.57	0.35-0.92
	PZAv/WGAv	1.43	0.62	0.0223	4.18	1.22-14.28
	PZAd/WGv	60.1	26.23	0.0219	129E+24	6058-2.77E+048
	PZAd	-1.34	0.67	0.0470	0.26	0.06-0.98
High grade model	Age	0.06	0.014	<0.0001	1.07	1.04-1.10
	DRE suspicious	0.61	0.240	0.0103	1.85	1.15-2.96
	DRE cancer	3.49	1.105	0.0015	33.08	3.79-288.7
	Ln PZad	0.98	0.134	<0.0001	2.67	2.05-3.49
	Previous negative biopsy	-0.69	0.330	0.0346	0.49	0.26-0.95

Variables not included in all grade model; DRE suspicious; PSA; Race Caucasian; Race Afro-Caribbean; PZD; TZD; PSAD (WGD)
Variables not included in high grade model; family history; PSA; PZAD/WGV; PZAV/WGAV; PZD; Race Caucasian; Race Afro-Caribbean; TZD; PSAD (WGD)

Table 3 - Positive and negative predictive values (PZad).

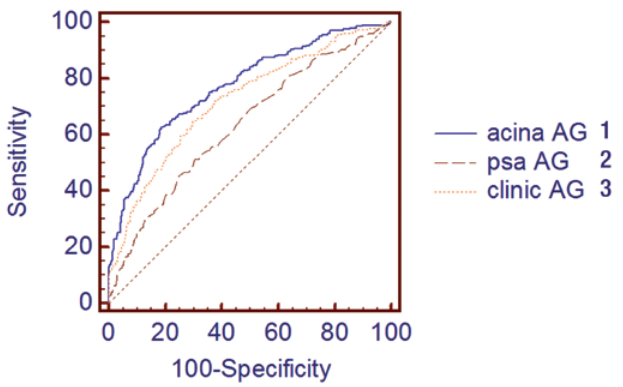
	High grade prostate cancer					All grades of prostate cancer			
Mean PZad	No cancer	Cancer	Total	PPV%	NPV%	No cancer	Cancer	PPV%	NPV%
0.043	128	7	135	5	95	104	31	23	77
0.082	118	17	135	13	87	101	34	25	75
0.121	115	19	134	14	86	92	42	31	69
0.197	106	29	135	21	79	73	62	46	54
0.775	74	61	135	45	55	40	95	70	30

range of influencing factors are categorised as intrinsic and extrinsic and there is no simple relation to androgen levels (17). We see in our cohort that the BPH glands are bigger overall, with larger transition zones, a similar size of peripheral zone and that the patients are younger than those with malignant glands (Table-1).

Ever since PSA was first localized to the gland (18) its exact use in diagnosis has been hampered by a lack of cancer specificity (1).

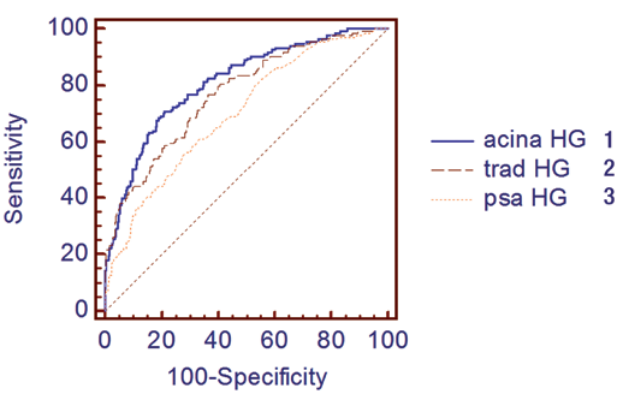
The relation of serum PSA to the entire gland volume was proposed (2) to allow an individualised approach to patients with intermediate PSA levels. The relation of PSA to zones of the gland was established using correlation coefficients (4). When reviewing patients who had undergone radical prostatectomy and cystectomy, it is apparent that the peripheral zone is significantly larger than the transition zone. It was found the average ratio of TZ to PZ was 3:1. Part of the

Figure 3 - ROC for all grades (AG) of cancer. Comparing acinar ratio (Prostatocrit) model with clinic model and PSA alone



1 - Acinar prostatocrit model AUC 0.7742; 2 - PSA AUC 0.636 P = 0.0001; 3 - Clinic model (PSAD) AUC 0.745 P = 0.01053

Figure 4 - ROC for high grade cancer. Comparing prostatocrit model with clinic model and PSA alone.



1 - Acina prostatocrit model AUC 0.811; 2 - Clinic (PSAD) model AUC 0.782 P = 0.005; 3 - PSA model AUC 0.700 P = 0.0001

problem with assessing the gland is the great variability in the TZ ranging from 2-80% of the total volume (19, 20). Recent studies (21) fail to differentiate cancer using PSAD. Further refinement was attempted with free/total ratio and TZAD (22) with PSA in the range of 2.5-4ng/mL. However, this has great limitations with small glands. The relation of the whole volume to PSAD and TZAD was proposed (23) and improves specificity and can limit unnecessary biopsies. The role of the PSATD has been recently strengthened by showing it had the most predictive power in diagnosing cancer (24). However, its use in predicting stage (25) reveals the confounding influence of the TZ. Regarding the PZ, it is suggested that the cancer which arises here, does so because of more cells of epithelial origin that are undergoing cell division and potential cancerous changes will be more numerous here. It appears there is a difference in the ratio of the two zones in cancer patients compared to benign patients. The peripheral zone is intrinsically richer in the acini that make PSA (5). The whole issue of PSA density and zonal densities has been dominated by the adoption of the term transition zone density which divides the entire serum PSA into the volume occupied by the inner gland (3, 26, 27). This is an incorrect use mathematically. Practically, it can contribute to diagnosis (26, 7), but ideally, the relative contribution of the separate zones should be accounted for. This is intuitively confusing otherwise and we end up with total densities greater than the original. The division of total serum PSA into the TZ ignores the contribution of the PZ to serum PSA. The corresponding lack of use of the TZAD highlights this (28). The peripheral zone has had limited application so far, although it has proved useful in men on alpha reductase inhibitors (29).

To illustrate the problem, we compared the two approaches below for a 40cc gland with equal components of peripheral and transition zones and with a serum PSA of 4ng/mL.

Thus, WGd=0.1ng/mL/cc

Traditional method

- TZ=20cc TZD=0.2ng/mL/cc
- PZ=20cc PZD=0.2ng/mL/cc
- Total density is now 0.4ng/mL/cc

Prostatocrit method

- We have to take into account for the relative contributions of each zone
- The TD is 0.1 x 20/40=0.05ng/mL/cc
- The PD is 0.1 x 20/40=0.05ng/mL/cc total=0.1ng/mL/cc

We now have a density attributable to the zonal volume. This can be refined estimating the bulk of epithelium/acini within each zone.

We are aware of packed cell volume in haematology. The acini are equivalent to red cells,

the stroma is equivalent to plasma and the PSA is equivalent to haemoglobin.

The gland is composed, overall, of 50-70% acini. Having accounted for different zonal volumes, we now need to account for the asymmetry of distribution of acini (Figures 1 and 2).

The peripheral zone is denser in acini by definition. Let us assume it is 80% acini. The transition zone must be less dense. For demonstration purposes, we choose 60% acini. The peripheral zone does not vary in its composition unlike the transition zone.

- For our 40cc gland with equal 20cc zones
- TZ=20cc x 0.6=12cc acinar volume
- PZ=20cc x 0.8=16cc acinar volume
- =28cc total acinar volume

This is the amount of epithelial tissue within the whole gland.

The density is derived by dividing the serum PSA into the relevant amount of acini.

- WGad=4/28=0.14ng/mL/cc of acini
- TZad=0.14 x 12/28=0.06ng/mL/cc
- PZad=0.14 x 16/28=0.08ng/mL/cc

Given that we can estimate the volume of acini within each zone, we can then by simple subtraction, estimate the amount of stroma, that is 1-acina %.

- TZsv=20-12=8cc
- PZsv=20-16=4cc
- 8+4=12cc stroma
- 12cc stroma + 28cc acini=40cc whole gland.

Using the model

Univariate logistic regression for prediction of all grades of prostate cancer were used to test significant predictors in a multivariate logistic regression. There were four significant zonal predictors. All involved the peripheral zone. The log of the peripheral zone acinar density, the ratio of the peripheral zone acina volume to the whole gland acinar volume, the peripheral zone acinal density to the whole gland volume and the peripheral zone acinar density. None of the conventional zone densities, whole gland, transition or peripheral zone densities were included.

We compared ROC for this model with a "clinic" model using the same information: PSA, age, family history, previous negative biopsy, rectal examination and overall gland volume (Table-2 and Figure-3). We also compared PSA on its own. There was a significant improvement in the area under the curve from PSA alone, to the conventional clinic model, to the prostatocrit model using ratio and acinar density of the PZ in four different combinations, all significant and all superior to traditional density measurements.

Most importantly, of practical clinical significance, it differentiated high grade (Gleason 7 and above) cancers better than traditional parameters. The only significant predictor was the peripheral zone acina density. None of the traditional densities were significant (whole gland, transition and peripheral zone densities) (Table-2 and Figure-4). The influence of the peripheral zone acinar density is further illustrated in Table-3, which shows the increasing positive predictive value as this density increases and the converse, the decreasing negative predictive value.

Limitations

TRUS is probably less accurate than MRI for measuring these volumes. These models will be improved with MRI and manual contouring of zones. We plan to do this in our next study. We also appreciate that other markers such as PCA3 and 4K could be included. The correlation of predicted acina density and final actual histological density will potentially strengthen this model.

Assumption of equal production of PSA in all types of acini in both periphery and transition zone. The formula is complex and will be part of a calculator, but this should not concern the physician.

Strengths

This is an intuitive use of density and accounts for the relative constant amount of peripheral zone epithelium within an easily measured zone. The peripheral acinar zone was consistently significant in predicting all grade cancer and high grade cancer. It revealed four significant parameters all superior to traditional density measurements.

CONCLUSIONS

When comparing the benign and malignant gland, the differences in ratio, with their acinal asymmetry, concentrated in the peripheral zone, enable the prostatocrit model to discriminate better between the two and hence who should have biopsies.

The absolute relation between zones, their acinal bulk and PSA production remains to be determined and may prove impractical, but this recognition of acinar contribution, may improve modelling of benign and cancerous disease, the response to drugs and need for surgery.

Take home message

PSA, density, zones, acinal asymmetry provides a new dimension to the analysis of the prostate gland. This prostatocrit model better predicts high grade cancer, all grades of cancer and it will help describe natural benign growth of the separate zones.

CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Current practice of antibiotic utilization for renal colic in the emergency room

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ABSTRACT

Introduction: Urinalysis (UA) in the emergency setting for patients with nephrolithiasis produces potentially confusing results leading to treatment of presumed urinary tract infections (UTIs). Our objective was to evaluate the use of antibiotics in patients with nephrolithiasis in a large network of emergency departments (EDs).
Methods: A retrospective analysis of all ED visits associated with an ICD-9 diagnosis of nephrolithiasis and a CT scan between 2010 and 2013 was performed. Urinalysis data, the use of IV and PO antibiotics during the ED visit and at discharge were assessed. The presence of fever, elevated serum WBCs, >5 WBCs per hpf, and/or dip positive nitrites were used as appropriate criteria for antibiotic use.
Results: Urinalysis data were available for 3,518 (70%) of 5,035 patients with an ED diagnosis of nephrolithiasis and CT imaging. Of these visits, 237 patients had positive nitrites (6.7%) and 864 had >5 WBCs per hpf (24.6%) with 158 (4.5%) having both findings for a total of 943 patients. Intravenous antibiotics were given to 244 patients (25.9%) and oral antibiotics were given to 629 patients (66.7 %) with positive UA findings. Of the 2,440 patients with a negative UA and no leukocytosis or fever, 86 patients (3.5%) received IV antibiotics and 533 patients (21.8%) received PO antibiotics upon discharge.
Conclusions: Proper treatment of nephrolithiasis in the ED includes the screening and diagnosis of concomitant UTIs. However, correct interpretation of UA studies is vital to the correct implementation of antibiotic therapy. This study suggests that 1/3 of patients were undertreated and 21.8% were over-treated.

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INTRODUCTION

Though nephrolithiasis continues to be one of the most common disorders treated by urologists, the urologist is rarely the first to diagnose and begin treatment of these patients (1). Due to the acute onset of symptoms, most patients with nephrolithiasis present to the emergency department (ED). A recent report showed there are an average of 700.000 ED visits annually for nephrolithiasis (2). This fact places ED physicians at the frontlines of diagnosis and treatment of nephrolithiasis.

The diagnosis of nephrolithiasis can be complicated by the concurrent presence of urinary tract infections (UTIs) or other urinary anomalies. Urinalysis (UA) is routinely performed as a screening test for nephrolithiasis and UTIs; however, interpretation of these results when both are present can be difficult. The presence of a non-obstructing stone in the ureter or kidney may lead to hematuria as well as inflammation. If present, this will

cause positive hemoglobin and leukocyte esterase on UA tests. These findings are often misinterpreted as positive for UTI or for "infected stones" in the ED. The actual incidence of infected stones, from urease-producing bacteria is 1-5% of all kidney stones (3). There does not appear to be standardized criteria for UTI diagnosis and treatment in the ED setting that takes the presence of nephrolithiasis into account.

The issue related to misdiagnosis of UTI in the patients with nephrolithiasis has two faces. First, if patients with actual UTIs in the setting of nephrolithiasis are not properly diagnosed and started on treatment or identified as needing urgent decompression, this may lead to a delay in definitive treatment for these patients. Second, the overtreatment of patients with nephrolithiasis without an actual UTI contributes to the growing number of multidrug resistant pathogens.

Our objective was to evaluate the current use of antibiotics in patients with diagnosis of nephrolithiasis in a large health system network of emergency departments (EDs).

MATERIALS AND METHODS

A retrospective analysis of all ED visits between December 2010 and March of 2013 at a large health system was conducted under IRB approval. Sixteen emergency department sites including academic programs with residents and community hospitals in two different states were included. Each site utilized the same electronic medical record (EMR), Epic Care (Epic Systems Corp., Madison, Wisconsin) and information was electronically extracted. Visits associated with an ICD-9 diagnosis of nephrolithiasis (592.0, 592.1, and 592.9) with an abdominal and pelvic computed axial tomography (CT) scan performed were evaluated. Inclusion criteria included all adult patients (>18 years old).

Patient demographic data were evaluated. Visual analog pain scores at admission as documented by nursing staff were assessed. Serum laboratory values and urinalysis data were reviewed when available. The administration of intravenous (IV) and oral (PO) antibiotics during ED admission was assessed via entries into the EMR. Antibiotic therapy initiated at discharge was assessed by reviewing medication orders at discharge within the EMR.

Appropriate criteria for antibiotic use was defined as patients found to be febrile (>101 degrees Fahrenheit) and/or UA findings of positive nitrites and/or presence of greater than five white blood cells per high power field (hpf) on microscopy. Given the lack of clear data in the literature regarding UA interpretation in the setting of nephrolithiasis, the definition of pyuria used in this study was based on expert opinion. Whether a urine culture was ordered during the ED visit was evaluated. Patients who had a negative UA or did not have a UA completed with concomitant finding of an elevated serum white blood count (WBC) greater than 11.000/µL or elevated temperature were excluded from the analysis. These patients were considered a "soft indication" for antibiotic administration, where clinical acumen beyond the scope of an EMR data analysis may play a role in decision making.

Statistics were performed using SAS software which included student t-test, ANOVA, and multi-variant analysis. Findings were considered significant if the p value was <0.05.

RESULTS

Data from 5.035 adult patient visits with ICD-9 codes for nephrolithiasis (592.0, 592.1, and 592.9) and CT imaging performed were identified. Urinalysis data were available for 3.518 patients, representing 70% of patients during the study time period. The mean age of patients with a UA performed was slightly younger at 45 years compared to those whom did not have a UA performed at 46.5. More females had a UA performed. About 5% of patients in both groups were found to have elevated serum WBC >11k/µL. Only a small number (<1%) of patients in both groups were found to have elevated temperatures. Initial pain scores were identical (See Table-1).

Of the visits with a UA performed (n=3.518), 102 patients (2.9%) had gross hematuria while 2.089 (59.4%) had >3 RBC/hpf on UA microscopy. Among patients with UA performed, 237 patients had positive nitrites (6.7%) and 864

Table 1 - Patient characteristics at ED visits for nephrolithiasis.

	UA Performed (n=3,518)	UA Not Performed (n=1,517)	P value
Age in years (mean, SD)	45.1 (±16.3)	46.5 (±15.6)	0.0034
Male (%)	1738 (49.1%)	845 (55.7%)	<0.0001
Elevated serum WBC > 11,000/µL (%)	191 (5.4%)	63 (4.2%)	0.058
Elevated temperature > 101ºF (%)	11 (0.3%)	4 (0.2%)	0.767
Initial pain score (median, IQR)	8 (7-10)	8 (7-10)	0.504
Gross hematuria on UA (%)	102 (2.9%)	NA	
>3 RBC/hpf on UA (%)	2089 (59.4%)	NA	
Nitrates on UA (%)	237 (6.7%)	NA	
>5 WBC/hpf on UA (%)	864 (24.6%)	NA	
Positive UA (%)*	943 (26.8%)	NA	

* Positive UA = nitrates and/or >5 WBC/hpf

had >5 WBCs per hpf (24.6%). Only 158 patients (4.5%) had both findings. A total of 943 patients were deemed to warrant antibiotic treatment based on UA analysis. 67 of these patients also had concurrent elevated serum WBC >11k/µL or elevated temperature. Of these 943, intravenous antibiotics were given to 244 patients (25.9%) and oral antibiotics were given to 629 (66.7%) at the time of discharge (Table-2).

Of the 2.575 patients with a negative UA finding, 135 patients were noted to have an elevated serum WBC >11k/µL or elevated temperature and therefore treatment may have been warranted, due to either a UTI or another infectious source (i.e. pneumonia), and as such were excluded. Of the remaining 2.443 patients, 86 patients (3.5%) received IV antibiotics and 533 patients (21.8%) received oral antibiotics that were not clearly warranted based on UA results upon discharge from the ED (Table-2).

Among the 1.517 patients seen for nephrolithiasis who did not have a UA performed, 64 were noted to have an elevated serum WBC>11k/µL or elevated temperature and were excluded as treatment may have been warranted due to another source of infection. Of the remaining 1.453 patients, 3.5% of these patients received IV antibiotics and 16.3% received oral antibiotics upon discharge (Table-2).

Table 2 - Utilization of antibiotics based on UA findings.

	UA Positive (n=943)	UA Negative (n=2,440)*	UA Not Performed (n=1,453)**	P value (ANOVA)
IV antibiotics in ED (%)	244 (25.9%)	86 (3.5%)	51 (3.5%)	<0.0001
Oral antibiotics at discharge from ED (%)	629 (66.7%)	533 (21.8%)	237 (16.3%)	<0.0001

* 135 patients were excluded with elevated temperature or elevated serum WBC as possible indication of antibiotics
** 64 patients were excluded with elevated temperature or elevated serum WBC as possible indication of antibiotics

Looking at the utilization of a confirmatory urine culture, only 570 of the 943 patients with positive UA findings (60.4%) had a culture sent. Of patients that received antibiotics during the ED encounter, 68.5% of those treated with IV antibiotics had a urine culture sent and 51.1% of those treated with PO antibiotics had a urine culture sent.

Multivariate analysis was performed for positive predictors of receiving oral antibiotics at discharge. Male gender, elevated serum WBC, positive UA findings, and having a UA performed were significant predictors for receiving oral antibiotics at discharge (Table-3).

DISCUSSION

The rate of nephrolithiasis is increasing throughout the United States (4-7). With the majority of episodes of kidney stones initially presenting to EDs, the burden of properly diagnosing and managing these patients falls to the ED physicians. One aspect of the diagnosis and management of stone patients is that of screening the urine for possible concomitant UTIs. As this study suggests, the interpretation of UA data may lead to both under and over utilization of antibiotics in these patients. To our knowledge, this is the first study to assess the antibiotic treatment pattern in the ED for patient with nephrolithiasis.

Recognizing the confounding results of a UA in the setting of nephrolithiasis is vital in the initial management of these patients. The presence of leukocyte esterase on dip UA without the presence of nitrites or visible white blood cells on microscopy is not specific for a UTI when inflammation from the stone is likely responsible. There is no consensus in the literature and a paucity of data regarding UA findings in the setting of nephrolithiasis which correlate with UTI. The definition of UA findings suggestive of pyuria used in this study was based on expert opinion, with the aim of accepting a liberal indication for antibiotic use. This definition provides a reference point only, the use of similar criteria in other settings has been shown to be nonspecific (8). Confirmatory urine culture should always be sent from patients with suspicious findings on UA. We found this was properly ordered in less than 2/3 patients.

The data presented in this study suggest that one third of patients presenting to the ED with nephrolithiasis had suggestive findings for pyuria and were not treated with home going antibiotic therapy. The ramifications for correct interpretation of UA results could affect the overall financial burden of nephrolithiasis on healthcare systems (9).

This study also suggests that a significant number of patients were treated with antibiotics that may not have warranted treatment based on UA results. The development of multidrug resistant pathogens has become a challenge to not only the urologist but almost all medical specialties. It is important to educate ED physicians on the interpretation of UA results in patients with nephrolithiasis, on the indications for appropriate

Table 3 - Multivariate analysis of positive predictors for receiving antibiotics at discharge from ED visit for nephrolithiasis.

Predictive factor	X^2	P value
UA performed	31.33	<0.0001
>5 WBC on UA	320.29	<0.0001
Positive Nitrites on UA	37.76	<0.0001
VAS Pain score at admission	9.4	0.67
Serum WBC level	24.92	0.0001
Initial temperature	0.58	0.45
Gender	20.49	0.0001
Age	0.50	0.48

antibiotic use and the importance of confirmatory urine cultures.

The limitations of this study include the inherent limitations associated with a large retrospective chart review. The data available were analyzed; however, data such as fever, anti-pyretic use, or antibiotic use prior to arrival was not available. Data regarding specific symptoms such as nausea and vomiting were also not available. Patients were excluded if another possible source of infection was present as evident by elevated WBC (WBC >11k/µL), however for those who were normopenic with a normal UA the rational of the ED provider's use of antibiotics was not evaluated. Further research is needed to define the precise UA criteria in the setting of nephrolithiasis which correlate with culture proven UTI.

Urologists should work with their local EDs to develop standardized criteria for diagnosing and initial treatment of nephrolithiasis. Patients who are properly diagnosed and have appropriate treatment underway when they are referred to the urologist are more likely to receive prompt and definitive treatment of the kidney stones regardless of which modality of treatment is chosen. Cooperation between the urologist and ED physician can also reduce duplicated investigations and delays, which could improve patient satisfaction and reduce costs. Care pathways may help facilitate the adaptation of best practices.

We propose the following algorithm for the administration of antibiotics in the ED. Firstly, antibiotics should be administered only after a urine sample for culture has been obtained. Secondly, clinical criteria for antibiotic administration include: WBC >15k/µL, UA >10WBC/hpf, temperature >101 degrees F, or nitrites positive on urine dip. Lastly, a urology consultation should be obtained in any of these situations if an obstructing stone is present, as the patient may require emergent decompression of the urinary system.

CONCLUSION

Proper treatment of nephrolithiasis in the ED includes the screening and diagnosis of potentially dangerous concomitant UTIs. However, correct interpretation of the urinalysis studies in these patients is vital to the correct implementation of antibiotic therapy. If a UTI is suspected based on UA results, a confirmatory culture should be sent and antibiotic treatment should be started. Care should be taken to practice antibiotic stewardship and ensure antibiotics are given only to patients who warrant them to prevent development of multidrug resistant pathogens. This study suggests that one third of patients were under-treated (satisfied criteria for antibiotics did not receive them) and nearly one fourth of patients were over-treated (received antibiotics despite normal urinalysis).

CONFLICT OF INTEREST

None declared.

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Evaluation of the learning curve for transurethral plasmakinetic enucleation and resection of prostate using a mentor-based approach

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ABSTRACT

Objective: To analyze the mentor-based learning curve of one single surgeon with transurethral plasmakinetic enucleation and resection of prostate (PKERP) prospectively.
Materials and Methods: Ninety consecutive PKERP operations performed by one resident under the supervision of an experienced endourologist were studied. Operations were analyzed in cohorts of 10 cases to determine when a plateau was reached for the variables such as operation efficiency, enucleation efficiency and frequency of mentor advice (FMA). Patient demographic variables, perioperative data, complications and 12-month follow-up data were analyzed and compared with the results of a senior urologist.
Results: The mean operative efficiency and enucleation efficiency increased from a mean of 0.49±0.09g/min and 1.11±0.28g/min for the first 10 procedures to a mean of 0.63±0.08g/min and 1.62±0.36g/min for case numbers 31-40 (p=0.003 and p=0.002). The mean value of FMA decreased from a mean of 6.7±1.5 for the first 10 procedures to a mean of 2.8±1.2 for case numbers 31-40 (p<0.01). The senior urologist had a mean operative efficiency and enucleation efficiency equivalent to those of the senior resident after 40 cases. There was significant improvement in 3, 6 and 12 month's parameter compared with preoperative values (p<0.001).
Conclusions: PKERP can be performed safely and efficiently even during the initial learning curve of the surgeon when closely mentored. Further well-designed trials with several surgeons are needed to confirm the results.

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INTRODUCTION

Transurethral resection of prostate (TURP) is the gold standard operation for symptomatic benign prostatic hyperplasia (BPH), but the complications such as transurethral resection syndrome (TURS) and blood loss still remain a problem, especially for the monopolar TURP with large prostates (1-3). Although there has been improvement in the TURP technique such as using the laser in the surgical treatment of BPH, the learning curve for the laser operation is longer and difficult to learn in a short period (4-6). Moreover, the expense of the laser may be higher and may not be widely available especially in the underdeveloped areas (7). Bipolar plasmakinetic TURP permits a longer operation time by saline irrigation instead of a mannitol solution, which significantly decreases the incidence of TURS (8). Plasmakinetic enucleation of prostate (PKEP) was developed to enucleate the prostate adenoma with the electrode loop and resectoscope tip without supernumerary

equipment. It is considered an effective alternative to TURP and superior to monopolar TURP and bipolar TURP but with lower morbidity and shorter hospital stay (9, 10). Compared with holmium laser enucleation of the prostate (HoLEP), PKEP is also an effective and safe treatment for BPH (9). Consequently, PKEP has aroused a great deal of interest in the urological community, and efforts to learn and adopt this technique are being made. Since the separate commercial morcellator is not widely available in China, the enucleaton tissue can be resected with an electrode loop without other additional instruments, which was used in our study as transurethral plasmakinetic enucleation and resection of prostate (PKERP). To our best knowledge, there is only one published retrospective study that had evaluated the learning curve of PKERP (11) and no prospective trials have been published by now. Thus, the learning curve of this procedure has not been clearly defined. In this study, we evaluated the learning curve of a resident in PKERP and compared experience with a senior urologist.

PATIENTS AND METHODS

Study design

All operative procedures were performed in our institution by a single senior resident (surgeon A) who had received adequate endourological training. Surgeon A had finished one year of basic endourological training in our hospital and passed the examination. He had previously performed 80 transurethral resections of bladder tumours. However, he was inexperienced with transurethral prostate surgery, and had previously only performed about 10 TURP cases. An experienced urologist (surgeon B) was the expert in PKERP and had performed more than 300 PKERP procedures at our institution since its inception. He served as a mentor for surgeon A. Surgeon A had not previously performed PKERP, thus he familiarized himself with the PKERP technique by viewing videos of surgeon B performing the procedure. The PKERP technique and the videos were then reviewed with surgeon B to discuss remaining questions. Surgeon A assisted surgeon B in 10 PKERP procedures, and participated during enucleation and resection. These 10 patients were excluded from the analysis. When he was judged to be reasonably confident with the technical dynamics, a prospective study was designed to assess his progress in learning PKERP. All patients gave written informed consent and the study protocol was approved by the Institutional Review Board of our hospital in compliance with the Declaration of Helsinki.

A total of 90 consecutive patients with symptomatic BPH had undergone PKERP which were performed by surgeon A, with supervision by surgeon B. Surgeon B gave advice when it was necessary, but did not replace surgeon A unless safety issues emerged during the procedure. This parameter was recorded as frequency of mentor advice (FMA). Pitfalls, tips, and tricks of the PKERP procedure were discussed pre and post surgery in detail. The patient's preoperative evaluations included transrectal ultrasonography with measurement of the total prostate size, serum prostate-specific antigen (PSA) assay and urine analysis. International Prostate Symptom Score (IPSS), quality of life (QoL) score, Qmax and postvoid residual (PVR) volume were recorded before and 3, 6, and 12 months after operation. Patients were excluded from the study if they had prostate cancer or neurogenic bladder or if they had undergone previous urethral or prostate surgery. Transperineal ultrasonography-guided prostate biopsies were performed to exclude prostate cancer when clinically necessary.

Data were collected during PKERP and included the total operative time, enucleation time, resection time, resected weight and FMA. The total operation time was defined as the interval between introducing the resectoscope and inserting the catheter. Operation, enucleation, and resection efficiency were calculated. The time to catheter removal and hospital stay were recorded after the operation. Complications were classified using the modified Clavien-Dindo classification of surgical complications and the data were also recorded.

To assess the number of procedures required to achieve competence in PKERP, the patients were first analyzed by divided with 10 cases depend on the time sequence. To assess the effect of the learning curve on the procedures outcome and

complications, the 90 patients treated by surgeon A were divided into two subgroups according to the competence time (Group1: patients 1-40; Group2: patients 41-90). The results of surgeon A were also compared with those from a cohort of 40 consecutive PKERP procedures performed by the department's senior urologist (surgeon C) during the study period, which formed a third group (Group 3). Surgeon C had more than 5 years of experience in PKERP surgery, and had performed more than 150 PKERP procedures.

Statistical analysis

Statistical analysis was performed using the statistical software program SPSS (SPSS, Inc., Chicago, IL, version 16.0) for Windows. Continuous variables were presented as the mean value±standard deviation and differences between group data were analyzed by one-way independent analysis of variance (ANOVA) for continuous variables. Differences with P values <0.05 were considered significant.

Surgical Techniques

PKERP was performed as previously described by Liu C (10) and Rao et al. (12). The procedure was performed with a 27Fr resectoscope with the loop of the bipolar PK system. The PK system uses 160W for cutting and 80W for coagulation. Physiologic saline was used as irrigation fluid. The ureteral orifices, bladder neck and verumontanum were identified preoperatively and incision was start close to the verumontanum from the 5 to the 7 o'clock positions. These grooves were deepened to the level of the surgical capsule. The tip of the resectoscope sheath was then inserted into the groove, which pushed the lobe along the surgical capsule line to create the cleavage plane between the detached lobe and the capsule. The bipolar plasmakinetic loop moved in exactly the same plane as the surgeon's index finger does when performing open prostatectomy. Middle lobe, left lobe, and right lobe were dissected off the surgical capsule in a retrograde fashion from the apex toward the bladder using the bipolar plasmakinetic loop with arrest of bleeding. The enucleated lobes were devascularized simultaneously but still attached at the bladder neck by a narrow pedicle (the "mushroom" technique (13)). The enucleation adenoma was resected into smaller prostatic chips by the plasmakinetic loop and extracted by Ellic. A 22Fr triple-lumen catheter was inserted and connected to straight drainage after the operation. Continuous bladder irrigation was necessary with physiologic saline and stopped when the urine cleared of hematuria. After catheter drainage became clear, bladder irrigation was stopped. If catheter drainage was still clear, the catheters were removed within 24 hours and the patients were then discharged from the hospital within 24h after decatheterisation.

RESULTS

Patients' Demographic and Perioperative Characteristics

All patients were successfully treated with PKERP. There were no perioperative deaths and no subject was converted to open prostatectomy. Of note, surgeon B did not take over any PKERP procedure in the 90 consecutive surgeries performed by surgeon A. Baseline and perioperative data are reported in Table-1. There were no statistically significant differences among the three groups (Group1, Group2 and Group3) with respect to age, prostate size, PSA level, IPSS, QOL score, Qmax, PVR, catheter time and hospital stay ($p>0.05$). The resection time was lower and the resected weight was higher in group 3 compared with group1 and group2, but the differences had no statistic significance ($p>0.05$). There were significant differences among the three groups with respect to the operation time, enucleation time, operation efficiency, enucleation efficiency and resection efficiency ($p<0.05$). The value of FMA in group 2 was significantly lower than group1 ($p<0.05$).

Follow-up Data

There were 5 (5/90, 5.6%) patients of surgeon A who were lost to follow-up. Eighty-five (85/90, 94.4%) completed the twelve months--follow-up. No patients in group 3 were excluded from the study. Table-2 lists changes in IPSS score, QOL, Qmax and PVR in the 3, 6 and 12 months after the operation. There were significant impro-

Table 1 - Patients' demographic and perioperative characteristics.

Parameters	Group 1 (n=40)	Group 2 (n=50)	Group 3 (n=40)	P value
Age(years)	72.8±7.3	70.9±8.1	72.1±8.7	0.528
Prostate size (mL)	75.2±28.1	81.8±23.9	78.9±24.9	0.476
PSA(ng/dL)	4.19±1.78	4.68±2.24	4.39±1.92	0.512
IPSS	21.8±2.3	22.5±1.9	21.7±1.4	0.110
QoL(score)	3.8±0.7	3.7±0.6	3.9±0.6	0.670
Qmax(mL/s)	7.6±2.5	7.7±2.6	6.9±2.2	0.252
PVR(mL)	86.6±37.7	101.7±47.8	94.7±40.7	0.211
Operation time(min)	79.2±24.6	71.3±23.9	67.9±18.8	0.040*
Enucleation time (min)	33.2±10.4	29.0±9.4	28.3±9.5	0.022*
Resection time (min)	45.9±17.1	42.0±15.4	39.8±11.8	0.115
Resected weight (g)	48.3±17.5	44.1±13.9	48.2±20.4	0.323
Operation efficiency (g/min)	0.59±0.09	0.61±0.08	0.68±0.13	0.036*
Enucleation efficiency (g/min)	1.49±0.39	1.56±0.30	1.71±0.44	0.019*
Resection efficiency (g/min)	1.04±0.17	1.12±0.15	1.29±0.35	0.027*
FMA	4.1±3.0	2.1±2.2	NA	<0.01*
Catheter time (d)	2.3±1.2	2.3±1.3	2.0±1.1	0.378
Hospital stay (d)	2.9±1.2	3.0±1.4	2.8±1.1	0.728

Data presented as mean±standard deviation. *p<0.05

PSA = prostate-specific antigen; **IPSS =** International Prostate Symptom Score; **QoL =** quality of life; **PVR =** postvoid residual volume; **FMA =** frequency of mentor advice; **Group 1 =** surgeon A, cases 1-40; **Group 2 =** surgeon A, cases 41-90; **Group 3 =** surgeon C, cases 1-40; **NA=** not applicable.

vements in 3, 6 and 12 month's parameters compared with preoperative values (p<0.001). There were no statistically significant differences among the three groups with respect to preoperative and postoperative values (p>0.05).

Complications

Complications are listed in Table-3. Capsule perforation occurred in 3 (3/40, 7.5%) patients in group 1. Bladder mucosa damage occurred in 2 (2/40, 5%) patients in group 1. All the damages were mild and treated with catheterization for 3-5 days. Transient urinary incontinence occurred in 3 (3/40, 7.5%) in group 1 and 2 (2/50, 4%) in group 2 and 1 (1/40, 2.5%) in group 3. No patient developed stress urinary incontinence persistent for more than three months. Totally there were 4 (4/130, 3.1%) patients who required blood transfusion because anemia existed preoperatively. Urinary tract infection occurred in 4 (4/130, 3.1%) patients, which were treated with antibiotics. There were 2 (2/130, 1.5%) patients who needed re-catheterization due to acute urinary retention after catheter removal, but these patients could self-void after bladder training for 5-7 days. Hematuria needing reoperation was observed in 1(1/40, 2.5%) patient

Table 2 - Preoperative characteristics and follow-up data.

Parameters	Group 1 (n=40)	Group 2 (n=50)	Group 3 (n=40)	P-value
IPSS				
Preop	21.8±2.3	22.5±1.9	21.7±1.4	0.110
3 month	8.1±2.8	8.2±2.7	7.5±2.5	0.432
6 month	7.3±2.4	7.7±2.1	7.1±2.1	0.395
12 month	6.8±1.9	6.9±1.8	6.7±1.8	0.925
P value	<0.001*	<0.001*	<0.001*	
QoL(score)				
Preop	3.8±0.7	3.7±0.6	3.9±0.6	0.670
3 month	1.7±0.7	1.8±0.7	1.7±0.6	0.878
6 month	1.5±0.5	1.6±0.7	1.5±0.5	0.789
12 month	1.4±0.5	1.4±0.4	1.4±0.5	0.786
P value	<0.001*	<0.001*	<0.001*	
Qmax(mL/s)				
Preop	7.6±2.5	7.7±2.6	6.9±2.2	0.252
3 month	20.2±2.8	20.7±2.9	20.9±2.6	0.523
6 month	21.2±2.3	21.3±2.3	21.4±2.1	0.883
12 month	21.5±1.8	21.6±1.7	21.7±1.9	0.935
P value	<0.001*	<0.001*	<0.001*	
PVR(mL)				
Preop	86.6±37.7	101.7±47.8	94.7±40.7	0.211
3 month	11.1±4.2	10.9±4.5	10.3±3.7	0.647
6 month	10.0±2.8	9.9±3.6	9.3±3.1	0.549
12 month	9.4±2.3	9.6±2.7	9.2±3.1	0.796
P value	<0.001*	<0.001*	<0.001*	

Data presented as mean±standard deviation. *p<0.001

Preop = preoperative; **IPSS =** International Prostate Symptom Score; **QoL =** quality of life; **PVR =** postvoid residual; **Group 1 =** surgeon A, cases 1-40; **Group 2 =** surgeon A, cases 41-90; **Group 3 =** surgeon C, cases 1-40.

in group 1, which underwent transurethral electric coagulation. The other postoperative complications included urethral stricture occurring in one patient in group 1 and in group 2, both requiring urethrotomy. Bladder-neck contracture occurred in 1 (1/40, 2.5%) patient in the group 1, which required transurethral resection of bladder neck.

Learning Curve

In the 90 procedures performed by surgeon A, the mean operative efficiency and enucleation efficiency gradually increased (Figures-1A, 1B). They ranged from a mean of 0.49±0.09g/min and 1.11±0.28g/min for the first 10 PKERP procedures to a mean of 0.63±0.08g/min and 1.62±0.36g/min

Table 3 - Complications of PKERP according to the CLAVIEN-DINDO grade system.

Complications (n%)	Group 1 (n=40)	Group 2 (n=50)	Group 3 (n=40)
Grade I			
Capsule perforation	3(7.5%)	0	0
Bladder mucosa damage	2(5%)	0	0
Transient incontinence	3(7.5%)	2(4%)	1(2.5%)
Grade II			
Blood transfusion	2(5%)	1(2%)	1(2.5%)
Urinary tract infection	2(5%)	1(2%)	1(2.5%)
Re-catheterization	1(2.5%)	1(2%)	0
Grade IIIa			
Hematuresis need reoperation	1(2.5%)	0	0
Grade IIIb			
Urethral stricture	1(2.5%)	1(2%)	0
Bladder-neck contracture	1(2.5%)	0	0

Data presented as n%.

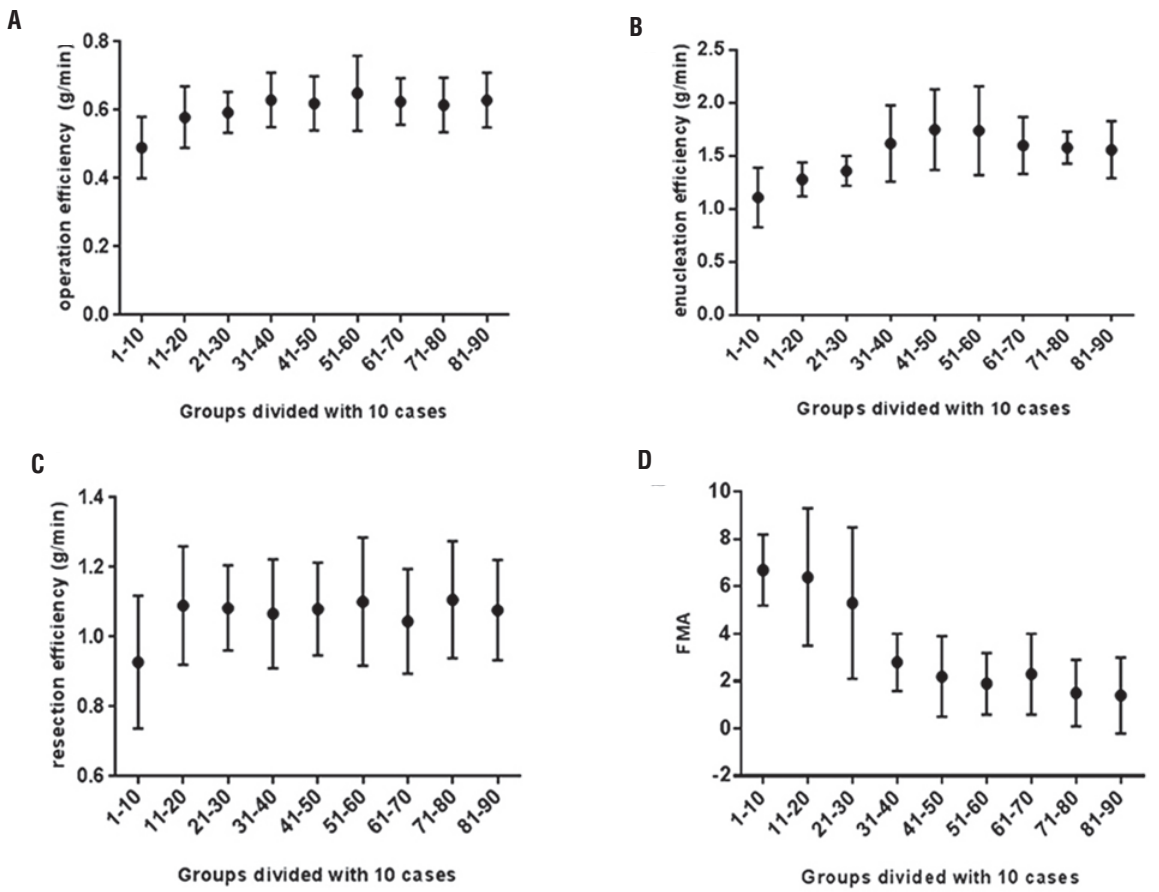
PKERP = transurethral plasmakinetic enucleation and resection of prostate; **Group 1 =** surgeon A, cases 1-40; **Group 2 =** surgeon A, cases 41-90; **Group 3 =** surgeon C, cases 1-40.

for case numbers 31-40. The increase in the mean operative efficiency and enucleation efficiency were statistically significant for the first 40 cases (p=0.003 and p=0.002). Then, few fluctuations were observed with respect to the mean operative efficiency and enucleation efficiency in the subsequent patient groups, indicating that a plateau had been reached (p=0.919 and p=0.232). Surgeon C had a mean operative efficiency of 0.68±0.13g/min and enucleation efficiency 1.71±0.44, which were similar to that achieved by surgeon A after 40 operations (Figure-2A and 2B; p=0.333 and p=0.473). The resection efficiency in surgeon A cases ranged from a mean of 0.91±0.09g/min for the first 10 PKERP procedures to a mean of 1.09±0.17g/min for case numbers 11-20. The increase in the mean resection efficiency was statistically significant for the first 20 cases (p=0.043). Then, few fluctuations were observed in the subsequent patient groups (Figure-1C, p=0.980). The resection efficiency in surgeon C was higher than surgeon A, however, the value had no statistic difference compared with group2 (Figure--2C, p=0.055). The value of FMA also reduced as experience in the procedure gradually increased (Figure-1D). The mean value of FMA was 6.7±1.5 for the first 10 cases, which decreased significantly to a mean value of 2.8±1.2 when the fortieth procedure had been completed (p<0.01). The value of FMA was maintained for case numbers 51-90 (group 2, p=0.246).

DISCUSSION

HoLEP has been proven to be an effective, minimally invasive procedure for the surgical treatment of BPH (14). PKEP is as safe and effective as HoLEP according to the previously reports (9, 15), although the PKEP remains less versatile than the holmium laser, particularly in terms of stone disease; however, the lower capital costs and ease of use for this technique makes it a good choice for BPH (6, 11). To our best knowledge, there is no

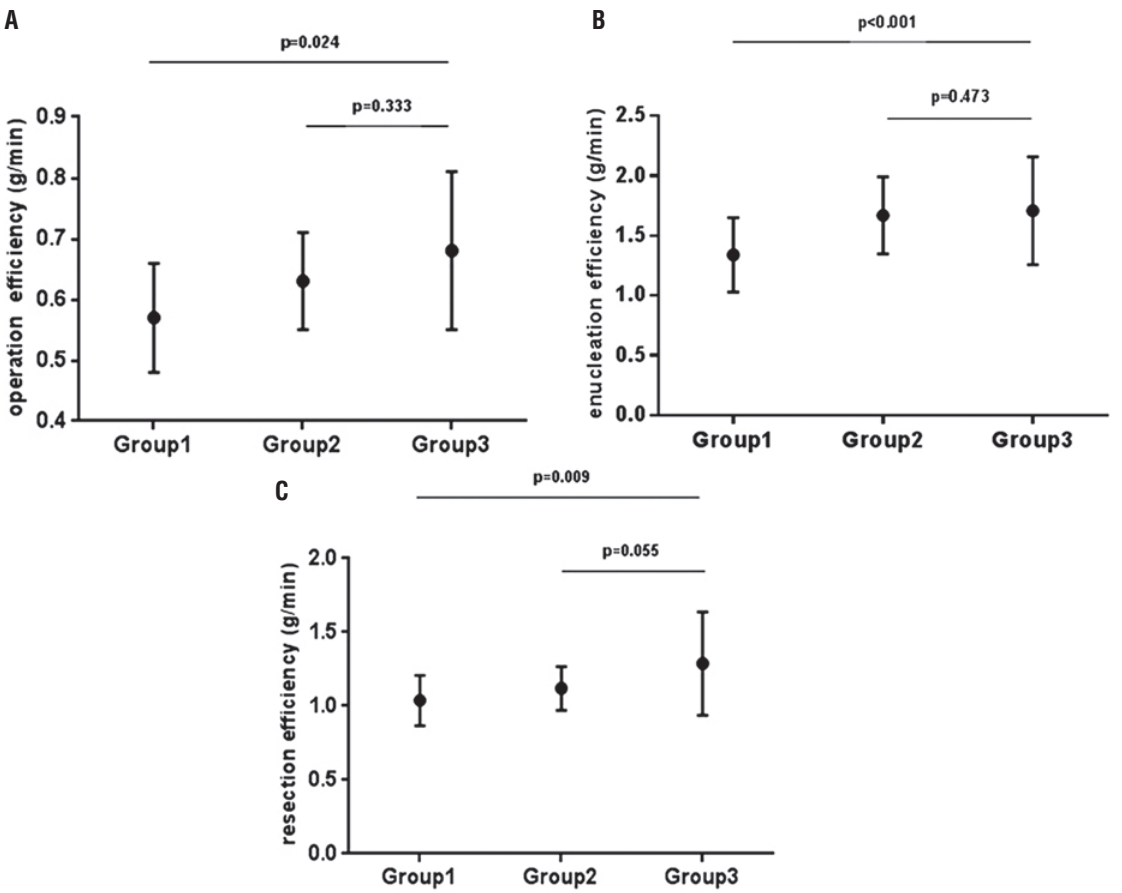
Figura 1 - (A): Comparison of operation efficiency over the number of PKERP cases performed by surgeon A (n=90); (B): Comparison of enucleation efficiency over the number of PKERP cases performed by surgeon A (n=90); (C): Comparison of resection efficiency over the number of PKERP cases performed by surgeon A (n=90); (D): Comparison of FMA over the number of PKERP cases performed by surgeon A (n=90).



prospective trial which has evaluated the learning curve of PKERP procedure. In the present study, using a mentor-based approach, we present the first prospective analysis of individual learning curves of the PKERP procedure.

Ideally the learning curve for surgery is completed during residency or fellowship training. However, the opportunity is limited. The concern is that a patient will probably be unwilling to be treated by a novice surgeon due to concern about adverse outcomes. Thus, mentoring with an expert is a desirable method to be used to acquire the technique. For learning the technique of PKERP, the mentor-based approach can help the novice surgeon determine the correct tissue plane, comment on the depth of incision and encourages the resident to proceed if the appropriate maneuvers are being done (16). The video based education before mentor based training operation is also important. The novice surgeon can familiarized himself with the PKERP technique by viewing videos and reviewed the videos with mentor to discuss remaining questions. The detailed discussion of critical or problematic operative steps with an expert allows the novice to learn the pitfalls, and tips and tricks of the procedure, thus improving the quality of the PKERP operation. In the learning curve of HoLEP procedures with a mentor-based approach, El-Hakim and Elhilali (17) found that the outcome in 27 HoLEP procedures performed by a senior resident was comparable to that of 118 done by an experienced urologist. They concluded

Figura 2 - (A): Comparison of operation efficiency between the three groups; (B): Comparison of enucleation efficiency between the three groups; (C): Comparison of resection efficiency between the three groups.



that extensive experience with transurethral surgery and the supervision by an experienced urologist were prerequisites for success. Our results also found that under the mentor supervision of an expert, the operation efficiency and enucleation efficiency of the resident were similar to that of a senior urologist after 40 cases. The resection weight of the prostate between the resident and the senior urologist were also similar. Using a mentor-based approach, a novice surgeon can perform PKERP with efficiency.

Compared with the monopolar TURP, the major benefits of bipolar TURP include the decreased elimination of dilutional hyponatremia even during longer operation time. The long safe operation time allows the beginner to recognize the anatomical landmarks meticulously and to enucleate the adenoma circumspectly. The anatomical landmarks that prompt the surgeon to identify the surgical plane mainly include capsule transverse fibers or fiber strands, capsule vessel reticula, capsule prostate calculi, and granular prostatic fluid retentates (10, 11). In retrograde fashion, the proximal and lateral margins of the verumontanum are the best sites for starting the enucleation of adenoma, where the plane between the surgical capsule and the hyperplasia adenoma is permanent (11). In our first 40 patients, more operation time and enucleation time were spent on identifying the anatomical landmarks. Therefore, the mean operation efficiency and enucleation efficiency was lower than in the later 50 cases.

In our series, there was a learning curve with this technique of at least 40 cases after the resident had experienced the procedure. The operation enucleation efficiency was increased to

stationary after 40 cases. Our results were similar to the findings from another study. Xiong W. et al. (11) stated that an inexperienced endourologist in plasmakinetic prostate enucleation can reach an efficiency plateau after 50 cases. However, there was no tutoring or mentor supervision in their study. The present study also showed that the mentor advice decreased to a stationary low frequency after 40 cases. The resident could independently complete the operation without a proper instructor after 40 cases, which was in accordance with the increase of surgical experience. The resection efficiency reached an efficiency plateau after 20 cases, which was faster than the enucleation efficiency. The reason might be the resident had previous experience in endourology. Although there was no statistic difference between the later 50 cases and the senior urologist, the value of the resident was still lower than the senior urologist. The results indicated that with the increased experience, the resection efficiency could be further increased and the operation time could be shortened.

Three cases of capsule perforation occurred in the initial 40 cases due to the unfamiliar experience of the identification of the surgical plane between prostate adenoma and prostate capsule. The three cases of perforation were minor and did not alter the clinical course. There were some reports indicating that in smaller fibrotic prostates, the surgical capsule was often less distinct and the plane of dissection more difficult than in larger glands, in which the greater degree of peripheral compression tended to create a more easily identifiable plane (18, 19). We also would not recommend a patient with a small size prostate as the primary choice for a novice's initial training. There were three bladder mucosa damage cases which occurred in the first 40 cases in this study. The enucleation tissue sometimes can affect the vision of the operator especially when the prostate volume is large and hematuria exists. The achievement of thorough hemostasis and bladder distension are essential to avoid this complication. In addition, no serious complications were experienced in our patients, and all transient urinary incontinence cases have completely recovered. At a 12 month's follow-up, our results showed a quick and durable improvement in IPSS, QOL, Qmax and PVR after operation, which agreed with those previously published reports (20, 21). The clinical efficacy of PKERP performed by the resident was also comparable with those of the senior urologist. The results indicated that PKERP was a safe and efficient treatment for urologists, even for an inexperienced surgeon.

There are still some limitations in our study that should be considered. First, this study describes the PKERP learning curve for only one surgeon. He had experience in endourology and his learning curve may not be applicable to someone emerging from their residency or someone who has limited endourological training. However, to the best of our knowledge, we provided the first prospective analysis of individual learning curves of the PKERP procedure and showed its safety and effectiveness during the initial learning experience of the surgeon when closely mentored. The mentor-based approach is recommended for an inexperienced surgeon to study the PKERP procedure. Secondly, the average follow-up was too short to demonstrate the long-term efficacy. Larger sample trials including more surgeons with longer follow--up are needed to further confirm our results.

CONCLUSIONS

The PKERP is a promising surgical treatment for safe and effective removal of prostatic tissue in patients with symptomatic BPH. PKERP can be performed safely and efficiently even during the initial learning curve of the surgeon when closely mentored. We found that performing the procedure in 40 cases is sufficient for a single operator to complete the learning curve. However, further well-designed trials with several surgeons are needed to confirm the results.

ABBREVIATIONS

TURP = transurethral resection of prostate
TURS = transurethral resection syndrome
PKEP = plasmakinetic enucleation of prostate
HoLEP = holmium laser enucleation of the prostate

PKERP = transurethral plasmakinetic enucleation and resection of prostate
TURBT = transurethral resection of the bladder tumor
FMA = frequency of mentor advice
PSA = prostate specific antigen
IPSS = International Prostate Symptom Score
QoL = quality of life
PVR = postvoid residual

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CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Correlation between overactive bladder symptom score and neuropsychological parameters in Alzheimer's disease patients with lower urinary tract symptom

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ABSTRACT

Purpose: To examine an association between the overactive bladder symptom score (OABSS) and neuropsychological parameters. Moreover, we investigate the factors that affect each item in the questionnaire.

Materials and Methods: A total of 376 patients (males: 184; females: 192) with probable Alzheimer's disease (AD) were recruited. Cognitive testing was conducted using the Mini Mental Status Examination (MMSE), Clinical Dementia Rating (CDR) scale, Global Deterioration Scale (GDS), and Barthel Activities of Daily Living (ADL). Lower urinary tract symptom (LUTS) was assessed using OABSS and voiding diary.

Results: The prevalence of overactive bladder (OAB) (defined as OABSS ≥3 with an urgency score of ≥2) in patients with AD was 72.6%. Among the OAB subjects, the most common severity of symptom was moderate (72.6%), followed by mild (21.2%), and severe (5.8%). It was found that OABSS had a very high correlation with aging ($r=0.75$; $p<0.001$). When compared with neuropsychological parameters, it was found that OABSS was highly correlated with the CDR scores ($r=0.446$; $p<0.001$). However, no significant correlation was found between the changes in OABSS scores and those in other neuropsychological parameters. Based on the individual symptom scores, urgency incontinence was highly correlated with the CDR scores ($r=0.43$; $p<0.001$).

Conclusions: OABSS is a useful tool in assessing AD patients with LUTS. There was a consistent positive association between OABSS severity, including urgency incontinence, and CDR scores.

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INTRODUCTION

Overactive bladder (OAB) is a symptom complex, comprising urinary urgency with or without urgency and incontinence, usually with urinary frequency and nocturia (1); it represents the storage component of lower urinary tract symptoms (LUTS) (2). The incidence of OAB increases significantly with age. The mechanisms underlying OAB in the elderly are multifactorial; the factors might include age-related changes in the bladder itself, or central nervous system changes innervating the bladder (3).

Alzheimer's disease (AD) is one of the most common neurodegenerative diseases and accounts for more than 80% of dementia patients among elderly people (4). The condition is associated with progressive memory loss, and impairment of cog-

nitive function and functional independence. Many affected patients will also have problems with bladder and bowel control (5, 6). Both OAB and AD are common, often coexisting in older patients (7). Although AD is known to be an independent risk factor for OAB or urinary incontinence, few studies have been published with regard to research on OAB of elderly patients with AD (3, 8).

As for the relationship between urinary incontinence and cognitive or functional measures in AD patients, we previously reported that severity of detrusor overactivity was linked to functional impairments, whereas there was no relationship between the Incontinence Questionnaire on Urinary Incontinence Short Form (ICIQ-UI) questionnaire and those (5). However, there has been a lack of study to investigate in detail the relationship between OAB with each symptom and cognitive or functional measures in AD patients with LUTS. Therefore, we assessed the OAB of patients with AD, examined the association between the Overactive Bladder Symptom Score (OABSS) questionnaire and neuropsychological parameters, and investigated the factors that affect each item of the questionnaire.

MATERIALS AND METHODS

Subjects who visited the dementia clinic were recruited sequentially. All patients met the National Institute of Neurological Communicative Disorders and Stroke (NINCDS) and the Alzheimer Disease and Related Disorders Association diagnostic criteria for probable AD. The diagnosis of probable AD was made by expert neurologists. Patients who were diagnosed with other dementia, including severe dementia in addition to behavioral disturbances, and inability to communicate were also excluded.

All participants underwent an extensive evaluation that included physical and neurological examinations and laboratory test. Cognitive tests were performed using the Mini Mental Status Examination (MMSE), Clinical Dementia Rating (CDR) scale, Global Deterioration Scale (GDS), and Barthel Activities of Daily Living (ADL), which have extensively been used in clinical and research settings to measure cognitive impairment. LUTS were assessed using the OABSS questionnaire and 3-day consecutive voiding diary with the Indevus Urgency Severity Scale (IUSS). OAB was defined as OABSS ≥3 with an urgency score of ≥2 (9, 10). In addition, scores on the OABSS of ≤5 were defined as mild, those of 6-11 as moderate, and those of ≥12 as severe (9, 10).

The institutional review board approved the study protocol, and informed consent was obtained from all patients or legal guardians in accordance with the Declaration of Helsinki. Patients were asked to complete by themselves a questionnaire and voiding diary. However, if they were unable to complete them due to behavioral disturbances and inabilities, educated caregivers asked them the questions from the questionnaire and assisted them in filling out the required forms.

Data are expressed as means and standard deviations. A p-value was calculated using the independent t-test, Pearson's chi-squared test, and one-way analysis of variance. A Pearson correlation analysis was used to determine the correlations between the individual symptom scores of OABSS and neuropsychological parameters. All tests with a p-value of <0.05 were considered as statistically significant. The Statistical Package for the Social Sciences Version 18.0 (SPSS, Chicago, IL) was used to carry out all statistical analyses.

RESULTS

A total of 376 patients (male 184, female 192, 56-92 years old), with probable AD, were included in the analysis. Of 430 patients screened, 54 were not included because of diagnosis of other dementia, any severe conditions of behavioral disturbances, and inability to communicate. The prevalence of OAB (defined as OABSS ≥3 with an urgency score of ≥2) in patients with AD was 72.6% (n=273; males: 42.1%; females: 57.9%). Of those, 260 patients (95.2%) complained of urinary leakage associated with urgency (urgency incontinence score

of ≥1) and 90 patients (33.0%) were incontinent more than once per day (urgency incontinence score of ≥3). However, only 56 patients (20.5%) used adult diapers or pads for incontinence or night wetting. Table-1 summarizes the variable parameters of patients with and without OAB. No statistical differences among age, sex, duration of disease, history of taking acetylcholinesterase inhibitors (AChEIs), and neuropsychological parameters were found between those with and without OAB.

When the severity of symptom was categorized as mild (OABSS: ≤5), moderate (OABSS: 6–11), and severe (OABSS: ≥12), the most common was moderate (72.6%), followed by mild (21.2%), and severe (5.8%). The mean age of patients with mild, moderate, and severe symptoms of OABSS was 70.8, 79.3, and 91.3, respectively. Urgency episodes and maximum urgency intensity were significantly increased in the severe group. However, there were no significant differences in the number of micturition, nocturia and mean voided volume among the groups. Table-2 summarizes the parameters of OAB patients that were classified into three groups in accordance with the severity of OAB. Among the neuropsychological tests, only CDR increased significantly with the severity of OAB. The MMSE, GDS, and ADL did not significantly differ among the three groups.

In the correlation analysis, OABSS had a very strong relationship with aging in AD patients with OAB (r=0.75; p<0.001). When compared with neuropsychological parameters, OABSS highly correlated with the CDR scores in AD patients with OAB. However, no significant correlation was found between changes in OABSS and the other three neuropsychological tests-MMSE, GDS, and ADL (Table-3).

Based on individual symptom scores, urgency incontinence highly correlated with the CDR scores (r=0.43; p<0.001). However, the frequency and nocturia scores indicated weak correlation (r=0.22 and 0.23). There was no correlation between individual symptom scores and other neuropsychological parameters (Table-4).

Table 1 - The prevalence of OAB on OABSS (n=376).

	OAB (n=273)	Non-OAB (n=103)	P-value
Age (years)	78.21±7.70	77.47±8.65	0.418
(range)	58–92	56–91	
Sex (M/F)	115/158	39/64	0.454
Hypertension	103 (37.7%)	43 (41.7%)	0.476
Diabetes	75 (27.4%)	22 (21.4%)	0.227
Dyslipidemia	91 (33.3%)	31 (30.1%)	0.550
Duration			
Of education (y)	6.57±4.36	7.14±5.02	0.693
Of dementia (m)	33.73±16.39	28.84±19.99	0.540
History of taking AChEIs (%)	115 (42.1%)	43 (41.7%)	0.947
MMSE	14.44±7.62	14.92±7.78	0.589
CDR	2.27 ± 0.97	2.21 ± 0.97	0.643
GDS	5.54 ± 0.98	5.57 ± 0.95	0.250
ADL	12.60±6.46	12.10±6.12	0.488

Values are presented as mean ± standard deviation.

OAB = overactive bladder; **AChEIs** = acetylcholinesterase inhibitors; **MMSE** = the Mini Mental Status Examination; **CDR** = the Clinical Dementia Rating scale; **GDS** = the Global Deterioration Scale; **ADL** = the Barthel Activities of Daily Living.

Table 2 - The severity of OAB on OABSS (n = 273).

	Mild	Moderate	Severe	P-value
	(OABSS ≤5)	(OABSS 6–11)	(OABSS ≥12)	
N (%)	58 (21.2%)	199 (72.6%)	16 (5.8%)	
Age (years)	70.8±6.2	79.3±6.2	91.3±0.79	<0.001
Sex (M/F)	30/28	81/118	4/12	0.118
Voiding diary (/24 hr)				
No. micturition	8.7±2.7	9.1±2.9	10.3±3.3	0.147
No. nocturia	1.2±0.8	1.2±0.9	1.6±1.0	0.144
No. urgency episodes	1.2±0.9	1.2±0.9	2.4±0.8	<0.001
Max. urgency intensity	1.6±0.9	1.6±0.9	2.2±0.9	0.038
Mean voided volume (mL)	127.3±45.7	122.9±41.7	118.1±28.0	0.675
Neuropsychological tests				
MMSE	14.36±7.31	14.59±7.74	12.94±7.51	0.705
CDR	1.76±0.79	2.32±0.94	3.38±0.72	<0.001
GDS	5.57±0.86	5.38±1.01	5.75±1.00	0.195
ADL	12.02±5.18	12.66±6.83	14.13±5.95	0.504

Values are presented as mean ± standard deviation.

MMSE = the Mini Mental Status Examination; **CDR** = the Clinical Dementia Rating scale; **GDS** = the Global Deterioration Scale; **ADL** = the Barthel Activities of Daily Living.

Table 3 - Correlations between neuropsychological parameters and OABSS scores.

	Pearson correlation with OABSS scores	P-value
MMSE	–0.027	0.65
CDR	0.446	<0.001
GDS	0.006	0.93
ADL	0.048	0.43

MMSE = the Mini Mental Status Examination; **CDR** = the Clinical Dementia Rating scale; **GDS** = the Global Deterioration Scale; **ADL** = the Barthel Activities of Daily Living.

Table 4 - The individual symptom scores of OABSS and correlations with neuropsychological parameters.

	Frequency	Nocturia	Urgency	Urgency Incontinence
MMSE	–0.16	–0.09	–0.03	0.03
CDR	0.22*	0.23*	0.11	0.43*
GDS	0.06	–0.07	–0.02	0.03
ADL	–0.02	0.05	0.11	–0.01

* $P < 0.001$

MMSE = the Mini Mental Status Examination; **CDR** = the Clinical Dementia Rating scale; **GDS** = the Global Deterioration Scale; **ADL** = the Barthel Activities of Daily Living.

DISCUSSION

OAB is a troublesome and extremely prevalent urinary symptom that causes a significant negative impact on the quality of life (QoL), associated with high economic costs or related comorbidities, particularly in elderly people. Many studies have shown that OAB can affect all aspects of QoL, including psychological, physical, sexual, domestic, social, and occupational aspects (11).

The incidence of OAB increases with aging, and central nervous system (CNS) degeneration in the elderly is proposed as one of the pathogenic factors of OAB (12). In our study, the overall prevalence of OAB between AD patients aged 56-92 was 72.6%, which is much higher than those of the general population, as reported in previous studies (13, 14) Furthermore, it is nearly twice those of the general population aged ≥75 in the studies. From the aspect of the severity of OAB symptom, elderly AD patients with OAB tend to have greater severe symptoms ($r=0.75$; $p<0.001$). However, there was no significant difference of age between AD patients with and without OAB.

We also analyzed the score of MMSE, CDR, GDS, and ADL of subjects to determine the relationship between OAB symptoms and the severity of AD. AD is diagnosed based on medical history, family history, and general behavioral observations. Diagnosis criteria require the presence of cognitive impairment and suspected dementia symptoms. It was confirmed through neuropsychological tests, such as MMSE, CDR, GDS, and ADL, which have shown good statistical reliability and validity compared with the definitive histopathological confirmation of brain tissues (15, 16).

MMSE is a 30-point questionnaire that is used in clinical and research settings to measure cognitive impairment (17). This test takes between 5–10 minutes and examines the following: registration, attention and calculation, recall, language, ability to follow simple commands and orientation (18). A previous study reported a correlation between nocturia and MMSE score, with a lower MMSE score being nocturia (17, 19). However, in our study, there was no significant difference of MMSE scores between AD patients with and without OAB. In addition, MMSE did not significantly differ with the severity of OAB or individual symptom scores, including nocturia. While the advantage of MMSE is its short administration period and ease of use, its disadvantage is that it is affected by educational level, age, and insensitivity to progressive changes (20).

CDR is a numeric scale used to quantify the severity of dementia symptoms. This scale assesses patient's cognitive and functional performance in six areas: memory, orientation, judgment and problem solving, community affairs, home and hobbies, and personal care. CDR could identify very mild impairments, but its drawbacks include its length of administration, ultimate reliance on subjective assessment, and relative inability to capture changes over time (21). We found a strong correlation between OABSS and CDR scores (Table-3). In addition, among OABSS scores, we also found a strong correlation between urgency incontinence scale and CDR scores (Table-4). It was well documented that among OAB symptoms, the urgency incontinence was the most frequent symptom in patients with AD (5, 22). Our study showed similar results with that of previous studies. CDR is being used as an effective tool for staging the severity of dementia, while MMSE scores are influenced by age, sex, and educational levels. Therefore, a correlation between OAB and CDR might be significant by itself. After all, our results suggest that patients with greater severity of AD also tend to have greater severity of OAB, including urgency incontinence.

GDS developed by Dr. Barry Reisberg provides caregivers an overview of the stages of cognitive function for those suffering from a primary degenerative dementia such as AD (23). It is subdivided into 7 different stages. Stages 1-3 are the pre-dementia stages. Stages 4-7 are the dementia stages. Beginning in stage 5, an individual can no longer survive without assistance (23). Clinicians broadly accept the GDS as a tool for staging severity of dementia. However, unlike CDR, GDS was not associated with OAB in

our study. It is because not all patients with AD follow the course described in the GDS (24).

We also used Barthel ADL to measure basic ADL. Barthel ADL was the scale of functional limitation which mainly evaluated the basic and physical activities of daily living, including dressing, using the toilet, getting about the house, getting in and out of bed, and bathing (22). Instrumental ADL (IADL) was the other scale of functional limitation, which mainly evaluated the more complex and higher-order skills (22). A previous study reported an association between urinary incontinence and IADL score in elderly women (25). However, although the basic ADL had more fundamental items, we could not find an association between OABSS and basic ADL in our study. This may be the case because we excluded very advanced severe dementia.

As far as we know, a few studies have been conducted in evaluating OAB of AD patients through a standardized questionnaire. This is possibly due to the difficulty of AD patients in completing a questionnaire or doubts about the inexactitude of their responses. Nevertheless, an assessment of symptoms is essential in clinical OAB diagnosis or treatment, and it is not exceptional in OAB management of AD patients (26, 27). Symptom questionnaires overcome historical drawbacks, such as missed questions, being led by questioners, or quantification difficulty because of the structured panel of questions in diagnosing OAB. OABSS was first introduced in 2006 and currently, it is one of the most widely used questionnaires for OAB treatment or research (10, 28). Among its advantages are exact compatibility with the ICS definition of OAB, as well as its simplicity and brevity. OABSS, which consists of only four questions, is nearly the simplest questionnaire available (10, 29-31). Thus, we thought that OABSS is very suitable in the evaluation of AD patients because many of them have cognitive impairment. Moreover, its simplicity can help in the reduction of confusion and in drawing exact responses as much as possible.

LUTS is a group of symptoms (voiding and storage) that is supposed to be expressed by the patients themselves. Thus, studying LUTS in patients with brain diseases is not easy as they are older or have cognitive disorders, which make them unable to fully cooperate. A standardized questionnaire could be helpful, and we tried to evaluate the OAB of AD patients using OABSS while remaining faithful to the definition of OAB. Still, we cannot be convinced that AD patients genuinely expressed OAB symptoms exactly as with the general population, even though we excluded patients with severe cognitive disorders. The result where the other three neuropsychological scores were not correlated with OABSS is another shortcoming of this study. Our limitations need to be solved through more novel research designs in the future.

CONCLUSIONS

The prevalence of OAB in AD patients is much higher than that of the general population. AD patients with OAB have more severe OAB symptoms as they age. Patients with more severe AD, based on CDR, tend to have more severe OAB symptoms. Thus, results of the present study suggest that OABSS could be a useful tool in the assessment of OAB symptoms of AD patients.

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CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Artificial urinary sphincter revision for urethral atrophy: comparing single cuff downsizing and tandem cuff placement

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ABSTRACT

Objective: To compare outcomes for single urethral cuff downsizing versus tandem cuff placement during artificial urinary sphincter (AUS) revision for urethral atrophy.
Materials and Methods: We identified 1778 AUS surgeries performed at our institution from 1990-2014. Of these, 406 were first AUS revisions, including 69 revisions for urethral atrophy. Multiple clinical and surgical variables were evaluated for potential association with device outcomes following revision, including surgical revision strategy (downsizing a single urethral cuff versus placing tandem urethral cuffs).
Results: Of the 69 revision surgeries for urethral atrophy at our institution, 56 (82%) were tandem cuff placements, 12 (18%) were single cuff downsizings and one was relocation of a single cuff. When comparing tandem cuff placements and single cuff downsizings, the cohorts were similar with regard to age (p=0.98), body-mass index (p=0.95), prior pelvic radiation exposure (p=0.73) and length of follow-up (p=0.12). Notably, there was no difference in 3-year overall device survival compared between single cuff and tandem cuff revisions (60% versus 76%, p=0.94). Likewise, no significant difference was identified for tandem cuff placement (ref. single cuff) when evaluating the risk of any tertiary surgery (HR 0.95, 95% CI 0.32-4.12, p=0.94) or urethral erosion/device infection following revision (HR 0.79, 95% CI 0.20-5.22, p=0.77).
Conclusions: There was no significant difference in overall device survival in patients undergoing single cuff downsizing or tandem cuff placement during AUS revision for urethral atrophy.

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Urinary Sphincter, Artificial; Urinary Incontinence; Male

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INTRODUCTION

While the use of an artificial urinary sphincter (AUS) in the management of severe male stress urinary incontinence has been associated with excellent long-term outcomes, many patients will experience recurrent incontinence (1-3). Notably, in several large series, urethral atrophy has been reported as the most common cause for non-mechanical failure or device revision (4, 5). It has been hypothesized that in this setting urethral atrophy occurs because the AUS achieves continence by applying constant circumferential compression of the corpus spongiosum, which over time leads to tissue atrophy (6).

Notably, several surgical options for AUS revision in cases of urethral atrophy have been reported, including: changing the location of the urethral cuff (7), downsizing the single urethral cuff (8), placement of a second (tandem) urethral cuff (9, 10), transcorporal cuff placement (11, 12) or revising the pressure-regulating balloon (5).

Unfortunately, there is a paucity of data comparing these management strategies, and those currently available are not in the setting of AUS revision for urethral atrophy (13). Given the lack of available data, the choice between these management options is based on the local tissue quality, location of the in-situ urethral cuff and surgeon preference.

Thus, we sought to compare outcomes for single urethral cuff downsizing versus tandem cuff placement during AUS revision for urethral atrophy.

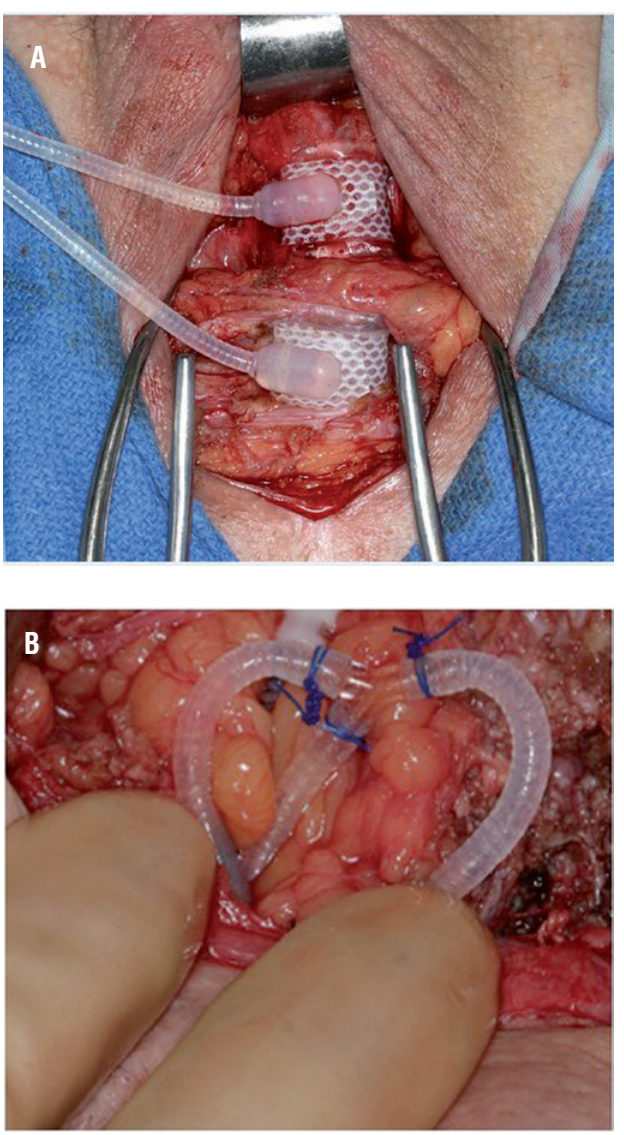
MATERIALS AND METHODS

After obtaining Institutional Review Board approval, we identified 1778 AUS surgeries at our institution from June 1st 1990 to December 31st 2014. Of those patients, 406 were the initial AUS revisions (i.e. secondary surgery), including 69 for urethral atrophy. Patients were excluded from analysis if they underwent revision surgery at another institution, underwent primary AUS placement secondary to neurogenic bladder dysfunction, were less than 18 years old at the time of primary AUS placement, or declined research consent. Both the primary implantation and revision surgery were performed at our institution in all cases. All implanted AUS devices were American Medical Systems 800 (AMS 800; American Medical Systems, Inc., Minnetonka, Minnesota, USA). The revision surgeries were performed by three surgeons.

With regard to our approach to evaluation of recurrent stress urinary incontinence after AUS placement, we typically obtain a history and physical, cystoscopy, and x-ray imaging (as contrast is instilled at the time of surgery in our primary placements). The diagnosis of urethral atrophy is confirmed during cystoscopy, when incomplete urethral coaptation is visualized with device cycling (with adequate fluid in the system on radiographic imaging). Patients confirmed to have urethral atrophy are considered for surgical AUS revision depending on symptom severity, patient preferences and comorbidities.

The decision to proceed with single cuff downsizing versus tandem cuff placement was at the discretion of the treating surgeon. Our tandem cuff placements are performed with the second cuff placed roughly 1-2cm distal to the primary cuff (Figure-1a). Additionally, we use a Y-shaped adapter, secured with free ties of non-absorbable suture to connect the pump tubing to both cuffs (Figure-1b). Furthermore, we add 1-3cc of fluid to the system to account for the additional volume sequestered in the cuff. In cases of severely atrophic urethral tissues (measurement <3.5cm), distal urethral tapering or difficult dissection planes (e.g. in some cases with prior pelvic radiation therapy or

Figure 1 - Tandem urethral cuff placement (a), connection of tandem urethral cuff to in-situ system (b).



urethral sling placement), we utilize a transcorporal approach, as previously described (11, 12). As all of the initial primary AUS placements in this study were performed at our institution, we are typically unable to relocate the cuff more proximally during subsequent revision surgery, as the primary cuff is placed as proximal as possible. Notably, we perform primary implantations with the cuff placed circumferentially around the bulbospongiosus muscle.

Individual charts were reviewed to evaluate pertinent clinical and surgical comorbidities, details of both the primary and secondary devices, primary device outcome including time to failure, revision management strategy (single cuff versus tandem cuff), and secondary device outcome. Given the retrospective study design patients, we did not have standardized follow-up. Instead, following device placement, patients are evaluated six weeks post-operatively for device activation. Following this, patients are followed via office evaluation on an as needed basis. Additionally, the Mayo Clinic AUS Registry monitors outcomes periodically by correspondence to the patient. Details regarding device survival were obtained from last office examination, subsequent operative report, written or telephone correspondence.

Statistical analysis was performed using the JMP 11 software package (SAS Institute, Inc.: Cary, NC). Patients were divided into cohorts based on management strategy, that is tandem cuff placements (including tandem and tandem transcorporal cuff placements) or single cuff downsizing. Continuous features were summarized with medians and interquartile ranges (IQRs); categorical features were summarized with frequency counts and percentages. Device survival was estimated as time from AUS revision for urethral atrophy to subsequent repeat (tertiary) surgery (including explantation or device revision for any reason), or last known follow-up, using the Kaplan-Meier method and compared with the log-rank test. All statistical tests were 2-sided, with a p-value <0.05 considered statistically significant.

RESULTS

Of the 406 patients undergoing first time revision surgery during the study timeframe, 69 patients (17%) underwent revision surgery for stress urinary incontinence secondary to urethral atrophy. Three surgeons performed the revision cases; with regard to distribution, individually the surgeons performed 41 (59%), 18 (26%), and 10 (14%) procedures. The median time from primary AUS placement to revision for atrophy was 4.92 years (IQR 2.67, 7.79). Of the 69 revision surgeries for urethral atrophy at our institution, 56 procedures (82%) were tandem cuff placements, 12 (18%) were single cuff downsizings and in one case we were able to relocate the cuff proximally (single cuff). Of the 56 tandem cuff placements, 8 (14%) were performed with a transcorporal approach. The distribution of surgeries by initial cuff size is shown in Figure-2. Notably, during primary implantation 87% of patients (60/69) had a 4.5cm cuff placed, 8.7% (6/69) had 4.0cm cuff, 2.9% (2/69) had a 5.0cm cuff placed and 1.4% (1/69) had a 5.5cm urethral cuff placed. No patients underwent implantation of 3.5cm urethral cuff with either primary or revisions surgery.

The demographics of patients undergoing single cuff downsizing compared to those undergoing tandem cuff placement are shown in Table-1. Notably, the cohorts were similar with regard to age (p=0.84), body-mass index (p=0.99), prior pelvic radiation exposure (p=1.00) and time from primary surgery (p=0.53). There was no significant difference in the length of follow-up after device revision between those that underwent single cuff downsizing, compared to tandem cuff placement (median 1.3 years versus 2.24 years; p=0.28).

Among patients undergoing revision for urethral atrophy (n=69), the median follow-up after revision surgery was 2.21 years (IQR 0.84, 6.76). During follow-up 19 patients underwent tertiary surgery, including 12 for device infection/urethral erosion, 3 for device malfunction and 4 for repeat urethral atrophy. All 4 cases of repeat urethral atrophy occurred in patients that underwent tandem cuff placement. Device infection or urethral erosion occurred in ten patients that underwent tandem cuff placement and two patients managed with cuff downsizing. Device malfunction occurred in two patients managed with tandem cuff placement and one treated with cuff downsizing.

Figure 2 - Distribution of cuff downsizings and tandem cuff placements by initial cuff size.

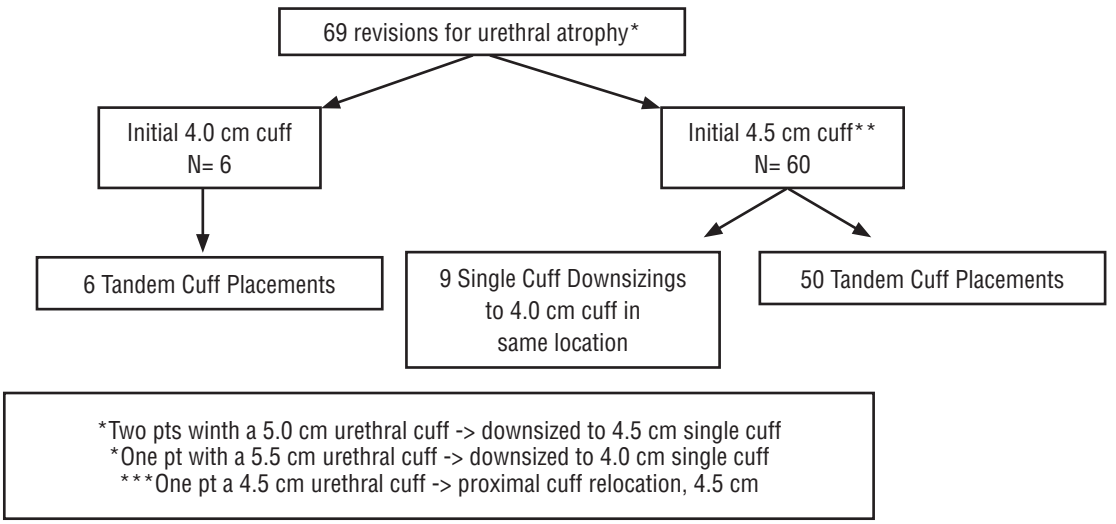


Table 1 - Clinical and demographic features of patients undergoing Artificial Urinary Sphincter Revision for mechanical failure stratified by revision technique.

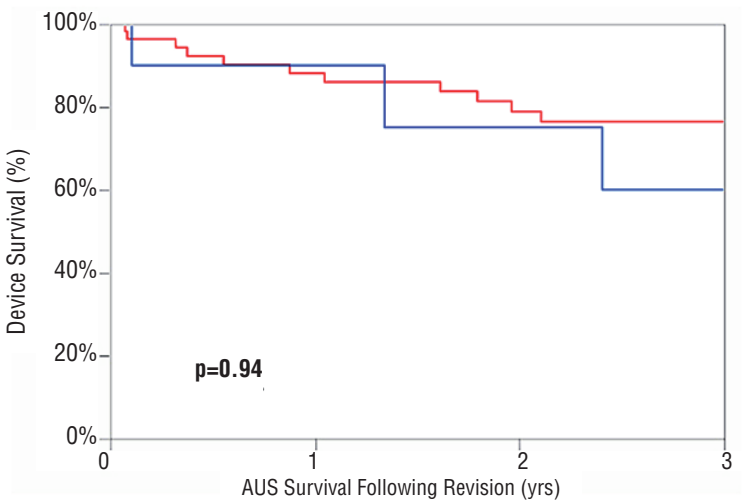
	Single Cuff Downsizing (n=12)	Tandem Cuff Placement (n=56)	p value
Age, years, median (IQR)	75.5 (71.8, 79.3)	74.6 (68.6, 80.6)	0.84
Body-mass index, kg/m^2, median (IQR)	28.7 (26.1, 29.9)	27.5 (25.3, 30.9)	0.99
Prior pelvic radiation	4 (33.3%)	19 (33.9%)	1.00
Coronary artery disease	4/9 (44.4%)	9/35 (25.7%)	0.41
Time to primary failure, years, median (IQR)	6.13 (3.77, 8.46)	4.92 (2.54, 7.59)	0.53

There was no difference in 3-year overall device survival compared between single cuff and tandem cuff revisions (60% vs. 76%, p=0.94) (Figure-3). Likewise, there was no association of tandem cuff placement (ref. single cuff) and the risk of tertiary surgery for any cause (HR 0.95, 95% CI 0.32-4.12, p=0.94). Furthermore, there was no association of the risk of urethral erosion/device infection and tandem cuff placement (ref. single cuff) (HR 0.79, 95% CI 0.20-5.22, p=0.77). However, among those undergoing tandem urethral cuff placement, a transcorporal approach (8/56) was associated with adverse 3-year device survival compared to those placed without a transcorporal approach (44% vs. 80%, p=0.0016). Specifically, four patients that had transcorporal cuff placement had a repeat revision surgery, including three for device infection/erosion and one for device malfunction.

DISCUSSION

We found here in a cohort of patients undergoing AUS revision for urethral atrophy, that

Figure 3 - Overall device survival following revision surgery for patients with single cuff downsizing or tandem cuff placement.



	% Survival (No. at Risk)		
	0 years	**1 years**	**3 years**
--- Tandem	100% (56)	88% (43)	76% (28)
--- Downsize	100% (12)	90% (8)	60% (5)

there was not enough evidence to prove a difference in overall device survival in patients undergoing single cuff downsizing versus tandem cuff placement. Furthermore, there was no significant difference in the risk of urethral erosion/device infection between the two cohorts. These results augment the existing literature by providing comparative data on these management options in the setting of AUS revision for urethral atrophy.

Unfortunately, recurrent stress urinary incontinence following AUS implantation and subsequent revision surgery is not uncommon, impacting roughly 26% of patients undergoing primary AUS placement (1). Of these cases, a rate of revision surgery of 7.9% (range 1.9-28.6%) for urethral atrophy was reported in a pooled analysis (1). Notably, several surgical options for AUS revision in these cases have been reported, including: changing the location of the urethral cuff (7), downsizing the single urethral cuff (8), placement of a second (tandem) urethral cuff (9, 10), transcorporal cuff placement (11) or revising the pressure-regulating balloon (5). Notably, each series demonstrates excellent results for the technique proposed. For instance, in a study of 17 patients treated for recurrent incontinence with cuff downsizing over a seven-year period, Saffrian et al. found improvements in pad use (from 3.9/day to 0.5/day) and patient satisfaction (from 15% to 80%) with this technique (8). However, there is no comparative group against which to evaluate the results. As such, the evidence base that could be used to guide surgical management in cases of AUS revision for urethral atrophy is limited.

The one area with comparative evidence is the use of a single versus double/tandem urethral cuff placement, however this is in a cohort of patients undergoing primary AUS implantation (13, 14). In their initial study of 56 patients, O'Connor et al. found that in a matched analysis, double cuff placement was associated with a significantly greater rate of complete continence (p=0.008) and improvement in IIQ-7 scores (p=0.03) compared to single cuff placement (14). However, with longer follow-up (n=47), an average of 74.1 months for single cuff and 58 months for double-cuff, there

was no significant difference in overall continence or quality of life between the two management strategies (13). We found similar results, with no evidence to support a significant difference in overall device survival or device infection/urethral erosion rates between single and double/tandem cuff placement in patients undergoing AUS revision for urethral atrophy.

Given these results, we have modified our practice and now, the typical initial approach to patients with recurrent bothersome stress urinary incontinence secondary to urethral atrophy is downsizing the urethral cuff, with tandem cuff reserved for cases of recurrent atrophy or when downsizing cannot be performed. An advantage to initial urethral cuff downsizing is that no additional periurethral dissection is necessary. However, it is worth noting that we found no difference in device infection/urethral erosion rates in our series between single and double cuff surgeries.

In cases where the smallest available cuff is already in-situ or cuff downsizing has previously failed, we proceed with additional urethral dissection and either moving the urethral cuff or placement of tandem urethral cuffs. As mentioned, all patients in this series underwent primary AUS placement at our institution, and thus relocation proximally was not physically possible. In this setting, tandem urethral cuff placement attempts to avoid increasing the pressure on the atrophic urethra segment and instead distributes additional compression to a second area of the urethra (9). It is worth noting that in order to account for the urethra tapering distally, we have used a transcorporal technique at times for added tissue bulk in the setting of revision for urethral atrophy (12). In the current series tandem transcorporal cuff placement was associated with adverse device survival compared to non-transcorporal tandem cuff placement. Given the study design it is difficult to discern if this is due to underlying factors that prompted a transcorporal approach, or the surgical technique. Similar to the experience of others (6, 15), we do not increase the pressure in the abdominal fluid reservoir in cases of urethral atrophy. Likewise, it is our preference to avoid downsizing to a 3.5cm urethral cuff, as there is little experience with this reported in the literature.

The limitations of our study, including its retrospective, non-randomized design should be noted. Given this, patient follow-up was not standardized and heterogeneous. While we verify follow-up through patient correspondence, some patients may undergo additional procedures with their local providers and may not be captured in our dataset. Additionally, we do not have functional outcomes available for patients that underwent revision for urethral atrophy, which limits our ability to determine if patient quality of life is different between these cohorts. As such, additional studies regarding the management of urethral atrophy following AUS placement, are needed to help define the optimal management strategy for these patients.

There was no significant difference in overall device survival in patients undergoing single cuff downsizing or tandem cuff placement during AUS revision for urethral atrophy. As such, we prefer to downsize the urethral cuff in the initial revision surgery to allow for tandem cuff placement during future revisions if needed.

CONFLICT OF INTEREST

None declared.

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EDITORIAL COMMENT

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EDITORIAL COMMENT: ARTIFICIAL URINARY SPHINCTER REVISION FOR URETHRAL ATROPHY: COMPARING SINGLE CUFF DOWNSIZING AND TANDEM CUFF PLACEMENT

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Several therapeutic options have been proposed for patients with recurrent or persistent post--prostatectomy urinary incontinence due to urethral atrophy: changing the balloon reservoir for a higher pressure one, downsizing the cuff diameter, or increasing the amount of fluid in the system (1-3). Theoretically, a transcorporal cuff could possibly provide some supplementary bulk of tissue to the circumference of the urethra, possibly decreasing the risk of erosion (4).

This article Linder, et al. (5) deals with a matter of great clinical interest. This is a retrospective series reporting 69 cases of revision surgeries for urethral atrophy, of which 56 (82%) underwent tandem cuff placements, 12 (18%) underwent single cuff downsizings and one case had a single cuff relocated proximally. There was no difference in 3-year overall device survival compared between single cuff and tandem cuff revisions (60% vs. 76%, p=0.94). Of the 56 tandem cuff placements, 8 (14%) were performed with transcorporal approach. Interestingly, these patients had adverse 3-year device survival compared to those without a transcorporal approach (44% vs. 80%, p=0.0016). Despite of the inherent limitations of this retrospective study, it seems that a transcorporal approach should be reserved for very selected patients (most probably in secondary or tertiary interventions). Randomized controlled trials are still needed to guide what is the best technique for each group of patients, taking into account anatomical characteristics, previous radiation therapy, the risk of urethral erosion and other local complications.

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Urethral pressure variation: a neglected contributing factor in patients with overactive bladder syndrome?

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ABSTRACT

Objective: To study urethral pressure variations during the whole filling phase among different groups of patients.
Material and Methods: We investigated 79 consecutive patients from January 2011 to June 2012. All patients were recruited within our routine practice in our continence clinic and were evaluated with urodynamic exam according to the standards of the International Continence Society (ICS) with an additional continuous measurement of the urethral pressure profile (cUPP) that was done in a supine position. Patients with genital prolapse >grade I, as well as patients with impaired cognitive function or neurogenic disorders were excluded. Bacteriuria at the time of investigation was excluded by urine analysis. Urethral pressure changes higher than 15cmH_2O were considered as 'urethral instability'.
Results: From 79 investigated patients, 29 were clinically diagnosed with OAB syndrome, 19 with stress urinary incontinence (SUI) and 31 with mixed (OAB and SUI) incontinence. The prevalence of 'urethral instability' as defined in this study was 54.4% (43/79). The mean Δp in patients with OAB (36.5cmH2O) was significantly higher ($p<0.05$) than in groups with pure stress (14.9cmH2O) and mixed urinary incontinence (19.3cmH2O).
Conclusions: Etiology of 'urethral instability' is unknown, but high prevalence among patients with overactive bladder syndrome, especially concomitant with detrusor activity can raise a fair question and direct further diagnostic as well as treatment efforts.

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INTRODUCTION

Overactive bladder (OAB) syndrome has a great impact on health-related quality of life with a high prevalence of up to 13%, in the female population over 18 years (1). According to the International Urogynecological Association (IUGA) and the International Continence Society (ICS), the term OAB describes the combination of symptoms consisting of urgency, with or without urge urinary incontinence, urinary frequency and nocturia, if there is no proven infection or other obvious pathological condition (2). But at the same time term OAB denotes a syndrome whose etiology is unknown, and it is believed that detrusor overactivity (DO) is a major factor and is currently the only accepted underlying pathophysiology of OAB (3). The fact that during classic urethral pres-

sure profile measurement catheter withdrawal along the urethra gives only a little information concerning external urethral sphincter behavior during the whole filling phase, which is according to "guarding reflex" theory by Park et al. can have a bladder--modulating role (4), as well as low efficacy of the existing drugs (anti-muscarinic, beta3-mimetics) targeting at the detrusor wall (5) – all that encouraged us to look closer if there is any consistent pattern of external sphincter functioning in different group of patients. In this regard, we investigated urethral sphincter pressure variations and in OAB, SUI and MUI patient's continuously measuring urethral pressure profile during the whole filling phase.

MATERIALS AND METHODS

Study Design

Ethical approval (EK 085/11-Universityclinic Aachen) and patient consent was obtained and studies were done in accordance to the declaration of Helsinki. The study evaluated 79 consecutive female patients with lower urinary tract symptoms (LUTS), including signs of overactive bladder syndrome (OAB), stress (SUI) and mixed urinary incontinence (MUI). Patients were referred to the Continence clinic of the University Hospital (RWTH) Aachen, an interdisciplinary unit for incontinence diagnostics. Period of enrollment was from January 2012 through June 2012.

Patients with genital prolapse >grade I, as well as patients with impaired cognitive function or neurogenic disorders were excluded. Bacteriuria at the time of investigation was excluded by urine analysis. All patients underwent pressure/flow studies followed by a conventional urethral pressure profile measurement with a triple lumen 9Fr catheter and a continuous urethral pressure profile (cUPP) registration for 60s during a second complete filling phase with the catheter positioned at the site of the maximum urethral closure pressure. The bladder was filled with medium filling speed of 15 to 30mL/min. The cUPP was done in a supine position to reduce movement artifacts and the patient was asked to lie relaxed without movement if possible. Throughout the investigation, pelvic floor electromyography (EMG) was registered by surface electrodes.

Evaluation of cUPP

The difference between the highest and lowest urethral pressure during cUPP was calculated. A urethral pressure drop with urgency to void before micturition was neglected, because focus of analysis was on the cUPP during filling phase. Urethral pressure variations exceeding 15cmH_2O were defined as 'urethral instability' (6-12). Urethral pressure variation (cmH_2O), maximum urethral closing pressure (cmH_2O), minimum urethral closing pressure (cmH_2O) and functional urethral length were determined for each patient group (OAB, MUI, SUI).

Area Under the Curve (AUC)

The 60s of raw data was obtained through the ASCII-exportation of Laborie UDS data into a standard spreadsheet program and included time and corresponding urethral pressure with a sample rate of 10Hz. Area under the curve (AUC) was calculated with the trapezoidal rule and corresponding minimum pressure values. Data were pooled for every patient group (OAB, MUI, SUI).

Fast Fourier Transform (FFT)

Raw data were used for the application of Fast Fourier Transform (FFT) and graphic functions. Basic control data (eg: known sinusoidal data) was also used to validate the FFT process to ensure that the frequency domain transformation was correct. Applying a Fast Fourier Transform (FFT) converted the temporal information of P_{ura} into the frequency domain and thereby allowed the reviewer to visualize, from the spectral density perspective, the frequency content of the signal under review. While no specific frequency component was expected to be identified during a 'urethral instability', we were looking for large clusters of frequency content to be uniquely differentiated from normal P_{ura} measurements. The FFT samples were calculated and plotted for a visual review.

Statistics

Statistical analysis was performed using NCSS (NCSS, LLC release 2007, Utah, USA) and GraphPad Prism Ver. 5 (GraphPad Software Inc., La Jolla, CA, USA). Data were analyzed using the

Shapiro-Wilk normality test and non-parametric Mann-Whitney u-test. Relationship between 'urethral instability' and functional urethral length was quantified by Spearman-rank test and Pearson r-squared correlation. A p-value of <0.05 was considered statistically significant. All statistical tests reported in this study were two tailed.

RESULTS

Of the 79 female patients, 29 presented with OAB syndrome, 19 with SUI and 31 with mixed (OAB and SUI) symptoms. Patient's characteristics are shown in Table-1.

The prevalence of 'urethral instability' (pressure variance over 15cmH_2O) in this cohort was 54.4% (43/79). For those with OAB syndrome, 'urethral instability' occurred in 23 of 29 (79.3%) patients, in 9 of 19 (47.4%) of those with stress incontinence and in 11 of 31 (36.6%) with mixed incontinence. Typical urodynamic traces of a patient with OAB and DO and a patient with SUI without DO are shown in Figure-1.

The mean Δp (delta pressure) in patients with a sensory component related to OAB syndrome (36.5cmH_2O) was significantly higher ($p<0.05$) than in groups with pure stress (14.9cmH_2O) and mixed urinary incontinence (19.30cmH_2O) (Figure-2). Patients of the OAB group were divided into sub-groups 'with DO' and 'without DO'. Mean urethral pressure of OAB patients with DO (85.5±37.0cmH_2O (mean±SD) was significantly higher than in OAB patients without DO (45.2±30.65cmH_2O) ($p<0.05$) (Figure-3).

The analysis of the area under the curve showed that urethral pressure curves of OAB patients are in sum either longer or higher than those of mixed or stress urinary incontinence (1195.7±985.6 (OAB) vs. 429.3±214.5 (MUI) vs. SUI 549.4±273.0 (SUI) (Figure-4).

Statistical analysis revealed significant differences with $p<0.005$ (OAB vs. MUI) and $p<0.01$ (OAB vs. SUI). Fast Fourier Transformation generated FFT-Plots for every patient and visual review revealed higher magnitudes and more peaks in the range of 0-0.5Hz within the group of patients with OAB (Figure-5).

DISCUSSION

Our study revealed that patients with OAB have statistically significant higher range of urethral pressure variation than in SUI and MUI patient groups, moreover we found that in patients with OAB and concomitant DO urethral pressure variation range was even higher than without DO ($p<0.05$) (Figure-3).

During urodynamic investigations (UDS), the focus regularly lies on the bladder pressure changes neglecting the fact that in the process of urine storage, the external urethral sphincter may play an important role. In 1959, the "guarding reflex" by the external urethral sphincter was brought into discussion by Garry et al. and

Table 1 - Main patient's characteristics.

Diagnosis	n	Age (years) (mean±SD)	Urethral pressure variation (cm H_2O)	Maximum urethral closing pressure (cm H_2O)	Minimum urethral closing pressure (cm H_2O)	Functional urethral length (mm)	Post Menop. %
OAB	29	60.2±13.6	36.5±28.3	202.1	1.0	38.0±7.9	79.3
Stress incontinence	19	62.8±12.1	14.9±6.6	114.5	3.0	33.9±8.7	84.2
Mixed incontinence	31	58.5±14.4	19.3±10.47	89.0	3.8	32.1±6.6	77.4

Figure 1 - Urodynamic evaluation in a patient with DO (left) and SUI (right).

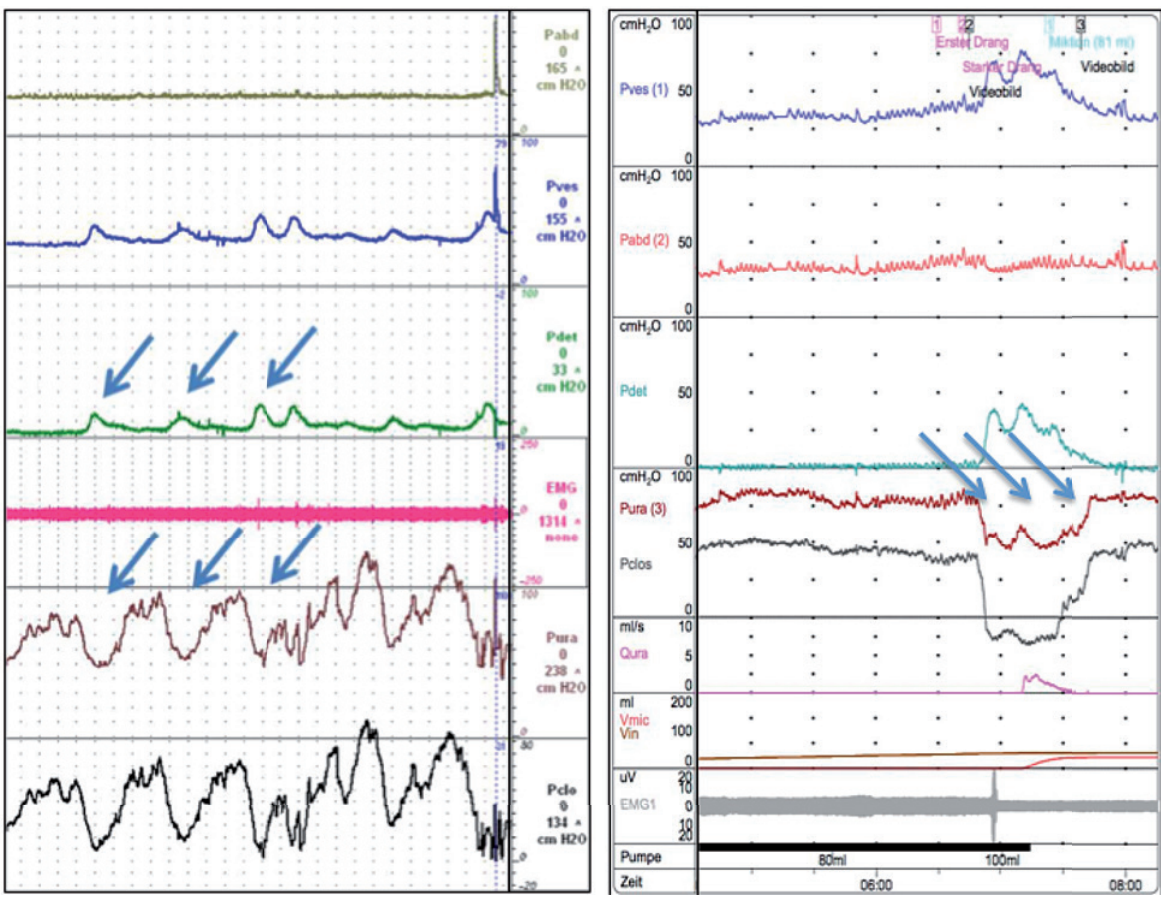


Figure 2 - Diagram of variance of delta pressure between the groups.

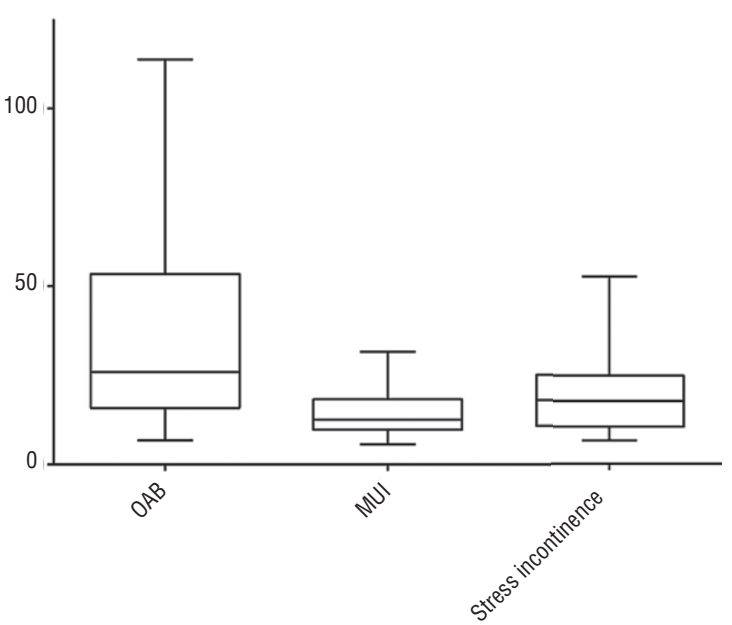


Figure 3 - Urethral Pressure in OAB patients with (left) or without (right) DO, single asterisk displays significance difference between the two groups determined with two-tailed t-test.

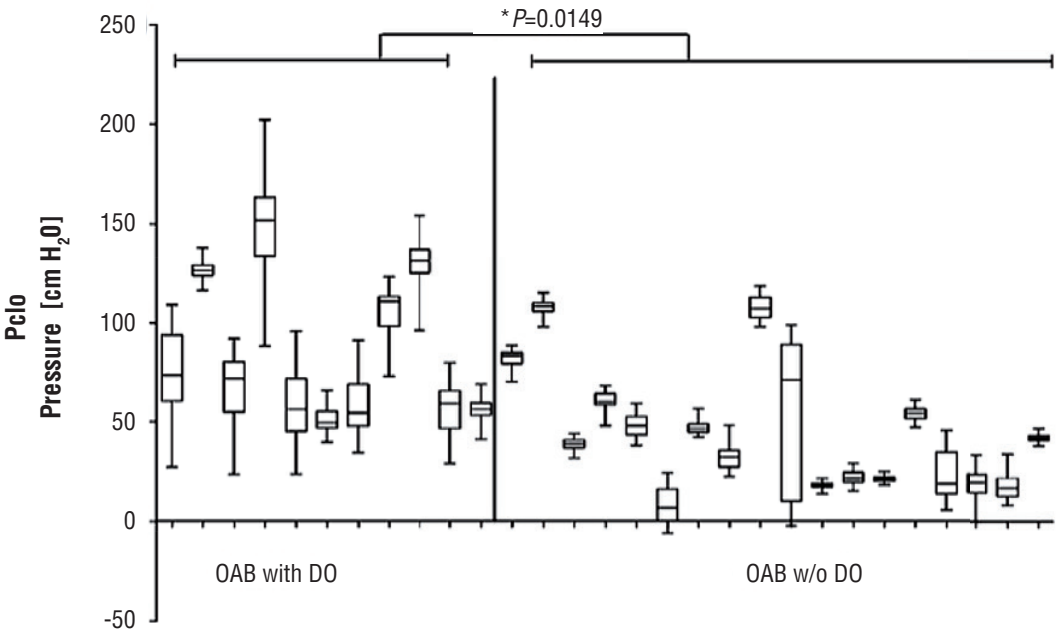


Figure 4 - Area under the curve of cUPP in patients with OAB, MUI and SUI, applied trapezoidal rule calculated from min. pressure.

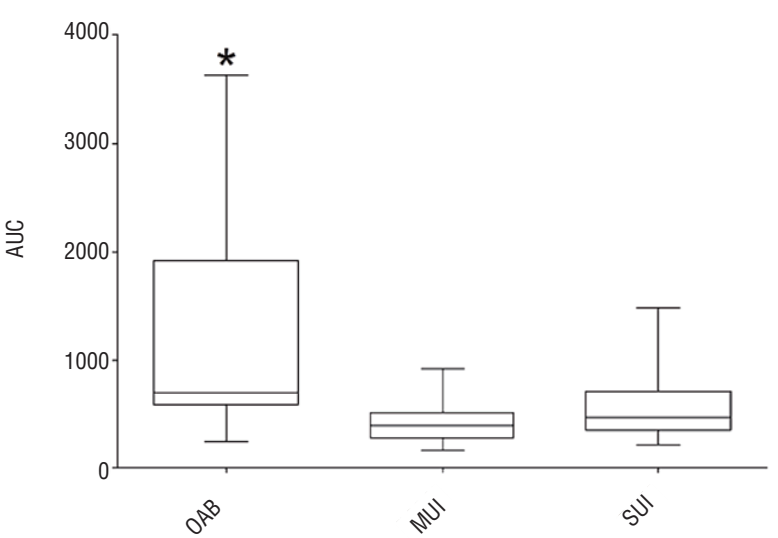
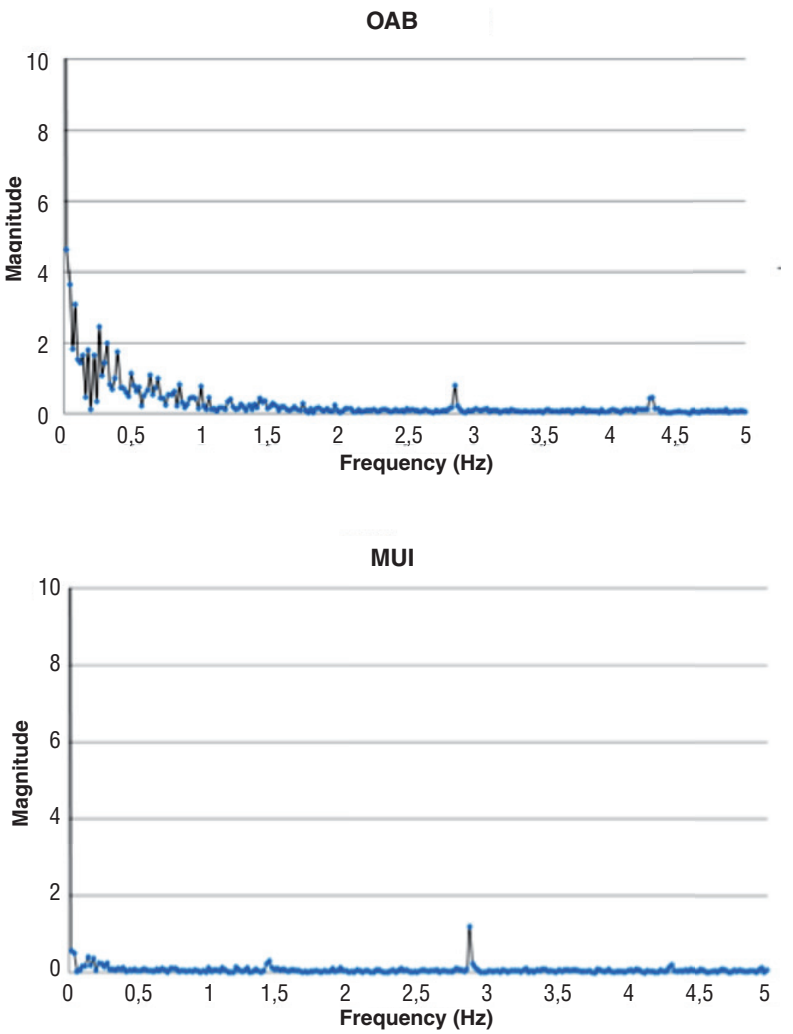


Figure 5 - Representative FFT-Transformation of urethral pressure measures in a patient with OAB (above) and MUI (bottom).



was attributed a main role in prohibiting stress incontinence (13). Referring to the "guarding reflex", Park et al. later stipulated a theory that the external sphincter has a bladder-modulating role (4). McGuire and Sørensen in the 70s and 80s concluded that urethral pressure variations seem to play an important role in normal urethral physiology, possibly contributing to continence and prevention of urinary tract infection (14, 15). In contrast, Vereecken et al. failed to demonstrate a difference between patients with urge and stress incontinence with regard to 'urethral instability', except urethral pressure variations of more than 35cmH_2O, which provoked urge (9). No difference in terms of prevalence or severity of urgency/frequency, nocturia or urge incontinence was reported between patients with or without 'urethral instability', and the symptom of stress incontinence was more common in women with 'urethral instability' (9, 16). Nevertheless, Matthiason et al. also came to the conclusion that women with stress, urge, and mixed urinary incontinence seem to have a primary neuromuscular disorder in the urethra. They described urethral pressure variation as an overactive opening mechanism with a fall in urethral pressure instead of a pressure increase

on provocation during the filling phase of the bladder, and during bladder emptying a more efficient opening of the bladder outlet than in normal women. They suggested that one and the same pathophysiological mechanism participates in female stress, urge, and mixed incontinence (17). Moreover in 2009, Groenendijk et al. stated that it makes sense to measure and register detrusor and urethral function during filling and voiding (18). Despite the fact that most authors consider urethral pressure variations, exceeding an amplitude of 15cmH_2O, as an abnormal finding (6-12) the significance of these fluctuations is still unclear, and the terms 'urethral instability' or 'unstable urethra' lack clarity and are not accurately defined by the ICS today.

In accordance with our own data, Sørensen et al. demonstrated that mean maximum urethral pressure and the mean maximum urethral closure pressure are significantly reduced in women with stress incontinence compared to women with detrusor overactivity (19).

Our study is not without limitations. First limitation is in the study design-small sample size, absence of assessors blinding, absence of a healthy patients group. Second is that the neurophysiologic explanation of urethral pressure variation is unclear. Urethral pressure variation may be caused by diminished sympathetic influence or by increased parasympathetic activity. It has been reported that neuronal nitric oxide (NO) synthase, known for its significant role in nociceptive pathways in the bladder has also been found in human female striated urethra sphincter (20). It may be that contraction of the detrusor is caused by a fall in urethral pressure (18). However, looking at the traces, it is very difficult to determine (Figure-1) which came first: detrusor overactivity, then urethral pressure drop or vice versa, or both appeared at the same time. Whatever concomitant finding of detrusor overactivity and urethral pressure variations may suggest a combined pathophysiology being a cofactor in some OAB patients. Considering that some DO might not have been detected, correlation should be even higher.

Further investigation of urethral pressure variation with high-speed urethral pressure urodynamics will let to sample at up to 1000Hz and not 10 to 50Hz used today in conventional equipment and possibly shed some more light on that issue.

CONCLUSIONS

Given the growing relevance of OAB syndrome and widely expanding armamentarium of treatment modalities and drugs as well the outcomes are still quite disappointing (21) and it seems that we are missing something important in patients with OAB. Possibly we should stress our attention on both: detrusor overactivity and urethral instability.

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CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Minimally invasive treatment of female stress urinary incontinence with the adjustable single-incision sling system (AJUST ™) in an elderly and overweight population

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ABSTRACT

Introduction: The prevalence of urinary incontinence is increasing. Two major risk factors are overweight and age. We present objective and subjective cure rates of elderly and overweight patients treated with an adjustable single-incision sling system (AJUST™, C.R. BARD, Inc.).

Materials and Methods: Between 04/2009 and 02/2012 we treated 100 female patients with the single incision sling. Patients were retrospectively evaluated by Stamey degree of incontinence, cough test, pad use, and overall satisfaction. The primary outcomes of the study were objective and subjective cure rates, secondary outcomes were the safety profile of the sling and complications.

Results: The overall success rate in this population was 84.6% with a mean follow-up of 9.3 months. The average usage of pads per day decreased from 4.9 to 1.6 and was significantly lower in patients with a BMI <30 (p=0.004). Postoperative residual SUI was also lower in patients with a BMI <30 (p=0.006). Postoperative satisfaction was better in patients with a lower BMI, but this difference did not reach a level of significance (p=0.055). There were no complications such as bleeding, bladder injury, or tape infection.

Conclusions: In elderly and obese patients a considerable success rate is achievable with this quick and minimal invasive procedure. However, the success rate shows a clear trend in favor of a lower body-mass-index. The cut-off point has been identified at a BMI of 30. The AJUST™ system can be regarded as safe and beneficial for elderly and obese patients.

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Keywords:
Body Mass Index; Obesity; Suburethral Slings; Urinary Incontinence, Stress

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INTRODUCTION

Stress urinary incontinence (SUI) is the involuntary loss of urine during coughing or physical activity, mostly due to a weak pelvic floor or urethral sphincter (1). SUI derogates social, physical, psychological, occupational, and sexual aspects of life (2). Worldwide median prevalence of female urinary incontinence is 27.6%, divided in stress (50%), mixed (32%), and urge incontinence (14%) (3). With the increasing number of elderly people in the developed countries the prevalence of SUI is growing (4). It is estimated that the number of women with urinary incontinence in the USA will increase by 55% until 2050, thus affecting one third of all American women (5). With better information and a decreasing fear to report such symptoms more and more women are seeking help. The annual number of continence operations increased by 28% between 1997 and

2007 in the UK (6). More than 200.000 incontinence procedures per year are currently performed in the USA (7). The annual costs of stress urinary incontinence management are estimated to be $19.5 billion in the United States and £740 million in the United Kingdom (8).

Obesity and older age are two major risk factors of SUI. Furthermore parity, prior surgical treatment of the pelvic floor, menopausal status, smoking, coffee and alcohol consumption, and several concomitant diseases, e.g. COPD, chronic pelvic pain and constipation were identified as independent risk factors (1). Especially obesity is a demographic challenge of the future and the incidence is increasing dramatically. Between 1980 and 2008, mean global BMI increased by 0.4kg/m² (men) and 0.5kg/m² (women) per decade. In 2008 1.46 billion adults had a BMI ≥25 and about 0.5 billion ≥30 worldwide (9). The rising percentage of obesity is alarming, ans also WHO is concerned with the development of overweight people. BMI correlates with intra-abdominal pressure, potentially increasing the risk for SUI (10). Richter et al. found that obese patients undergoing SUI surgery complain of more incontinence episodes, more symptom distress, and worse quality of life (11).

With the growing number of elderly and overweight female patients with SUI a safe treatment procedure with little invasiveness, high efficacy, and good reproducibility is in great demand. Conventional tension free tapes show inferior results in older patients with a low pressure urethra and a higher morbidity in obese patients (12). Particularly the treatment of SUI in (mostly elderly) ISD (intrinsic sphincter deficiency) patients can mean a challenge. The TVT (tension free vaginal tape) procedure showed inferior results in Type III SUI compared to Type I/II SUI (13).

In our patient population we observed a high proportion of elderly and overweight females and therefore intended to review our results systematically concerning the outcome of an adjustable single incision sling (SIS) system (AJUST™, C.R.BARD Inc.) with particular focus on the correlation with obesity and age. The AJUST system offers adjustability of the tension applied to the urethra and minimal morbidity for no retrobubic or transobturator needle passage is necessary. Secondary outcomes included the safety profile of the sling device and possible complications, which are particularly important in an elderly population.

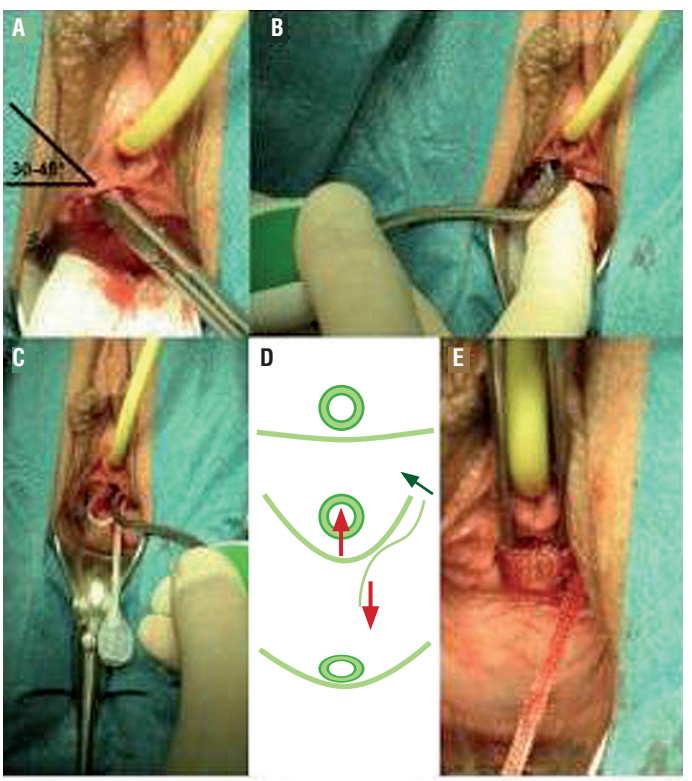
MATERIAL AND METHODS

This is a retrospective analysis of 100 female patients with SUI treated between 04/2009 und 02/2012 with the adjustable single-incision sling system (AJUST™). All procedures were performed by the same surgeon (R.A.) with the experience of approximatly 400 tape procedures since the year 2000 including the first SIS (TVT Secur) in 2006.

The primary outcomes of the study were objective and subjective cure rates, evaluated by Stamey degree of incontinence (Grade 1: loss of urine with sudden increases of abdominal pressure: e.g. coughing, sneezing or laughing. Grade 2: loss of urine with lesser degrees of stress: e.g. walking or standing up. Grade 3: loss of urine without any relation to physical activity or position, e.g. while lying in bed.), cough test, pad use, and overall satisfaction. In accordance with the current literature and guidelines (21, 31) we do not systematically perform urodynamic testing in clearly demonstrable stress urinary incontinence on physical examination when it does not change management. Urodynamic testing was performed when other influencing factors were present, e.g. prior failed SUI surgery or neurological diseases. Postoperative evaluations were performed through clinical examination (60 patients) and telephone interview in all but one patient (1 to 4 times) to obtain subjective outcome. Patients were asked for continence status, pad use, urge symptoms, side effects (pain, bleeding), and overall satisfaction. The mean follow-up period was 9.3 months (1 to 23 months). The study protocol was approved by the local ethics committee (vote number 286/16).

Procedure (Figure-1): A small incision underneath the mid-urethra is performed, the length must not be little more than 1cm what matches the width of the tape. This prevents dislocation of the tape during tensioning and in the postoperative period. A bilateral small tunnel towards the inner

Figure 1 - A) A bilateral small tunnel towards the inner margin of the inferior ramus pubis is created; B) Firstly the introducer is gently forwarded through the tunnel until the right obturator membrane is fully perforated (2 steps of resistance); C) The contralateral obturator membrane is perforated. The introducer shape is helpful in preventing a too deep insertion; D, E) A gentle tightening of the sling follows until no further leakage occurs through the Valsalva manoeuvre with 300cc bladder filling.



margin of the inferior ramus pubis is created. The introducer is gently forwarded through the tunnel under digital control until the right obturator membrane is fully perforated in the medial corner (2 steps of resistance related to the obturator fascia). Then, the contralateral obturator membrane is perforated. The introducer shape is helpful in preventing a too deep insertion, digital control prevents perforation of the vaginal skin in the sulcus. There follows a gentle tightening of the sling by pulling on the adjustment mesh arm until no further leakage occurs during the Valsalva manoeuvre with 300cc bladder filling. Under general or spinal anesthesia this is done through a Credé manoeuvre to create an intravesical pressure that approximately correlates with the cough pressure in an upright position. Special attention is paid to the prevention of overcorrection. If there is still some dribbling under adequate tension this is rather accepted than pulling too hard on the adjustment mesh what might end in extraction of the anchor and disruption of the obturator membrane. After adjustment a 14Fr. catheter must still smoothly pass the urethra. Certainly this testing adds to the operating time.

Statistical analysis

Numerical variables are presented with mean and standard deviations. Comparative analysis of these variables were made with the Student`s t-test. Nominal or ordinale variables are presented as relative values. Chi-square and Fisher`s exact test were used to compare these statistics. Significance of all p-values was achieved at 0.05 in a two-tailed test. All statistical analysis were performed with SPSS statistical software (version 21.0).

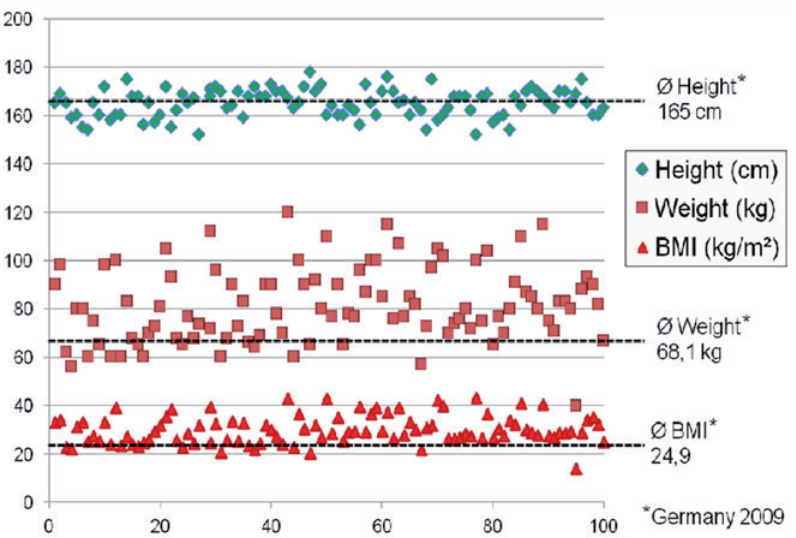
RESULTS

Mean age of the patients was 70.3±8.7 years, mean parity was 2.5±1.7. 16/100 patients presented with I SUI according to the Stamey classification, 43/100 with II SUI, 18/100 with III SUI, and 23/100 with mixed incontinence and a predominant stress component (Table-1). Mean body--mass-index (BMI) was 28.7, 29.8, 30.9, and 30.2, for I, II, III SUI, and mixed incontinence, respectively, compared to an average BMI of 24.9kg/m² in Germany 2009 (Figure-2). 88/100 patients had prior pelvic floor surgery (mean 2.2 (0-13) operations). The patients stayed in hospital for 3.1±1.8 days as usual in the German health system where these procedures are typically not performed as 1-day-surgery due to patient safety and reimbursement aspects (Table-1). Nearly all procedures were done under general or spinal anesthesia, only few patients opted for local anesthesia. No major concomitant procedures were performed, in 12 cases a tape resection or urethrolysis due to prior failed tape insertions was done, in 1 case a posterior mesh for rectocele correction was inserted at the same time. The procedures lasted 24.9±13.1 min on average and were easily feasible in all patients despite considerable periurethral scarring in many patients with prior interventions (Table-1). There were no complications like bleeding, bladder injury, or tape infection that required any kind of treatment. However, in 11 cases we observed urinary retention due to a suspected hypocontractile detrusor muscle, 7 temporary and 4 persistent. One of the latter was treated with a suprapubic catheter, three had a sling transection after 4, 5, and 11 months, respectively. Two of the

Table 1 - Pre-, intra-and postoperative data. Postoperative SUI and postoperative pad use are significant lower in patients with BMI <30. Data presented as mean value±standard deviation or relative frequency.

	All patients	BMI < 30	BMI ≥ 30	p
Preoperative data				
Parity	2.5 ± 1.7	2.3 ± 1.5	2.7 ± 1.9	0.297
Patient age [a]	70.3 ± 8.7	71.8 ± 7.8	68.3 ± 9.4	0.055
Intraoperative data				
Operation time [min]	24.9 ± 13.1	23.8 ± 11.8	26.3 ± 14.7	0.352
Postoperative data				
Hospital stay [d]	3.1 ± 1.8	3.3 ± 2.2	2.9 ± 1.0	0.284
Urge	20/99	12/56	8/43	0,804
SUI				0.006
- no	50/71	35/41	15/30	
- I°	10/71	3/41	7/30	
- II°	8/71	3/41	5/30	
- III°	3/71	0	3/30	
Positive stress test	14/91	5/54	9/37	0.075
Satisfaction				0.055
- very good	33/100	24/56	9/44	
- good	30/100	17/56	13/44	
- fair	11/100	5/56	6/44	
- poor	26/100	10/56	16/44	
Pad use	1.6 ± 2.3	1.3 ± 2.2	2.8 ± 2.9	0.004

Figure 2 - Age ranged from 43 to 86 years (mean 70.3 years). The body-mass-index (BMI) of the patients is predominantly clear above the German average.



latter remained completely dry, one re-developed first degree stress urinary incontinence.

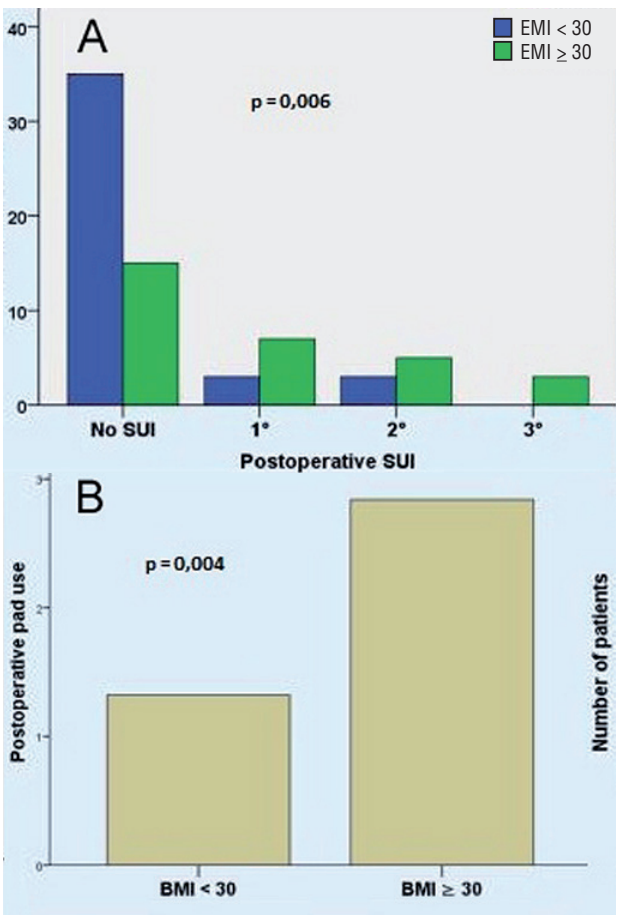
77 of 91 clinically evaluable patients (84.6%) had a negative postoperative stress test what we considered objective cure. 9 patients were not available for postoperative reexamination for various reasons. We observed a distinct correlation with the BMI value: if BMI was <30 the pad test was negative in 90.7%. If BMI was >30 it was negative in 75.7%. The Stamey degree of SUI after the procedure was 0° in 50/99 (50.5%), I in 10/99 (10.1%), II 8/99 (8.1%) and III 3/99 (3.0%). 8/99 patients (8.1%) presented with mixed incontinence, 20/99 (20.2%) with isolated urge incontinence, 1 patient was not available for analysis due to language difficulties. 10/99 (10.1%) patients developed de novo urge symptoms, 6 of those with incontinence that was linked to poor satisfaction. 4 patients with de novo urge symptoms but without incontinence reported good or very good satisfaction. Comparative analysis showed a significantly better degree of SUI in patients with a BMI <30 (p=0.006, Figure-3a). No significant differences between patients with BMI <30 and patients with BMI ≥30 could be detected in pre-and intraoperative demographic data. Similarly, no significant difference between these two groups were found in postoperative length of stay and postoperative frequency of urge symptoms (p=0.804). The average usage of pads per day decreased from 4.9 (1-13) to 1.6 (0-10) and was significantly lower in patients with a BMI <30 (1.3±2.2 vs. 2.8±2.9; p=0.004, Figure-3b). 65/100 patients had a pad reduction of at least 50%.

Overall satisfaction with the result, based on a subjective assessment of the patients was very good in 33/100 patients, good in 30/100, fair in 11/100, and poor in 26/100. The average BMI of the patients with a very good, good, fair, and poor result was 28.0, 29.8, 30.3, and 31.9, respectively (Figure-4). In general, postoperative satisfaction was better in patients with a lower BMI, but this difference did not quite reach a level of significance (p=0.055). 4 of the 26 patients who were regarded as failure underwent a surgical revision, 2 with sling shortening, 2 with a different sling.

DISCUSSION

The proper treatment of stress urinary incontinence is continuously under debate. With the high number of procedures performed today, a highly competitive market for different tape implants has evolved. The now available longer-term data

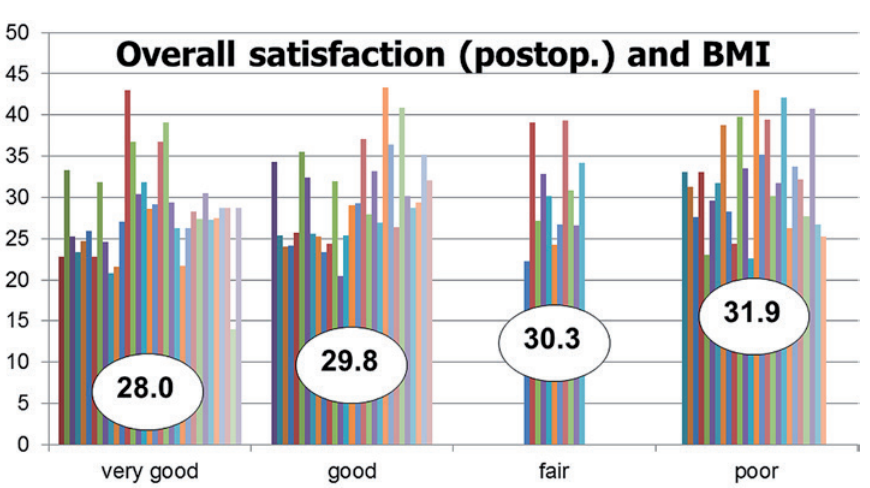
Figure 3 - A) Postoperative SUI is significant lower in patients with BMI <30 (p=0.006); B) Postoperative pad use is significant lower in patients with BMI <30 (p=0.004).



of implants have to compete with proven standard procedures. Jonsson et al. investigated long-term outcomes after different types of SUI surgeries. The Burch procedure had the lowest incidence of repeat SUI surgery (10.8%), followed by slings (13.0%), needle suspension (22.2%), and bulking agents (61.2%) (14). Novara et al. found similar results in a systematic review and meta-analysis (15). They reported higher objective continence rates using midurethral retropubic tapes compared to Burch colposuspension (odds ratio [OR]=0.38, p <0.0001), but with a high risk of bladder perforation (OR=4.94, p=0.00003). Midurethral tapes and pubovaginal slings showed similar results. Retropubic slings went along with slightly higher objective cure rates compared to transobturator tapes (TOT) (OR=0.8, p=0.04), but subjective cure rates were similar. Retropubic tapes had a higher risk of bladder perforation (OR=2.5, p <0.0001). With regard to the increasingly overweight population Subak et al. demonstrated a reduced frequency of self-reported urinary incontinence episodes in obese women after an intensive weight-loss program (16). Apparently the willingness for life style changes to reduce weight is commonly low, therefore treatment is requested.

Postoperative results of SUI surgery in obese patients are rated differently. The TOT procedure, for instance, is regarded as safe and successful. It avoids the retropubic space and causes fewer outflow

Figure 4 - The average BMI of the patients with a very good, good, fair, and poor result was 28.0, 29.8, 30.3, and 31.9, respectively. A clear trend in favor of a lower BMI is obvious.



obstructions (17). Frohme et al. did not find any influence of the BMI value on the outcome of TOT (18). Similarly Zivkovic et al. did not find any outcome differences between normal and overweight patients undergoing anterior colporrhaphy, anterior colporrhaphy with needle suspension of the bladder neck, and Burch colposuspension (19). Mukherjee et al. reported an equal effectiveness of TVT in obese women compared to lower BMI rates (20).

The current German interdisciplinary guidelines accept the use of single-incision slings due to the benefits of less invasiveness, less blood loss, and less postoperative pain compared to traditional slings. Complications with the blind TVT passage through the retropubic space (bladder, bowel, vascular injuries) can be avoided. Also injuries of the obturator nerve branches or groin pain caused by lesions of the adductor muscles by transobturator slings do not occur (15, 21-25).

In short term studies the outcome results of single incision slings are similar to retropubic or transobturator slings. Barber et al. found similar subjective cure rates after the prototype of mini-slings (TVT Secur, J&J Gynecare) or TVT after one year, (25) but this device failed to proof efficacy in later studies. In a meta-analysis by Abdel--Fattah et al. single-incision slings were inferior to standard midurethral slings concerning patient reported outcomes and objective cure rates and also showed higher reoperation rates (26). Similar results were reported by Djehdian et al., but noninferiority of the single-incision sling compared to a transobturator midurethral sling could not be demonstrated (27). These studies all include the learning-curve with SIS, longer term results considerably exceeding two years are still missing.

The adequate fixation by means of the anchoring systems is the crucial point of single--incision slings (28). Inferior results in the early studies can obviously be attributed to the immature technique of the TVT Secur that was not yet provided with a barbed hook (22). Such a barb was introduced in the Miniarc sling (AmericanMedicalSystems Inc.) that is designed to enter the obturator membrane, but not to fully perforate it. The adjustable single-incision sling system (AJUST™) provides a different anchoring system that is secured beyond the obturator membrane. Adjustment of the sling tension is made possible by a variable anchor after insertion. Midterm results after two years are promising and continence rates of 82.4% are comparable to TVT/TOT results, with fewer complications and significant improvements in quality of life indeces (23). In rare cases vaginal bleeding and erosion can occur (29, 30). Mostafa et al. described postoperative urinary retentions with a need for catheterization in 4.3% of patients (30). Meschia et al. mentioned 1/102 patients with postoperative urinary retention, resulting in tape resection (29). Naumann et al. described postoperative de novo urgency after AJUST™ insertion in 7.8% of patients. Only 1/52 patients complained of groin pain (23).

Our series demonstrates that the use of the adjustable single incision sling is particularly beneficial for obese patients as it avoids the passage through the retropubic and groin area. The design of the instrument allows a secure tape placement as obesity has usually no influence on vaginal anatomy. The minimal invasiveness allows the gentle treatment of elderly or frail elderly patients that are bothered by SUI. The crucial point is that both obese and older patients benefit from the adjustability of the tape. Only a careful and controlled degree of tension can make the difference for this 'risk group' of patients, especially when a low pressure urethra is present (Type III SUI) and the tension free principle has its limitations.

There is an ongoing debate if this condition has to be confirmed by urodynamic testing (UDS), but for straight forward SUI there is now consensus that UDS is not necessary as it has no impact on the outcome of surgery (31). Accordingly success rates in SUI studies are more defined by clinical parameters as mentioned above (32). In this context ample experience and a flair for the adequate tentioning is necessary, in particular with these apparently small procedures. The adjustment is supported by a 'stress test' with a filled bladder, an intraoperative cough test is mostly insufficient when the patient is under sedation and in a supine position. With an increasing degree of tension the risk of erosion or loosening of the anchors might also increase. Such complications did not occur in our series, but longer term studies should also focus on this issue.

In this regard we consider an objective cure rate of 84.6% and a subjective cure rate of 74% as reasonable in a group of the patients that might otherwise only be treated with absorbents. A 10% difference between objective and subjective cure rate is a well-known phenomenon in this field. Only a complete success meets the expectations of many patients despite all unfavorable conditions.

The retrospective design and the incomplete postoperative clinical examinations are the major limitations of the study. Nevertheless, this observational study obviously reflects real-life challenges of the health system with changing demographic parameters, in particular age and body weight. Ultimately, patient satisfaction is of paramount importance in SUI treatment.

CONCLUSIONS

In our single-institution, single-surgeon experience the success rate shows a clear trend in favor of a lower body-mass-index. The cut-off point has been identified at a BMI of 30. But even in obese and morbidly obese patients a considerable success rate is achievable with this quick and minimal invasive procedure. The procedure can be regarded as safe as no complications were observed in this elderly group of patients in part with multiple risk factors. The individual adjustability of the sling in order to achieve an adequate degree of tension to the urethra is the key factor in restoring continence in this selected cohort of elderly and overweight patients. Further prospective studies comparing different types of sling systems are necessary to define the best approach for elderly and overweight patients.

ABBREVIATIONS

BMI = Body Mass Index (kg/m^2)
SUI = Stress Urinary Incontinence
UK = United Kingdom
USA = United States of America
COPD = Chronic Obstructive Pulmonary Disease
WHO = World Health Organisation
ISD = Intrinsic Sphincter Deficiency
TVT = Tension free Vaginal Tape
SIS = Single Incision Sling
Fr. = French (1/3mm)
OR = Odds Ratio
TOT = Transobturator Tape
UDS = Urodynamics

CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Research prioritization of men's health and urologic diseases

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ABSTRACT

Objectives: We sought to determine whether disease representation in the Cochrane Database of Systematic Reviews (CDSR) reflects disease burden, measured by the Global Burden of Disease (GBD) Study as disability-adjusted life-years (DALYs).
Materials and Methods: Two investigators performed independent assessment of ten men's health and urologic diseases (MHUDs) in CDSR for systematic review and protocol representation, which were compared with percentage of total 2010 DALYs for the ten conditions. Data were analyzed for correlation using Spearman rank analysis.
Results: Nine of ten MHUDs were represented by at least one CDSR review. There was a poor and statistically insignificant positive correlation between CDSR representation and disease burden (rho = 0.42, p = 0.23). CDSR representation was aligned with disease burden for three conditions, greater than disease burden for one condition, and less than disease burden for six conditions.
Conclusions: These results yield high-quality estimates to inform future research prioritization for MHUDs. While prioritization processes are complex and multi-faceted, disease burden should be strongly considered. Awareness of research priority setting has the potential to minimize research disparities on a global scale.

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INTRODUCTION

In order to achieve effective clinical research, scarce research funds must be distributed to appropriate diseases in order to maximize health benefits to the represented population. Systematic approaches to inform research prioritization include identifying and prioritizing research questions, recognizing existing research, and setting goals for primary research (1, 2). A derivative of this approach is to value major diseases, injuries, and risk factors based on their burden to society (3). Spearheaded by the Institute for Health Metrics and Evaluation (IHME), the Global Burden of Disease (GBD) 2010 Study estimates the burden of 291 diseases and injuries across 187 countries from 1990 to 2010 (4, 5). The metric of disability--adjusted life years (DALYs), in which 1 DALY is

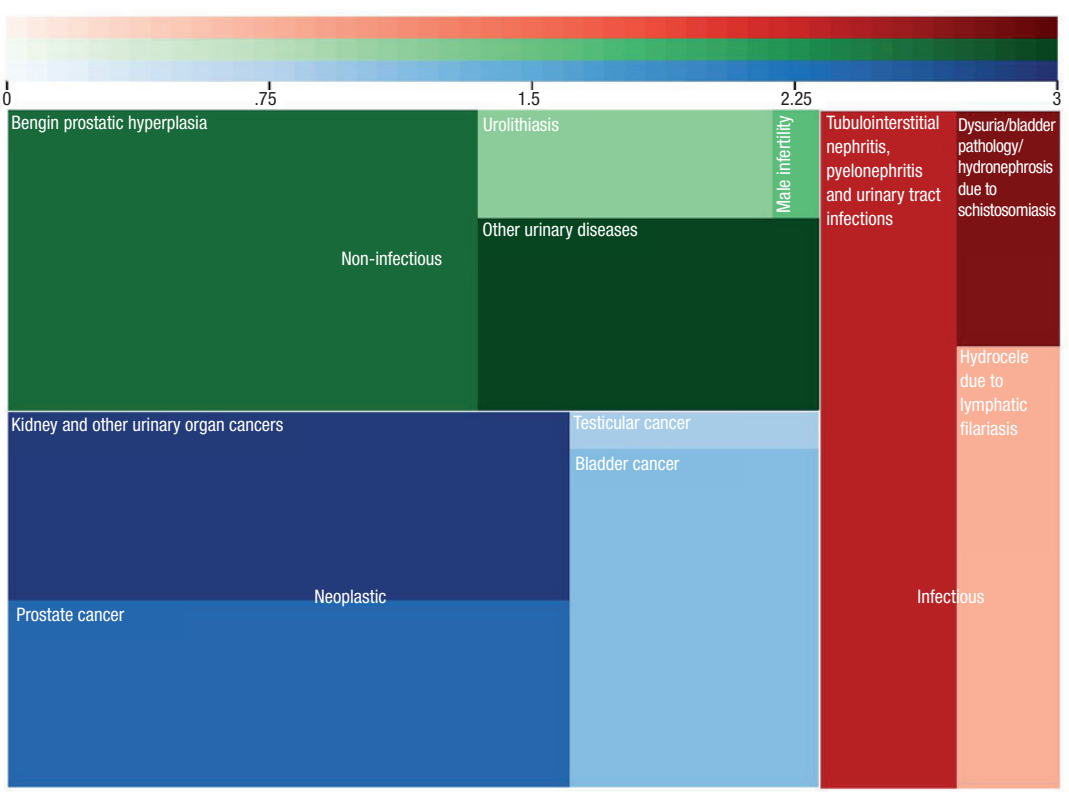
equivalent to 1 year of healthy life lost, allows for descriptive global epidemiology of a wide array of disease states. The following ten men's health and urologic diseases (MHUDs) were studied by GBD on the basis of prevalence, common case definitions, and data availability: tubulointerstitial nephritis, pyelonephritis, and urinary tract infections; kidney and other urinary organ cancers; urolithiasis; male infertility; benign prostatic hyperplasia; prostate cancer; testicular cancer; hydrocele due to lymphatic filariasis; dysuria/bladder pathology/hydronephrosis due to schistosomiasis; and bladder cancer (Figure-1).

Systematic reviews are the cornerstone of evidence-based medicine, yet few efforts have been made to assess whether the prioritization of systematic reviews reflect global disease burden (6). The Cochrane Database of Systematic Reviews (CDSR) produces systematic reviews and protocols (published proposals for future systematic reviews) across all medical specialties as well as health systems, public health, and child development. Cochrane systematic reviews undergo exhaustive editorial processing, are more methodologically rigorous, and are updated more frequently than non-Cochrane reviews and paper-based journals (7, 8). Prior studies have evaluated the association between broad categories of disease burden with randomized trials and Cochrane systematic reviews (9-12). This study will assess whether the CDSR representation of ten MHUDs corresponds to GBD 2010 disability estimates.

MATERIALS AND METHODS

ICD-10 code definitions for the ten MHUDs have been previously published and were used to generate search terms, which were entered into the "title, abstract, keywords" CDSR search function (5, 13). Systematic reviews and protocols were

Figure 1 - Square pie chart representing percent of total DALY for ten men's health and urologic diseases; area of each square/rectangle represents percentage of total burden.



green = non-infectious conditions, **red** = infectious conditions, and **blue** = neoplastic conditions

considered to determine MHUD representation in CDSR, according to abstract subject content. Online publication date, the number of studies included in each systematic review, and the particular Cochrane review group that published the review or protocol were collected.

Two authors (T.O and H.P.) collected data independently during February 2015. DALY metrics for each of the ten MHUDs, expressed as percentages of total DALY's of all 291 conditions measured in GBD 2010, were obtained from the GBD Compare interactive time plot, available at <http://viz.healthmetricsandevaluation.org/gbd-compare/>. Spearman rank correlation analysis was performed to assess statistical dependence between CDSR representation and disease burden. Rho, a coefficient ranging from-1 (strong negative correlation) to +1 (strong positive correlation), is interpreted with a two-tailed p-value. A line-of-best-fit was also generated between CDSR representation and % of total DALYs.

As this study did not involve human subjects, institutional review board approval was not necessary.

RESULTS

Nine of the ten MHUD conditions studied in GBD 2010 were represented by at least one systematic review. A total of 116 systematic reviews and protocols published by nine Cochrane review groups represented the ten MHUDs (Supporting Tables 1 and 2 for included and excluded titles, respectively). The majority of reviews and protocols covered tubulointerstitial nephritis, pyelonephritis, and urinary tract infections (n=46). Hydrocele due to lymphatic filariasis had no representation in CDSR. Of the ten MHUDs, benign prostatic hyperplasia had the greatest global disease burden (0.2%) while male infertility had the lowest (0.007%) Table-1.

Reviews and protocols representing the ten MHUDs were published by the following Cochrane review groups: Prostatic Diseases and Urologic Cancers Group (n=43); Renal Group (36); Incontinence Group (14); Menstrual Disorders and Subfertility Group (8); Pregnancy and Childbirth Group (7); Infectious Diseases Group (3); Pain, Palliative and Supportive Care Group (2); Gynecological Cancer Group (2); and Neonatal Group (1).

Spearman rank correlation testing between CDSR representation and DALY metrics revealed poor positive correlation that was statistically insignificant (rho=0.41, p=0.21). The majority of the MHUDs (6) were under-represented in CDSR as compared to GBD DALY (Figure-2). Most of the systematic reviews and protocols (58.6%) were published from 2011 to 2015 while 37.9% were published from 2000 to 2010; only 4 reviews were published prior to 2000. Maintaining systematic reviews up-to-date is critical to deliver consensus statements on current world literature that ultimately impact clinical decisions and patient outcomes.

Representation of tubulointerstitial nephritis, pyelonephritis, and urinary tract infections exceeded GBD disease burden. This disease category also had the greatest number of cumulative studies informing its systematic reviews (529). The one systematic review representing testicular cancer, entitled "Screening for testicular cancer," found no randomized controlled trials in the literature. Systematic reviews that find no suitable trials to address their objectives uncover areas for much-needed, high-quality research.

The World Health Organization (WHO) classifies two of the MHUDs as neglected tropical diseases: dysuria/bladder pathology/hydronephrosis due to schistosomiasis and hydrocele due to lymphatic filariasis (14). It is important to note that just as the DALY metrics reported for these two diseases include only burden due to the MHUD morbidity (dysuria, bladder pathology, hydronephrosis, hydrocele), systematic reviews were only considered representative if they included assessment of the MHUD pathology.

DISCUSSION

We acknowledge several limitations of our study. The scope of CDSR systematic reviews is subject to variability. For instance, authors may prepare one large review of multiple interventions (lumping) or several reviews of individual interventions (splitting). Therefore, treating a systematic review or protocol as one measurement unit may not be entirely accurate for every topic.

Supporting Table 1

Injury or Trauma	Review (R) or Protocol (P) Title	Cochrane Group	Number of Studies	Year of Online Publication
Tubulo-interstitial nephritis, pyelonephritis, and urinary tract infections	Antibiotics for acute pyelonephritis in children (R)	Renal Group	27	2007
	Antibiotics for asymptomatic bacteriuria in pregnancy (R)	Pregnancy and Childbirth Group	14	2007
	Treatments for symptomatic urinary tract infections during pregnancy (R)	Pregnancy and Childbirth Group	10	2011
	Duration of treatment for asymptomatic bacteriuria during pregnancy (R)	Pregnancy and Childbirth Group	13	2011
	Routine blood cultures in the management of pyelonephritis in pregnancy for improving outcomes (R)	Renal Group	0	2015
	Procalcitonin , C-reactive protein , and erythrocyte sedimentation rate for the diagnosis of acute pyelonephritis in children (R)	Renal Group	17	2015
	Duration of antibacterial treatment for uncomplicated urinary tract infection in women (R)	Renal Group	32	2005
	Interventions for preventing recurrent urinary tract infection during pregnancy (R)	Pregnancy and Childbirth Group	1	2012
	Long-term antibiotics for preventing recurrent urinary tract infection in children (R)	Renal Group	12	2011
	Treatments for symptomatic urinary tract infections during pregnancy (R)	Pregnancy and Childbirth Group	10	2011
	Prophylactic antibiotics to reduce the risk of urinary tract infections after urodynamic studies (R)	Incontinence Group	9	2012
	Types of indwelling urethral catheters for short-term catheterization in hospitalized adults (R)	Incontinence Group	26	2014
	Antibiotics for treating lower urinary tract infection in children (R)	Renal Group	16	2012
	Methenamine hippurate for preventing urinary tract infections (R)	Renal Group	13	2012
	Estrogens for preventing recurrent urinary tract infection in postmenopausal women (R)	Renal Group	9	2008
	Short versus standard duration oral antibiotic therapy for acute urinary tract infection in children (R)	Renal Group	10	2003
	Urinary catheter policies for long-term bladder drainage (R)	Incontinence Group	8	2012
	Antimicrobial agents for treating uncomplicated urinary tract infection in women (R)	Renal Group	21	2010
	Modes of administration of antibiotics for symptomatic severe urinary tract infections (R)	Renal Group	15	2007

Antibiotic duration for treating uncomplicated , symptomatic lower urinary tract infections in elderly women (R)	Renal Group	15	2008
Antibiotic prophylaxis for short-term catheter bladder drainage in adults (R)	Incontinence Group	6	2013
Cranberries for treating urinary tract infections (R)	Renal Group	0	1998
Antibiotics for preventing recurrent urinary tract infection in non-pregnant women (R)	Renal Group	19	2004
Cranberries for preventing urinary tract infections (R)	Renal Group	24	2012
Routine neonatal circumcision for the prevention of urinary tract infections in infancy (R)	Neonatal Group	0	2012
Urinary catheter policies for short-term bladder drainage in adults (R)	Incontinence Group	17	2005
Intermittent catheterization for long-term bladder management (R)	Incontinence Group	31	2014
Antibiotic prophylaxis for transrectal prostate biopsy (R)	Prostatic Diseases and Urologic Cancers Group	19	2011
Short term urinary catheter policies following urogenital surgery in adults (R)	Incontinence Group	39	2006
Washout policies in long-term indwelling urinary catheterization in adults (R)	Incontinence Group	5	2010
Routine intraoperative ureteric stenting for kidney transplant recipients (R)	Renal Group	7	2013
Interventions for primary vesicoureteric reflux (R)	Renal Group	20	2011
Types of indwelling urinary catheters for long-term bladder drainage in adults (R)	Incontinence Group	3	2012
Laser prostatectomy for benign prostatic obstruction (R)	Prostatic Diseases and Urologic Cancers Group	20	2000
Quinolones for uncomplicated acute cystitis in women (R)	Renal Group	11	2006
Drugs for treatment of urinary retention after surgery in adults (R)	Incontinence Group	7	2010
Indwelling bladder catheterization as part of intraoperative and postoperative care for caesarean section (R)	Pregnancy and Childbirth Group	5	2014
Dietary interventions for preventing complications in idiopathic hypercalciuria (R)	Renal Group	5	2014
Interventions for covert bacteriuria in children (R)	Renal Group	3	2012
Pharmacological interventions for preventing complications in idiopathic hypercalciuria (R)	Renal Group	5	2009
Different antibiotic regimens for treating asymptomatic bacteriuria in pregnancy (R)	Pregnancy and Childbirth Group	5	2010
Urinary alkalization for uncomplicated urinary tract infection (P)	Renal Group	N/A	2013

	Dimercaptosuccinic acid scan versus ultrasound in screening for vesicoureteral reflux among children with urinary tract infections (P)	Renal Group	N/A	2013
	Chinese herbal medicine for treating recurrent urinary tract infections in women (P)	Renal Group	N/A	2013
	Probiotics for preventing urinary tract infection in people with neuropathic bladder (P)	Renal Group	N/A	2013
	Probiotics for preventing urinary tract infections in adults and children (P)	Renal Group	N/A	2010
Kidney and other urinary organ cancers	Targeted therapy for advanced renal cell carcinoma (R)	Prostatic Diseases and Urologic Cancers Group	25	2008
	Immunotherapy for advanced renal cell cancer (R)	Prostatic Diseases and Urologic Cancers Group	37	2004
	Surgical management of localized renal cell carcinoma (R)	Prostatic Diseases and Urologic Cancers Group	0	2010
	Surgical management for upper urinary tract transitional cell carcinoma (R)	Prostatic Diseases and Urologic Cancers Group	1	2011
Urolithiasis	Extracorporeal shock wave lithotripsy (ESWL) versus percutaneous nephrolithotomy (PCNL) or retrograde intrarenal surgery (RIRS) for kidney stones (R)	Renal Group	5	2014
	Dietary interventions for preventing complications in idiopathic hypercalciuria (R)	Renal Group	5	2014
	Extracorporeal shock wave lithotripsy (ESWL) versus ureteroscopic management for ureteric calculi (R)	Renal Group	7	2012
	Pharmacological interventions for preventing complications in idiopathic hypercalciuria (R)	Renal Group	5	2009
	Fluids and diuretics for acute ureteric colic (R)	Renal Group	2	2012
	Water for preventing urinary stones (R)	Renal Group	1	2012
	Alpha-blockers as medical expulsive therapy for ureteral stones (R)	Renal Group	32	2014
	Percussion , diuresis , and inversion therapy for the passage of lower pole kidney stones following shock wave lithotripsy (R)	Renal Group	2	2013
	Analgesia for patients undergoing shockwave lithotripsy for urinary stones (P)	Renal Group	N/A	2012
	Interventions for treating urinary stones in children (P)	Renal Group	N/A	2013
Male infertility	Intra-uterine insemination for male subfertility (R)	Menstrual Disorders and Subfertility Group	8	2007
	Antioxidants for male subfertility (R)	Menstrual Disorders and Subfertility Group	48	2014
	Surgery or embolization for varicoceles in subfertile men (R)	Menstrual Disorders and Subfertility Group	10	2012
	Regular (ICSI) versus ultra-high magnification (IMSI) sperm selection for assisted reproduction (R)	Menstrual Disorders and Subfertility Group	9	2013

	Intra-uterine insemination for unexplained subfertility (R)	Menstrual Disorders and Subfertility Group	8	2012
	Techniques for surgical retrieval of sperm prior to intra-cytoplasmic sperm injection (ICSI) for azoospermia (R)	Menstrual Disorders and Subfertility Group	2	2008
	Cervical insemination versus intra-uterine insemination of donor sperm for subfertility (R)	Menstrual Disorders and Subfertility Group	4	2008
	Gonadotrophins for idiopathic male factor subfertility (R)	Menstrual Disorders and Subfertility Group	6	2013
Benign prostatic hyperplasia	*Pygeum africanum* for benign prostatic hyperplasia (R)	Prostatic Diseases and Urologic Cancers Group	18	1998
	Finasteride for benign prostatic hyperplasia (R)	Prostatic Diseases and Urologic Cancers Group	23	2010
	Beta-sitosterols for benign prostatic hyperplasia (R)	Prostatic Diseases and Urologic Cancers Group	4	1999
	Serenoa repens for benign prostatic hyperplasia (R)	Prostatic Diseases and Urologic Cancers Group	32	2012
	Naftopidil for the treatment of lower urinary tract symptoms compatible with benign prostatic hyperplasia (R)	Prostatic Diseases and Urologic Cancers Group		
	Microwave thermotherapy for benign prostatic hyperplasia (R)	Prostatic Diseases and Urologic Cancers Group	15	2012
	Phosphodiesterase inhibitors for lower urinary tract symptoms consistent with benign prostatic hyperplasia (P)	Prostatic Diseases and Urologic Cancers Group	N/A	2012
	Laser prostatectomy for benign prostatic obstruction (R)	Prostatic Diseases and Urologic Cancers Group	20	2000
	Bipolar versus monopolar transurethral resection of the prostate for lower urinary tract symptoms secondary to benign prostatic obstruction (P)	Prostatic Diseases and Urologic Cancers Group	N/A	2014
Prostate cancer	5-alpha-reductase inhibitors for prostate cancer prevention (R)	Prostatic Diseases and Urologic Cancers Group	9	2008
	Screening for prostate cancer (R)	Prostatic Diseases and Urologic Cancers Group	5	2013
	Lycopene for the prevention of prostate cancer (R)	Prostatic Diseases and Urologic Cancers Group	3	2011
	Psychosocial interventions for men with prostate cancer (R)	Prostatic Diseases and Urologic Cancers Group	19	2013
	Radical prostatectomy versus watchful waiting for prostate cancer (R)	Prostatic Diseases and Urologic Cancers Group	2	2010
	Bisphosphonates for advanced prostate cancer (R)	Prostatic Diseases and Urologic Cancers Group	10	2006
	Cryotherapy for localized prostate cancer (R)	Prostatic Diseases and Urologic Cancers Group	8	2007

	Neo- adjuvant and adjuvant hormone therapy for localized and locally advanced prostate cancer (R)	Prostatic Diseases and Urologic Cancers Group	21	2006
	Early versus deferred androgen suppression in the treatment of advanced prostatic cancer (R)	Prostatic Diseases and Urologic Cancers Group	4	2001
	Chemotherapy for hormone-refractory prostate cancer (R)	Prostatic Diseases and Urologic Cancers Group	47	2006
	Maximal androgen blockade for advanced prostate cancer (R)	Prostatic Diseases and Urologic Cancers Group	20	1999
	Adjuvant radiotherapy following radical prostatectomy for prostate cancer (R)	Prostatic Diseases and Urologic Cancers Group	3	2011
	Intermittent versus continuous androgen suppression for prostatic cancer (R)	Prostatic Diseases and Urologic Cancers Group	5	2007
	Low-dose rate brachytherapy for men with localized prostate cancer (R)	Prostatic Diseases and Urologic Cancers Group	1	2011
	Interventions for sexual dysfunction following treatments for cancer (R)	Pain, Palliative and Supportive Care Group	11	2007
	Selenium for preventing cancer (R)	Gynaecological Cancer Group	55	2014
	Green tea *(Camellia sinensis)* for the prevention of cancer (R)	Gynaecological Cancer Group	51	2009
	Exercise for the management of cancer-related fatigue in adults (R)	Pain, Palliative and Supportive Care Group	56	2012
	Laparoscopic versus open prostatectomy for the treatment of localized prostate cancer (P)	Prostatic Diseases and Urologic Cancers Group	N/A	2012
	Non-steroidal antiandrogen monotherapy compared with luteinizing hormone –releasing hormone agonists or surgical castration monotherapy for advanced prostate cancer (R)	Prostatic Diseases and Urologic Cancers Group	11	2014
	Conservative management for postprostatectomy urinary incontinence (R)	Incontinence Group	50	2015
	Surgery for stress urinary incontinence due to presumed sphincter deficiency after prostate surgery (R)	Incontinence Group	1	2014
	Antibiotic prophylaxis for transrectal prostate biopsy (R)	Prostatic Diseases and Urologic Cancers Group	19	2011
Testicular cancer	Screening for testicular cancer (R)	Prostatic Diseases and Urologic Cancers Group	0	2011
Bladder cancer	Intravesical gemcitabine for non-muscle invasive bladder cancer (R)	Prostatic Diseases and Urologic Cancers Group	6	2012
	Intravesical Bacillus Calmette-Guérin versus epirubicin for Ta and T1 bladder cancer (R)	Prostatic Diseases and Urologic Cancers Group	5	2011
	Gemcitabine for unresectable , locally advanced or metastatic bladder cancer (R)	Prostatic Diseases and Urologic Cancers Group	6	2011
	Intravesical Bacillus Calmette-Guérin in Ta and T1 bladder cancer (R)	Prostatic Diseases and Urologic Cancers Group	6	2000

	Neo-adjuvant chemotherapy for invasive bladder cancer (R)	Prostatic Diseases and Urologic Cancers Group	11	2004
	Intravesical Bacillus Calmette-Guérin versus mitomycin C for Ta and T1 bladder cancer (R)	Prostatic Diseases and Urologic Cancers Group	7	2003
	Surgery versus radiotherapy for muscle invasive bladder cancer (R)	Prostatic Diseases and Urologic Cancers Group	3	2001
	Adjuvant chemotherapy for invasive bladder cancer (individual patient data) (R)	Prostatic Diseases and Urologic Cancers Group	6	2006
	Green tea (*Camellia sinensis*) for the prevention of cancer (R)	Gyanecological Cancer Group	51	2009
	Perioperative nutrition for the treatment of bladder cancer by radical cystectomy (P)	Prostatic Diseases and Urologic Cancers Group	N/A	2012
	Urinary diversion and bladder reconstruction / replacement using intestinal segments for intractable incontinence or following cystectomy (R)	Incontinence Group	5	2012
Hydrocele due to lymphatic filariasis				
Dysuria/bladder pathology/ hydronephrosis due to schistosomiasis	Drugs for treating urinary **schistosomiasis** (R)	Infectious Diseases Group	30	2014
	Therapeutic and prophylactic drug interventions for **Schistosomiasis** japonicum (P)	Infectious Diseases Group	N/A	2012
	Rapid screening and diagnostic tests for human **schistosomiasis** in endemic areas (P)	Infectious Diseases Group	N/A	2012
	Urinary diversion and bladder reconstruction/replacement using intestinal segments for intractable incontinence or following cystectomy (R)	Incontinence Group	5	2012

Supporting Table 2:

Condition	Review (R) or Protocol (P)
Tubulo-interstitial nephritis, pyelonephritis, and urinary tract infections	Interventions to improve professional adherence to guidelines for prevention of device-related infections (R)
	Mechanical dilatation of the cervix at non-labour caesarean section for reducing postoperative morbidity (R)
	Antibiotic prophylaxis versus no prophylaxis for preventing infection after cesarean section (R)
	Traditional suburethral sling operations for urinary incontinence in women (R)
	Antibiotic prophylaxis for surgery for proximal femoral and other closed long bone fractures (R)
	Mupirocin ointment for preventing Staphylococcus aureus infections in nasal carriers (R)
	Rituximab for relapsing-remitting multiple sclerosis (R)
	Beta lactam antibiotic monotherapy versus beta lactam -aminoglycoside antibiotic combination therapy for sepsis (R)
	Habit retraining for the management of urinary incontinence in adults (R)
	Preoperative skin antiseptics for preventing surgical wound infections after clean surgery (R)
	Antibiotic prophylaxis for cirrhotic patients with upper gastrointestinal bleeding (R)
	Antimicrobial therapy for chronic bacterial prostatitis (R)
	Cyanoacrylate microbial sealants for skin preparation prior to surgery (R)
	Perioperative increase in global blood flow to explicit defined goals and outcomes following surgery (R)
	Interventions to improve antibiotic prescribing practices in ambulatory care (R)
	Teriflunomide for multiple sclerosis (R)
	Laquinimod for multiple sclerosis (R)
	Mitoxantrone for multiple sclerosis (R)
	Efficacy and safety of cesarean delivery for prevention of mother-to-child transmission of HIV-1 (R)
	Surgical approach to hysterectomy for benign gynecological disease (R)
	Interventions for preventing mastitis after childbirth (R)
	Laparoscopy versus laparotomy for benign ovarian tumor (R)
	Valproate preparations for agitation in dementia (R)
	Regional versus general anesthesia for caesarean section (R)
	Interventions for promoting the initiation of breastfeeding (R)
	Protocolized versus non- protocolized weaning for reducing the duration of mechanical ventilation in critically ill adult patients (R)
	In-hospital care pathways for stroke (R)
	Schedules for home visits in the early postpartum period (R)
	Short term benefits for laparoscopic colorectal resection (R)
	Diaphragm versus diaphragm with spermicides for contraception (R)
	Cervical cap versus diaphragm for contraception (R)
Kidney and other urinary organ cancers	Early and late renal adverse effects after potentially nephrotoxic treatment for childhood cancer (R)
	Urate oxidase for the prevention and treatment of tumor lysis syndrome in children with cancer (R)
	Bisphosphonates and other bone agents for breast cancer (R)
	Interventions for preventing non-melanoma skin cancers in high-risk groups (R)
	Perioperative blood transfusions and recurrence of colorectal cancer (R)
	Medical interventions for the prevention of platinum-induced hearing loss in children with cancer (R)

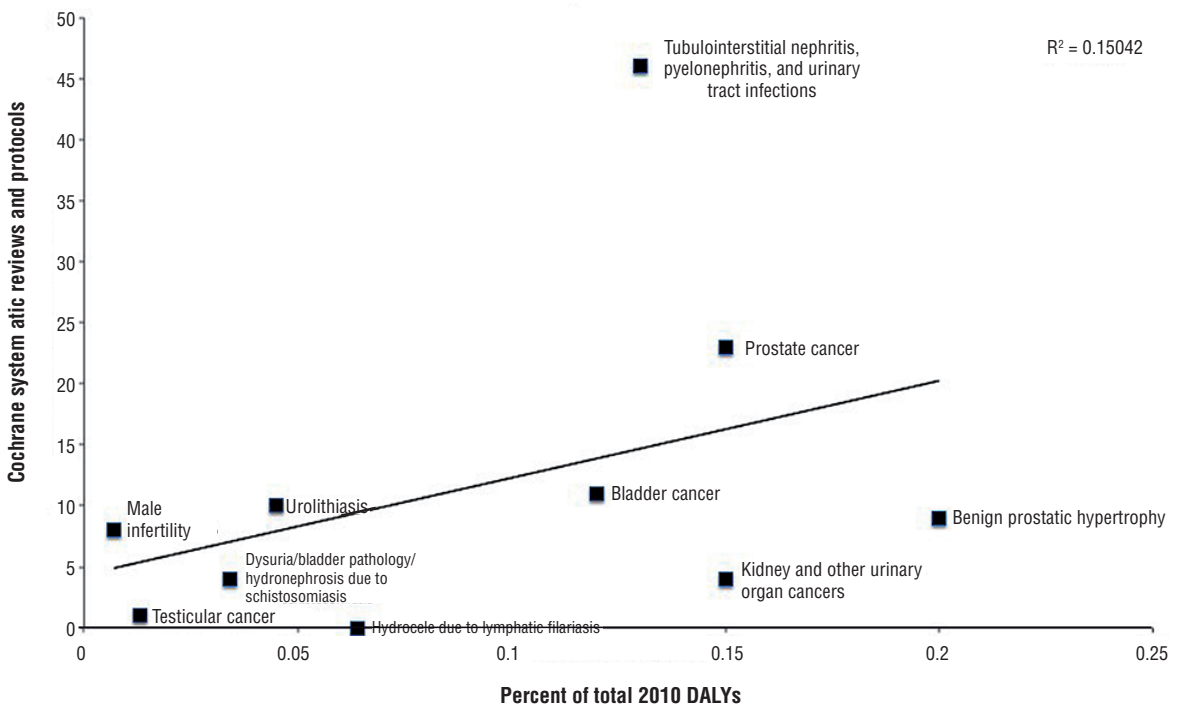
	Cisplatin versus carboplatin in combination with third-generation drugs for advanced non-small cell lung cancer (R)
	Immunosuppressive T-cell antibody induction for heart transplant recipients (R)
	Concomitant hydroxyurea plus radiotherapy versus radiotherapy for carcinoma of the uterine cervix (R)
	Amphotericin B versus fluconazole for controlling fungal infections in neutropenic cancer patients (R)
	HMG CoA reductase inhibitors (statins) for dialysis patients (R)
	Urinary diversion and bladder reconstruction /replacement using intestinal segments for intractable incontinence or following cystectomy (R)
	Pharmacological interventions for pruritus in adult palliative care patients (R)
	Homocysteine-lowering interventions for preventing cardiovascular events (R)
	HMG CoA reductase inhibitors (statins) for kidney transplant recipients (R)
	Vitamin D supplementation for prevention of mortality in adults (R)
	Vitamin D and vitamin D analogues for preventing fractures in post-menopausal women and older men (R)
	Antibody induction therapy for lung transplant recipients (R)
	First-line tandem high-dose chemotherapy and autologous stem cell transplantation versus single high-dose chemotherapy and autologous stem cell transplantation in multiple myeloma , a systematic review of controlled studies (R)
	Antibiotic prophylaxis for preventing post solid organ transplant tuberculosis (R)
	Tacrolimus versus cyclosporin as primary immunosuppression for lung transplant recipients (R)
	Bisphosphonates in multiple myeloma: a network meta-analysis (R)
	Darbepoetin for the anemia of chronic kidney disease (R)
	Selenium for preventing cancer (R)
	Hyperbaric oxygenation for tumor sensitization to radiotherapy (R)
	Adjuvant radiotherapy following radical prostatectomy for prostate cancer (R)
	Exenterative surgery for recurrent gynecological malignancies (R)
	Laparoscopically assisted radical vaginal hysterectomy versus radical abdominal hysterectomy for the treatment of early cervical cancer (R)
	Laparoscopy versus laparotomy for the management of early stage endometrial cancer (R)
	Laparoscopic versus open total mesorectal excision for rectal cancer (R)
	Adjuvant radiotherapy for stage I endometrial cancer (R)
	Cryotherapy for localized prostate cancer (R)
	Spinal cord stimulation for cancer -related pain in adults (R)
	Chemoradiation for advanced primary vulval cancer (R)
	High dose rate versus low dose rate intracavity brachytherapy for locally advanced uterine cervix cancer (R)
	Surgical management for upper urinary tract transitional cell carcinoma (R)
	Cholecystectomy for patients with silent gallstones (R)
	Drugs for treating urinary schistosomiasis (R)
	Non-surgical interventions for late radiation cystitis in patients who have received radical radiotherapy to the pelvis (R)
	Cranberries for preventing urinary tract infections (R)
	Radioiodine therapy for differentiated thyroid carcinoma with thyroglobulin positive and radioactive iodine negative metastases (R)
	Conservative management for postprostatectomy urinary incontinence (R)
	Surgery for stress urinary incontinence due to presumed sphincter deficiency after prostate surgery (R)
	The role of alpha blockers prior to removal of urethral catheter for acute urinary retention in men (R)
Urolithiasis	Pegloticase for chronic gout (R)

	Fluids and diuretics for acute ureteric colic (R)
	Vitamin D supplementation for prevention of mortality in adults (R)
	Chinese medicinal herbs for cholelithiasis (R)
	Thyroid hormones for acute kidney injury (R)
	Vitamin D and vitamin D analogues for preventing fractures in post-menopausal women and older men (R)
Male infertility	Intra-cytoplasmic sperm injection versus conventional techniques for oocyte insemination during in vitro fertilisation in couples with non-male subfertility (R)
	Oral 5-aminosalicylic acid for induction of remission in ulcerative colitis (R)
	Oral 5-aminosalicylic acid for maintenance of remission in ulcerative colitis (R)
	Intrauterine insemination versus fallopian tube sperm perfusion for non-tubal infertility (R)
	Vasectomy occlusion techniques for male sterilization (R)
	Steroid hormones for contraception in men (R)
Benign prostatic hyperplasia	Interventions for chronic abacterial prostatitis (R)
Prostate cancer	Interventions for improving the adoption of shared decision making by healthcare professionals (R)
Testicular cancer	Chemotherapy for malignant germ cell ovarian cancer in adult patients with early stage, advanced and recurrent disease (R)
Bladder cancer	Cholecystectomy for patients with silent gallstones (R)
	Hyperbaric oxygenation for tumor sensitization to radiotherapy (R)
	Drugs for treating urinary schistosomiasis (R)
	High dose rate versus low dose rate intracavity brachytherapy for locally advanced uterine cervix cancer (R)
	Adjuvant radiotherapy for stage I endometrial cancer (R)
	Laparoscopy versus laparotomy for the management of early stage endometrial cancer (R)
	Selenium for preventing cancer (R)
	Radioiodine therapy for differentiated thyroid carcinoma with thyroglobulin positive and radioactive iodine negative metastases (R)
	Chemoradiation for advanced primary vulval cancer (R)
	Laparoscopic versus open total mesorectal excision for rectal cancer (R)
	Spinal cord stimulation for cancer-related pain in adults (R)
Hydrocele due to lymphatic filariasis	Albendazole for lymphatic filariasis (R)
	Diethylcarbamazine (DEC)-medicated salt for community-based control of lymphatic filariasis (R)
Dysuria/bladder pathology/ hydronephrosis due to schistosomiasis	Metrifonate for Alzheimer's disease (R)
	Drugs for treating *Schistosoma mansoni* infection (R)

Table 1 - Men's health and urologic diseases studied by GBD 2010 with corresponding ICD-10 codes, search terms, number of systematic reviews (R) and protocols (P) in CDSR, percent of total DALYs (arranged in order of decreasing % of total DALY), and number of studies included in Cochrane reviews.

Condition	ICD-10 code	Search terms	Number of cochrane reviews (R) & protocols (P)	% total 210 dalys (out of 291 conditions)	Number of studies in cochrane review
Benign prostatic hyperplasia	N40	"benign prostatic hyperplasia" "median bar" "prostatic hyperplasia" "adenofibromatous hypertrophy of prostrate" "hypertrophy of prostate "prostatic obstruction"	9 (7 R, 2 P)	0.2%	35
Prostate cancer	C61, D07.5, D40.0	"prostate cancer" "prostatic carcinoma in situ" "prostate neoplasm"	23 (22 R, 1 P)	0.15%	411
Kidney and other urinary organ cancers	C64-C66, D41.0-D41.2	"kidney cancer" "neoplasm of the ureter" "neoplasm of the kidney" "neoplasm of the renal pelvis"	4 (4 R)	0.15%	63
Tubulointerstitial nephritis, pyelonephritis, and urinary tract infections	N10-N12, N15.1-N15.9, N30, N34, N39.0	"tubulointerstitial nephritis" "pyelonephritis" "urinary tract infections" "infectious interstitial nephritis" "pyelitis" "balkan nephropathy" "renal and perinephric abscess" "cystitis" "trigonitis" "urethral abscess" "urethritis"	46 (41 R, 5 P)	0.13%	529
Bladder cancer	C67, D09.0*, D41.4	"bladder cancer" "bladder carcinoma" "bladder neoplasm"	11 (10 R, 1 P)	0.12%	106
Hydrocele due to lymphatic filariasis	B74 (except B74.3, B74.4, B74.8, B74.9)	"lymphatic filariasis" [hydrocele]	0	0.064%	0
Urolithiasis	N20-N23	"urolithiasis" "urinary stones" "nephrolithiasis" "kidney stones" "ureterolithiasis" "cystolithiasis" "bladder stones"	10 (8 R, 2 P)	0.045%	59
Dysuria/bladder pathology/ hydronephrosis due to schistosomiasis	B65	"schistosomiasis" [dysuria, bladder, hydronephrosis]	4 (2 R, 2 P)	0.034%	35
Testicular cancer	C62, D40.1	"testicular cancer" "malignant neoplasm of testis"	1 R	0.013%	0
Male infertility	N46	"azoospermia" "oligospermia"	8 R	0.007	95

Figure 2 - Comparison of men's health and urologic disease representation in the Cochrane Database of Systematic Reviews with percent of 2010 DALYs from 291 conditions studied by GBD 2010.



While beyond the scope of this limited study, further exploration is warranted into potential underrepresentation of certain conditions.

There remains a lack of transparency in publications and databases on the quality of data and criteria involved in prioritization decisions (15). Other important factors in priority setting include availability of research funds, knowledge gap, and impact on disadvantaged populations. Research prioritization is also inherently political and dependent on financial backing, which further demonstrates the importance of a transparent process. Attention and awareness of priority setting has the potential to minimize research disparities and, ultimately, impact populations at a global scale.

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CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Assessment of satisfaction and Quality of Life using self – reported questionnaires after urethroplasty: a prospective analysis

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ABSTRACT

Objectives: To assess patient satisfaction and quality of life after urethroplasty using two different self-reported outcome measures and to compare it with objective clinical data.

Materials and Methods: We prospectively collected data from 35 consecutive patients who underwent urethroplasty from January 2013 to September 2014. Patient demographics, International Prostate Symptom Score (IPSS), quality of life score, urethral stricture surgery patient-reported outcome measure (USS-PROM), maximum flow rate (Qmax) and post-void residual urine were collected before, two and eight months after surgery. Failure occurred when any postoperative instrumentation was performed. General estimation equation was used to compare the results and linear regression analysis to correlate both questionnaires with objective data.

Results: Mean age was 61 years. Urethroplasties were equally divided between anastomotic and buccal mucosa grafts and 19 patients (59.3%) had a previous urethral procedure. Overall success rate was 87.5%. IPSS improved from a mean 19 at baseline to 5.32 at 8 months ($p < 0.001$). The mean USS-PROM score also improved from 13.21 preoperatively to 3.36 after surgery ($p < 0.001$) and 84.3% of patients were satisfied or very satisfied with surgical results. Mean Qmax increased from 4.64mL/s to 11mL/s ($p < 0.001$). Strong negative correlation was found respectively between flow rate and USS-PROM ($r=-0.531$, $p < 0.001$) and with IPSS ($r=-0.512$, $p < 0.001$).

Conclusions: Significant improvements in urinary symptoms and in quality of life are expected after urethroplasty and they are correlated with objective measures.

ARTICLE INFO

Keywords:
Urethral Stricture; Surveys and Questionnaires; Quality of Life; Cost-Benefit Analysis

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INTRODUCTION

Urethral strictures are a high complexity disease that impacts on quality of life (QoL) with an increasing reported incidence in elderly population (1). The aim of any intervention is to restore patient's normal pattern of voiding while maintaining a good QoL (2). Urethroplasty is considered the gold standard for the management of urethral stricture disease with excellent and durable successful reported rates (3, 4). It has already been shown that urethroplasty is the most cost-effectiveness strategy compared to minimally invasive procedures in the treatment of urethral stricture disease (5, 6).

The definition of what constitutes an urethroplasty success varies widely in the literature

as well as the methods and frequency used to follow-up these patients. This lack of standardization makes comparisons between different studies difficult (7). Strategies of surveillance can range from subjective symptoms questionnaires to more invasive testing such cystoscopy and cystourethrography, that impact in the costs and the risks involved in this process. Despite reports that voiding symptoms have a good accuracy in predicting stricture recurrence, most outcome measures are clinician-driven indicators of technical success (8). Little has been published using patient-perceived symptoms and QoL outcomes after urethral reconstruction, although the recent development of a urethral stricture surgery patient - reported outcome measure (USS-PROM) is gaining considerable importance in the evaluation of patients' perception of surgical success (9-11). The purpose of the present study was to prospectively analyze the pre- and post-operative patient-reported outcomes measures describing patient's satisfaction and QoL after open urethral reconstruction and to compare these results with objective data.

MATERIALS AND METHODS

We prospectively collected data from a cohort of consecutive patients older than 18 years old, Portuguese speakers who underwent urethroplasty from January 2013 to September 2014. The study was approved by the institutional review board of our hospital. Patients were excluded if they had not undergone formal reconstruction (i.e. perineal urethrostomy). Patients with previous open irreversible reconstruction, with evident cognitive impairment, or who refused to participate were also excluded. Patients lost to follow-up were excluded from analysis.

Preoperative evaluation included history taking for demographic characteristics. Stricture etiology, location and extension of the stenosis and previous treatments were collected. Retrograde and voiding cystourethrography were done preoperatively in all subjects to assess stricture length and site. If the patient had a suprapubic catheter for urinary diversion an anterograde cystourethrography was performed concomitantly to verify the urethral defect. The International Prostate Symptom Score (IPSS), QoL score of the IPSS, uroflowmetry, post-void residual urine (PVR) and the USS-PROM were collected preoperatively, 2 and 8 months after urethroplasty at the follow-up visits. The USS-PROM was developed in 2011 (11) as the first questionnaire specifically designed for patients with urethral stricture disease. This instrument is comprised of a LUTS domain and a health-related quality of life domain. The LUTS domain is composed by a six-item LUTS bother questions that generates a total score that varies from 0 (asymptomatic) to 24 (most symptomatic); by a separated LUTS-specific QoL question; and by the Peeling's voiding picture, an illustration of a man voiding scored between 1 (best) and 4 (worst). The postoperative USS-PROM is supplemented with a treatment satisfaction question. The patients were invited to complete the LUTS domain and the overall satisfaction question.

All urethroplasties were performed by a single surgeon, and the surgical technique was at surgeon's discretion. Urethral catheter was usually removed three weeks after surgery. Cystourethrography was done postoperatively only in patients who complained of urinary symptoms and was used to assess urethral patency and to direct further treatment if necessary. The surgery was considered a failure when any postoperative instrumentation or reoperation was performed.

The follow-up scores of IPSS, QoL, USS-PROM, Qmax and PVR were compared with preoperative scores and among them. The results of the IPSS and USS-PROM were also correlated with Qmax using linear regression analysis. To show an improvement of 10 points in the IPSS and 7mL/s in Qmax with a study power of 80% and p value <0.05, a sample size of 18 patients was estimated. General estimation equation was used to assess statistical significance between the baseline and postoperative time points. All statistical analysis was done using SPSS® 18.0 with 2-sided significance considered at $p<0.05$.

RESULTS

A total of 35 consecutive patients were included, of whom 3 were lost to follow-up and excluded from analysis. The mean age was 61

years (range 24-82). The average stricture length was 4.2cm (range 1-13) and strictures were located mainly in the bulbar urethra. The most commonly identifiable etiology was trauma in 11 patients (34.4%). In 16 patients (50%) a suprapubic urinary diversion was required due to complete urinary retention, thus obviating the preoperative assessment of subjective and objective data. A total of 18 (56.2%) substitution dorsal onlay repairs using buccal mucosa and 14 (43.7%) excision and primary anastomosis urethroplasties were done. Nineteen patients (59.3%) had undergone a previous urethral procedure. The overall success rate in the 8-month follow-up period was 87.5%, with all 4 urethroplasty failures presenting before the 2-month schedule visit with progressive worsening of voiding symptoms. Baseline data are summarized in Table-1.

Mean preoperative IPSS score was 19 and significantly decreased to 4.96 and 5.32 at the 2 and 8-months visit respectively (p <0.001). This small raise in IPSS in the follow-up period was not statistically significant. Patients also showed a significant improvement in QoL scores of the IPSS from 4.71 to 1.17 in the 2 and 8-month consultation (p <0.001). The mean improvements for IPSS and QoL score in individual patients at 8-months were respectively 13.64 (95% CI 18.14–9.13, p <0.001) and 3.43 (95% CI 4.20–2.65, p <0.001, Table-2).

The mean six-item LUTS score of the USS--PROM questionnaire was 13.21 at baseline, 2.46 two months after urethroplasty, and 3.36 eight months after urethroplasty (p <0.001). This worsening in LUTS score between the 2 and 8 month's evaluation was not significant (p=0.193). The LUTS score showed a mean decrease of 9.21 points (95% CI 12.70–5.71, p <0.001, Table-2). The mean Peeling's voiding picture score improved from 3.64 at baseline to 1.79 two months after urethroplasty (p <0.001). The 8-month score was 2.04 and was a trend towards worsening between the 2 and 8 month's score, but without significance (p=0.064). The mean LUTS-specific QoL question was 3.21 at the preoperative evaluation and dropped to 1.36 at the 8-month follow-up (p <0.001). Overall 27 of 32 men (84.3%) of the patients were "satisfied" or "very satisfied" with the results of their urethroplasty at 8 months. From the 5 patients that were unsatisfied, 4 had recurrence of the stricture with worsening of their symptoms, requiring surgical reintervention.

Table 1 – Baseline characteristics.

Mean age, year (range)	61 (24 – 82)
Mean stricture length, cm (range)	4.2 (1 – 13)
	n (%)
Stricture site	
Penile	8 (25)
Bulbar	14 (43.7)
Bulbo-penile	4 (12.5)
Membranous	4 (12.5)
Panurethral	2 (6.2)
Etiology	
Trauma	11 (34.4)
Iatrogenic	7 (21.8)
Infectious	4 (12.5)
Idiopathic	10 (31.2)
Suprapubic catheter	**16 (50)**
Procedure performed	
Buccal mucosa graft	18 (56.6)
Anastomotic	14 (43.7)
Previous Intervention	**19 (59.3)**
DVIU	6 (18.7)
Dilatation	10 (31.2)
Urethroplasty	5 (15.6)

DVIU = Direct vision internal urethrotomy

The mean Qmax at baseline was 4.64mL/s and significantly increased to 11.11mL/s and 11mL/s, respectively at two and eight months (p <0.001). The mean improvement in Qmax from preoperative assessment to the 8-month evaluation was 7.02 (95% CI 3.22–10.77, p <0.001, Table-2). Mean PVR decreased from 41.04mL preoperatively to 5.07mL at the final visit of the study (p=0.017). The preoperative and postoperative USS-PROM and IPSS scores were compared with Qmax using linear regression analysis and a strong negative correlation was found for both (r=-0.531, p <0.001; r=-0.512, p=0.001, respectively; Figure-1). When

Table 2 – Baseline to 8-month postoperative differences.

	Baseline Mean (95% CI)	8-mo Mean (95% CI)	P Value	Mean Difference (95% CI)	P Value
USS-PROM	13.21 (10.19 – 16.24)	3.36 (2.22 – 4.49)	<0.001	-9.21 (-12.70 to -5.71)	<0.001
IPSS	19 (14.45 – 23.55)	5.32 (3.87 – 6.78)	<0.001	-13.64 (-18.14 to -9.13)	<0.001
QoL	4.71 (4.05 – 5.37)	1.17 (0.71 – 1.85)	<0.001	-3.42 (-4.20 to -2.65)	<0.001
Qmax (mL/s)	4.64 (3.59 – 5.70)	11 (8.65 – 13.35)	<0.001	7.02 (3.22 to 10.77)	<0.001
PVR (mL)	41.04 (12.33 – 69.69)	5.07 (1.55 – 8.60)	0.017	-35.92 (-63.90 to -7.95)	<0.001

CI = Confidential Interval; **USS-PROM =** Urethral stricture surgery patient-reported outcome measure; **IPSS =** International Prostate Symptom Score; **QoL =** quality of life; **Qmax =** maximun flow rate; **PVR =** postvoid residual urine

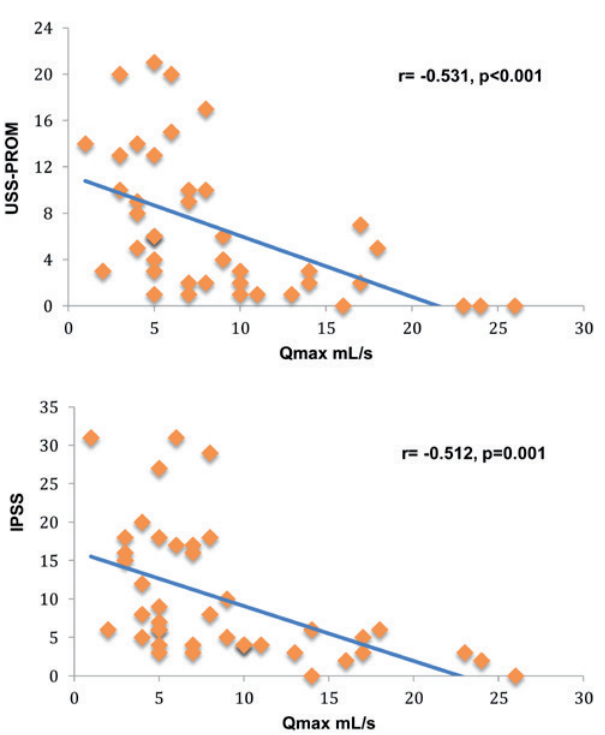
patients were divided and analyzed by procedure type or by the presence of preoperative suprapubic urinary catheter, no significant differences were seen for IPSS, QoL, Qmax and USS-PROM in the post-urethroplasty assessment.

DISCUSSION

This study presents patient-reported outcomes measures after urethroplasty using two different questionnaires for this purpose with each patient as his own control. We demonstrated continuous relief in IPSS, QoL and USS-PROM scores 8-months after urethroplasty with 84.3% of patients being satisfied or very satisfied with surgical results. For many surgeries the motivating factor is QoL, thus it is critical to know how satisfied patients are after undergoing urethroplasty both for counseling and following these patients up. Kessler et al. was among the first to highlight that subjective measures should be included in the assessment of urethroplasty outcomes (9). They noted that of 30 patients who were considered a failure by the surgeon's perspective, 24 were subjectively satisfied or very satisfied with the surgical outcome, showing that patients may consider the outcomes differently than surgeons.

In addition to symptoms questionnaires we used uroflowmetry and PVR to evaluate surgical outcomes, and also demonstrated significant improvements after urethral reconstruction. Many investigators have used uroflowmetry to determine the success of urethroplasty, but usually do not correlate it with subjective findings (8, 12, 13). In our study, the mean Qmax improved from 4.64mL/s

Figure 1 – Correlations between USS-PROM and IPSS scores with Qmax showing strong negative correlation.



preoperatively to 11mL/s 8 months after surgery. These improvements in Qmax were more modest when compared with those reported in other series (12, 14). Maybe this occurred because our patients were older (mean age of 61 years), a group where a large number of men have a component of benign prostatic hyperplasia or have a long-standing urethral obstruction with detrusor dysfunction. Accordingly, DeLong et al. found a median improvement in Qmax of 12mL/s after surgery, but when splitting the cohort by age, patients with less than 45 years experienced an improvement of 16mL/s vs. 8mL/s achieved in those older than 45 years (15). Both studies highlight that the differences between the preoperative and postoperative data using the patient as his own control is, perhaps, more important than setting a Qmax cutoff at which all men should be evaluated for stricture recurrence. Differently, our self-reported outcomes represented by the USS-PROM, IPSS and QoL scores were not diminished by the lesser improvement in Qmax and the results were in accordance with the few studies that analyzed these questionnaires pre- and postoperatively (10, 14-16).

Monitoring patient symptoms should be a crucial part in any surveillance protocol for stricture recurrence. It is easy, readily available, cheap and with no adverse events reported. IPSS, although developed to assess treatments for benign prostatic hyperplasia, is the most frequently used questionnaire in the evaluation of urethroplasty outcomes (7). It was first used by Morey et al. who demonstrated significant improvements in IPSS after successful reconstruction and a strong negative relationship between the IPSS and Qmax (16), a finding also demonstrated in a subsequent study (13). Voelzke et al. in a systematic review of the literature examining the use of patient-reported outcome measures after anterior urethroplasties found only 4 articles that used a LUTS instrument (2). The development of a specific instrument to assess urethroplasty outcomes was necessary, a step made by Jackson et al., who in 2011 developed and validated a USS-PROM as an attempt to standardize patient-centered evaluations of interventions for urethral strictures (11). In 2013 the same group presented the first paper that prospectively evaluated the USS-PROM reporting continuous relief of patient's symptoms and QoL in the 2-year follow-up period, setting a reference point which other groups can compare their performances with (10). The USS--PROM was also recently validated to Italian and German (17, 18).

Our cohort was only the second to report prospectively the results of the USS-PROM after urethroplasty and shows comparable outcomes. Overall 84% of our patients were satisfied or very satisfied with the results of urethroplasty at 8 months, compared to the 87% satisfaction rate reported by Jackson et al. (11). We found that the self reported six-item LUTS score significantly decrease postoperatively from 13.21 to 3.36 at 8 months, a better result than the 5.4 reported by the same group at a 2-year follow-up visit, but similar to the 3.4 score described by them at the 6-month survey. Moreover, we observed a strong negative correlation between the LUTS score and Qmax ($r=-0.531$, $p<0.001$), resembling the correlations described for Qmax and IPSS in other series (13, 16). The changes in Peeling's voiding picture and in the LUTS-specific QoL question in our study also did not differ from that reported by Jackson.

To date the best strategy to evaluate stricture recurrence is not clear. Instead of this there are many different protocols varying from invasive testing such cystoscopy and voiding cystourethrography to non-invasive like questionnaire symptoms and uroflowmetry employed in surveillance regimens after urethroplasty. This is demonstrated by Meeks et al. who performed a meta-analysis and found an average of 3.15 different diagnostic tests for this purpose after surgery (19). This lack of standardization makes comparisons across different studies difficult as well as the ability to perform meta-analysis difficult, highlighting that follow-up protocols remains extremely variable.

Our study has some limitations, including that half of our population had a suprapubic tube, and this might have overestimated the results. We tried to minimize this effect using the General Estimation Equation in the statistical analysis. We also recognize that Qmax and especially PVR may not be enough to determine the anatomical success of the surgical repair. On the other hand, one

of the objectives of our study was to correlate this non-invasive methods with subjective measures of success. For this purpose, further studies with more patients and longer follow-up are necessary. Additionally, the USS-PROM is not still formally validated to Portuguese.

In our practice, we use a multi-tier approach to screen patients after urethroplasty. We start with symptoms questionnaires, uroflowmetry and PVR comparing the results with the data collected preoperatively. If symptoms of voiding difficult or changes in uroflowmetry are present, we than proceed to more invasive evaluation. We found this strategy helpful in identifying recurrences while keeping patients comfort, favoring a less-expensive regimen. Furthermore, there is no evidence in the literature that early treatment of an asymptomatic recurrence is beneficial. Another issue of growing importance is the cost involved in the follow-up strategy in an era of increasing health care costs. Belsante et al. reported that a symptom-based, risk-stratified follow-up protocol would be far most cost-effective than close follow-up in all patients after urethroplasty, missing less than 1% of an asymptomatic recurrence (20). It has also been demonstrated that the first year charges of anterior urethroplasty surveillance can range from $205 to $1784 depending on the strategy adopted (21). It is our belief that urethral reconstruction is a quality of life surgery, and as long as the patient is satisfied with his symptoms, perform routinely invasive tests is, sometimes, overly aggressive, exposing patients to unnecessary risks and will usually not change management until patient feels symptomatic. The adoption of a disease-specific instrument like the USS-PROM questionnaire is of great value since it could help patients and physicians predict their outcomes and satisfaction.

CONCLUSIONS

We have demonstrated that urethroplasty is a well-tolerated and worthwhile procedure by patient's point of view. We have shown with both patient-reported outcomes and objective measures a significant improvement in symptoms, QoL scores and Qmax after urethral reconstruction using patients as their own control. Harmonization of surveillance protocols is clearly necessary as a method to more effectively compare the results between different reconstructive procedures and institutions. The use of a tool specifically designed to access urethral stricture disease is a great advance in this field and should be encouraged as an attempt to minimize costs and to incorporate the patients perspective in this process. Further studies are necessary to establish the optimal surveillance protocol for stricture recurrence and the place of PROMs in this setting.

CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Assessment of hormonal activity in patients with premature ejaculation

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ABSTRACT

Purpose: Premature ejaculation is considered the most common type of male sexual dysfunction. Hormonal controls of ejaculation have not been exactly elucidated. The aim of our study is to investigate the role of hormonal factors in patients with premature ejaculation.

Materials and Methods: Sixty-three participants who consulted our outpatient clinics with complaints of premature ejaculation and 39 healthy men as a control group selected from volunteers were included in the study. A total of 102 sexual active men aged between 21 and 76 years were included. Premature ejaculation diagnostic tool questionnaires were used to assessment of premature ejaculation. Serum levels of follicle stimulating hormone, luteinizing hormone, prolactin, total and free testosterone, thyroid-stimulating hormone, free triiodothyronine and thyroxine were measured.

Results: Thyroid-stimulating hormone, luteinizing hormone, and prolactin levels were significantly lower in men with premature ejaculation according to premature ejaculation diagnostic tool (p=0.017, 0.007 and 0.007, respectively). Luteinizing hormone level (OR, 1.293; p=0.014) was found to be an independent risk factor for premature ejaculation.

Conclusions: Luteinizing hormone, prolactin, and thyroid-stimulating hormone levels are associated with premature ejaculation which was diagnosed by premature ejaculation diagnostic tool questionnaires. The relationship between these findings have to be determined by more extensive studies.

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INTRODUCTION

Premature ejaculation (PE) is the most frequent male sexual disorder. According to different criteria, its prevalence might widely vary, ranging from 8 to 30% (1) up to 22-38% (2). Over the past decade, increasingly assertive efforts have been assumed to explain the etiology of PE. Genetic, neurobiological and somatic etiologies for PE have been hypothesized: disruptions in central serotonergic neurotransmission (3), potent cortical representation of the pudental nerve (4), prostatitis (5), hypersensitivity of the penis (6), recreational drugs (7), and hormonal disorders (8-11). Although it is well described that male sexuality and reproduction are hormonally regulated, the

association between endocrine control and ejaculatory reflex is still not completely elucidated. It is well reported that sex steroids play a role in the regulation of ejaculation (8) and demonstrated similar effects of thyrotropin and prolactin, but in the against direction (12). Current guidelines on PE supply little information on this circumstance (13, 14).

Stopwatch measures of IELT are commonly used in clinical trials of PE. However, they have not been recommended for use in routine diagnosis of PE (15). The International Society for Sexual Medicine (ISSM) described three common structures used in the most definitions of PE: (1) a short ejaculatory latency time (prior to or within about 1 minute of vaginal penetration (lifelong PE) or a clinically significant and bothersome reduction in latency time, often to about 3 minutes or less (acquired PE); (2) the inability to delay ejaculation on all or nearly all vaginal penetrations; (3) distress and/or avoidance of sexual intimacy (16). The requirement to evaluate PE objectively has led to the development of some questionnaires based on the use of patient-reported outcome. The premature ejaculation diagnostic tool (PEDT) was developed and validated by Symonds et al. to standardize the diagnosis of PE in clinical trials (17). In the current study, we used PEDT questionnaires to assesssment of the relationship between PE and hormonal functions.

MATERIALS AND METHODS

The present study involved an observational and prospective design. It was conducted on a series of 102 sexual active men aged between 21 and 76 years between May 2014 and June 2015. Sixty three participants who consulted our outpatient clinics with complaints of PE and 39 healthy men as a control group selected from volunteers enlisted through an announcement about the research project were included in the study. We included patients with monogamous and heterosexual relationship with the same partner for at least 6 months.

PE was evaluated by using the Turkish version of the PEDT questionnaires which provides scores for five subdomains: control, frequency, minimal stimulation, distress, and interpersonal difficulty. Each question is scored 0-4 and higher scores indicate higher severity. Total PEDT score is calculated as the sum of the scores of the five subdomains. A PEDT score 42.6±11.8 11 indicates PE (17, 18).

Patient's age, body mass index (BMI), smoking history, alcohol consumption and the International Index of Erectile Function Erectile Domain (IIEF-ED) score were recorded. Blood samples to evaluate the routine laboratory tests and serum levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin, total testosterone, free testosterone, thyroid-stimulating hormone (TSH), free triiodothyronine (T3), free thyroxine (T4) were measured using immunoassay method, except free testosterone measurement that was performed by the enzime-linked immunosorbent assay method in all participants. Blood samples were obtained in the morning after an overnight fasting. For abnormal laboratory findings, a second blood sample was taken in a few days.

We excluded men with moderate or severe erectile dysfunction, any sexual dysfunction of partner, a sexual intercourse frequency less than once a week, any medication history due to PE, any history of neuropathic or psychological disease, hypogonadism, and concomitant chronic diseases. Participants who had used drugs that might disturb hormonal values, and who had been diagnosed with prostatitis were also excluded from the study.

Committee of Ethics approved the protocol and all participants provided written informed consent. Collected data were analyzed with Statistical Package for Social Sciences (SPSS) version 17. Statistical analysis were performed using Student t test, Mann-Whitney U test and Chi-square test. P values lower than 0.05 were considered as significant.

RESULTS

The baseline characteristics of the 102 participants are described in Table-1. The mean ages of the participants evaluated in the PE group (n=63) and control group (n=39) were 42.6±11.8 years (23-76) and 40.3±10.7 (21-65), respectively (p=0.065). There was no significant difference be-

Table 1 - Characteristics and hormonal status of the studied participants.

	PEDT score ≥11 (PE group)	PEDT score <11 (Control group)	p*
	n=63	n=39	
Age	42.6±11.8	40.3±10.7	0.065
BMI (kg/m²)	26.6±4.2	27.7±5.0	0.333
Smoking history (n, %)	32, 50.8%	20, 51%	0.962
Alcohol consumption (n/%)	8/12.7%	3/7.69%	0.428
IIEF–ED score	25.2±3.7	25.7±3.5	0.491
Glucose	113.4±43.4	95.0±8.0	0.162
Creatinine	0.8±0.1	0.9±0.1	0.186
Total cholesterol	180.2±43.4	182.7±42.3	0.635
LDL cholesterol	112.8±31.1	118.2±36.7	0.517
HDL cholesterol	41.2±9.3	43.5±10.0	0.219
Triglycerides	169.9±157.8	154.5±63.4	0.474
Free T3 (pg/dL)	3.5±0.6	3.6±0.4	0.067
Free T4 (ng/dL)	1.3±0.2	1.3±0.1	0.251
TSH (mIU/L)	1.7±1.4	2.1±1.2	0.017
FSH (mIU/mL)	7.6±7.1	5.5±3.9	0.054
LH (mIU/mL)	6.5±2.8	5.2±2.0	0.007
Prolactine (ng/mL)	9.5±6.1	11.6±5.0	0.007
Total Testosterone (ng/dL)	4.4±1.7	4.5±1.5	0.598
Free Testosterone (pg/dL)	12.3±4.0	12.2±3.3	0.920

* **T** test/Mann-whitney U test/Chi-square test. **PEDT** = Premature Ejaculation Diagnostic Tool; **BMI** = Body mass index; **IIEF-ED** = International Index of Erectile Function-Erectile Domain; **LDL** = *Low Density Lipoprotein;* ***HDL*** = *High Density Lipoprotein;* **TSH** = Thyroid-stimulating hormone; **FSH** = Follicle-stimulating hormone; **LH** = Luteinizing hormone.

tween the BMI, smoking status, alcohol consumption, glucose, creatinine and lipid profiles between the two groups (p>0.05).

The mean PEDT score in patients with PE was found to be 15.31 and in the control group, the mean PEDT score was 5.43. Of the participants, 63 scored 11 and higher (61.7%), 39 scored lower than 11 (38.2%).

The mean IIEF-ED scores did not differ between the groups. Based on IIEF-ED evaluation, the mean scores of the subjects in PE group was 25.2±3.7, while in the control group was 25.7±3.5 (p=0.491).

There was no significant difference between the levels of FSH, total testosterone, free testosterone, free T3 and free T4 between the two groups, whereas lower levels of TSH, LH and prolactin were reported in the PE group (p=0.017, 0.007 and 0.007, respectively). In the PE group mean serum TSH, LH and prolactin levels were 1.7±1.4mIU/mL, 6.5±2.8mIU/mL and 9.5±6.1ng/mL, respectively. LH level (OR, 1.293; p=0.014) was found to be an independent risk factor for premature ejaculation Table-2.

DISCUSSION

Although PE is a common type of male sexual disorder, it is poorly understood and patients may be misdiagnosed and mistreated (19). The pathophysiology of PE is largely unknown and hormones might have a potential role in the

Table 2 - Multivariate model for PEDT score and LH level.

PEDT		
	Reference	p*
LH	1.293 (1.054-1.585)	0.014

* Spearman correlation. **PEDT =** Premature Ejaculation Diagnostic Tool; **LH =** Luteinizing hormone.

pathophysiology of PE. The main aim of our study was to investigate the role of several hormones in the etiology of PE.

Corona et al. reported for the first time that several testosterone levels might lead to different severities of ejaculatory disorders. They reported higher testosterone levels in their youngest subjects suffering from PE while the elder group with delayed ejaculation had lower testosterone levels (20). In our study, nevertheless, total testosterone and free testosterone levels were not significantly different in the two groups. A reasonable clarification for this is related to our inadequate sample size. Therefore, androgen suppression is not an acceptable treatment for PE yet.

In the current study, lower levels of prolactin were associated with PE; however, low prolactin levels are not a risk factor for PE. The effect of high prolactin levels has been extensively researched, but the effects of low prolactin levels have not received similar interest. In the European Male Ageing Study (EMAS) showed that low prolactin levels were associated with lower ejaculatory time on almost 3000 subjects (21). Low prolactin levels can be the outcomes of an increased dopamine activity. The use of dopamine antagonists (haloperidole etc.) induces a dramatic increases ejaculatory latency time in a dose dependent manner (22). Therefore, an increased dopaminergic tone (reflected by low prolactin levels) can explain the association with PE. Otherwise, men with lower levels of prolactin showed the highest level of free-floating anxiety and related to psychobiological features such as anxiety symptoms and PE (23).

The role of thyroid hormones in PE has been assumed. Associations between premature and delayed ejaculation with hyperthyroidism and hypothyroidism, respectively, have been recorded in animal models and humans (24-26). Treatment of hyperthyroidism has verified to be effective for PE; the prevalence of PE fell from 50 to 15%, after normalizing thyroid function in men with hyperthyroidism (24). In our study, lower levels of TSH were reported in the participants with PE than control group. We suggest that laboratory confirmation should be advised in men with PE inasmuch as hyperthyroidism increased the risk of all-cause mortality (27) .

Mohseni et al. found no correlation between LH level and PE, even though men with PE had significantly higher levels of FSH (9). However, in our study there was no correlation between PE and FSH level, but men with PE had lower levels of LH than control group. Moreover, we found that LH is an independent risk factor for PE. The literature evidence regarding the gonadotropins related to men with PE is still lacking.

In the present study, we used the PEDT score which was developed and validated by Symonds et al. for diagnosis of PE (17). Rowland and Kolba advocated that clinicians should approach the 1 minute IELT criterion with flexibility, considering IELTs up to 2 minutes for a PE. Finally, PE cannot be diagnosed simply by the IELT alone (28). Kam et al. and Pakpour et al. demonstrated that the PEDT was highly effective in detecting the presence of PE and the result of their study supports its validity as a diagnostic tool in the clinical setting (29, 30). To the best of our knowledge, this is the first study to investigate the association of hormones and PE in which was used PEDT questionnaires.

Relatively small sample size is the major limitation of our study. Furthermore, patients with delayed ejaculation were not included in the study. It is obvious that there is a need for further large-scale studies which include other hormones such as oxytocin and adrenal steroids.

CONCLUSIONS

We showed an association between hormonal activity and PE. Patients with PE have lower LH, TSH, and prolactin levels compared with normal men and the lower levels of LH is an independent risk factor for PE. The results of this study showed that the role of endocrinologic factors should be more investigate in the etiology of PE. We think that hormones might also have a potential role in the pathophysiology of PE. Perhaps hormonal therapy of PE will ameliorate the sexual and the general health of these patients. Furthermore, we think that laboratory confirmation for hyperthyroidism should advise in men with ejaculatory disorders.

CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Effect of tadalafil 5mg daily treatment on the ejaculatory times, lower urinary tract symptoms and erectile function in patients with erectile dysfunction

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ABSTRACT

Objective: To investigate the effect of a 5mg daily tadalafil treatment on the ejaculation time, erectile function and lower urinary tract symptoms (LUTS) in patients with erectile dysfunction.
Materials and Methods: A total of 60 patients diagnosed with erectile dysfunction were retrospectively evaluated using the international index of erectile function questionnaire-5 (IIEF-5), intravaginal ejaculatory latency time (IELT) and international prostate symptoms scores (IPSS). After the patients were treated with 5mg tadalafil once a day for three months, their erection, ejaculation and LUTS were assessed again. The fasting levels of blood glucose, total testosterone, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol and total cholesterol were measured. The independent-samples t-test was used to compare the pre- and post-treatment scores of the patients.
Results: The mean age of the 60 participants was 50.4±7.9 and the mean baseline serum total testosterone, total cholesterol, and fasting blood sugar were 444.6±178.6ng dL^{-1}, 188.7±29.6mg/dL^{-1},104 (80-360) mg dL^{-1}, respectively. The mean baseline scores were 2.2±1.4 min for IELT, 9.5±3.7 for IIEF-5 and 14.1±4.5 for IPSS. Following the three-month daily 5mg tadalafil treatment, the scores were found to be 3.4±1.9 min, 16.1±4.7, and 10.4±3.8 for IELT, IIEF and IPSS, respectively. When the baseline and post-treatment scores were compared, a statistically significant increase was observed in the IELTs and IIEF-5 values whereas there was a significant decrease in IPSS ($p<0.01$).
Conclusion: A daily dose of 5mg tadalafil can be safely used in the treatment of erectile dysfunction and LUTS, that prolongs the ejaculatory latency time.

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Tadalafil; Ejaculation; Erectile Dysfunction; Therapeutics

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INTRODUCTION

Premature ejaculation (PE) is considered one of the most common sexual function disorders in men with a prevalence of 9-30% (1-5). PE is defined as ejaculation with minimal sexual stimulation before or shortly after penetration, resulting in anxiety and distress. Patients have minimal or no voluntary control over PE (5). There are two types of PE: lifelong or primary, and acquired or

secondary (5). Based on modern evidence, the causes of PE have been found to be psychogenic and performance anxiety (1, 5). Organic factors have been suggested as significant predictors of PE (2, 6). Genetic factors have also been listed among the factors affecting lifelong PE (7). Other common organic factors that have an impact on acquired PE include hormonal abnormalities (2), prostatitis (6), and erectile dysfunction (ED) (8). It has been reported that in many cases of lifelong PE, the men do not suffer from ED (9); however, approximately one third of the patients with ED have PE (10). Similarly, in a recent large-scale survey in the Asian-Pacific region administered to 4997 heterosexual men aged 18 to 65 years in a stable sexual relationship, ED was found to accompany PE in more than 30% of the respondents (11).

Many studies have suggested that assessing the effect of PE treatment is to measure the time taken to achieve ejaculation using the intra-vaginal ejaculation latency time (IELT). IELT is based on self-report and measured by a chronometer. It has 80% specificity and sensitivity for PE (12). Behavioral and pharmacological therapies are the common treatment options for PE. Behavioral therapy includes several techniques such as squeezing and start-stop methods but many couples have reported these to be inadequate. The first choice in pharmacological therapy is the use of serotonin reuptake inhibitors (SSRIs) (e.g., citalopram, sertraline, fluoxetine, dapoxetine or paroxetine); however, other options include phosphodiesterase type 5 (PDE5) inhibitor therapy (tadalafil or sildenafil), topical desensitizing agents (prilocaine or lidocaine) and other agents (tramadol or pindolol) (13). PDE5 inhibitors are frequently used in the treatment of ED and clinical studies have reported their positive effect on patients with PE (14-16). In a recent study, a daily dose of 5mg tadalafil has been shown to significantly increase IELT in patients diagnosed with lifelong PE (17). However, to our knowledge, there is no study in the literature that determined the effect of tadalafil 5mg daily on ejaculatory time in patients with ED.

The current study investigated the effect of 5mg daily tadalafil treatment on the time taken to achieve ejaculation, erectile function and lower urinary tract symptoms in patients diagnosed with ED.

MATERIALS AND METHODS

A total of 60 patients who were referred to the urology policlinic of the hospital with the complaint of erectile dysfunction between January 2015 and January 2016 were included in the study. The study was approved by the local ethics committee of Erzincan University and all patients gave informed consent for the treatment. All patients reported to be heterosexual and in a stable sexual relationship for more than six months. The exclusion criteria were neurological disorders such as depression, Parkinson's disease, diabetic neuropathy, and cerebrovascular damage, an active urinary system infection, history of chronic prostate; alcohol, drug or substance abuse, organic diseases limiting the use of PDE5 inhibitors, pelvic trauma, anemia, thyroid disease, hypogonadism (total testosterone) end-stage renal failure; and having used medication affecting the sex hormone and/or vitamin metabolism or for the treatment of PE and ED within the last three months. The information related to patient's age, duration of sexual dysfunction, smoking status, and sexual and medical history was obtained and a complete physical examination was performed on all patients. PE was assessed by IELT, which is defined as the time from vaginal intromission to intravaginal ejaculation (18). IELT was measured using a self-report method. It was measured by the female sexual partner using a stopwatch and expressed in minutes. If ejaculation occurred before or during penis vaginal intromission, it was defined as 0 minute. The same company calibrated all the stopwatches (12). The erectile functions of the patients were evaluated using the five-item international index of erectile function questionnaire (IIEF-5). According to their IIEF-5 scores, the ED patients were divided into three groups as severe ED (score: 1-7), moderate ED (8-11) and mild ED (11-21). The patient's intra-vaginal ejaculation times were recorded. The lower urinary system symptoms (LUTS) of the patients were assessed using the international prostate symptom score (IPSS). Following fasting for 12 h, at 8 a.m.,

blood samples for the laboratory tests were obtained to measure the levels of fasting blood glucose (FBG), total testosterone (TT), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C). The accepted normal values were: TT: 271-965ng dL^{-1}, FBG: 70-110mg dL^{-1}, TG: <150mg dL^{-1}, LDL-C: <130mg dL^{-1} and HDL-C: >40mg dL^{-1}.

For the treatment of ED, the patients were prescribed 5mg tadalafil daily for three months. At the end of this period, the patients were re-evaluated using IIEF-5, IELT and IPSS. In addition, the side effects of the treatment were recorded and the patient's baseline and post-treatment scores were compared.

Statistical analysis

A power analysis was conducted, in which the Biostatistics power of 80.193% was evaluated and the sample width was determined as a minimum of 19 individuals in each group. The statistical software SPSS (Statistical Package for Social Sciences, Version 20, Chicago IL, USA) was used for calculations. All values were presented as mean±standard deviation, means (maximum-minimum), percentages and frequencies. The results of the homogeneity (Levene's Test) and normality tests (Shapiro Wilk) were used to decide which statistical methods had to be applied in the comparison of the study groups. Groups that were normally distributed and those with homogeneous variances were compared using the Student's t test, and three or more groups were compared by the Analysis of Variance. According to the results of these tests, parametric test assumptions were not available for some of the variables and therefore the comparisons of two independent groups were performed by Mann-Whitney U test, and the comparisons of three independent groups were performed using Kruskal-Wallis test. For the multiple comparison tests, the adjusted Bonferroni method was used. The repeated measures of analysis of variance were analyzed by Mauchy's sphericity test and Box's Test of Equality of Covariance Matrices. For comparisons of the means of repeated measures, the Repeated Measures Analysis of Variance was used. When the parametric tests (factorial design for repeated measures analysis) did not meet the preconditions, methods by Greenhouse-Geisser (1959) or Huynh-Feldt (1976) were used for corrections to the Degrees of Freedom or Friedman Test. The Corrected Bonferroni test was used in multiple comparisons. The categorical data was analyzed with Fischer's Exact Test and chi-square test. p values of <0.05 and <0.01 were considered statistically significant.

RESULTS

The mean age of the 60 participants was 50.4±7.9 (range 36–67). The mean serum total testosterone, fasting blood sugar, total cholesterol, LDL-C, HDL-C were found to be 444.6±178.6ng/dL^{-1} (310–900), 104 (80-260) mg/dL^{-1},188.7±29.6mg/dL^{-1}, 111.9± 32.4mg/dL^{-1}, and 43.2±9mg/dL^{-1}, respectively (Table-1). The mean baseline scores were 2.2±1.4 for IELTs, 9.5±3.7 for IIEF-5, and 14.1±4.5 for IPSS. At the end of the three-month tadalafil treatment, the patient's scores were found to be 3.4±1.9, 16.1±4.7 and 10.4±3.8 for IELTs, IIEF-5 and IPSS, respectively (Table-2). The results indicated a statistical improvement in all parameters

Table 1 - Clinical data and fasting endocrine values of the participants.

Characteristic	Patients (n:60)
Age (year) *	50.4±7.9
Total Testosterone (ng dL^{-1}) *	444.6±178.6
Total Cholesterol (mg/dL^{-1}) *	188.7±29.6
Fasting blood sugar (mg dL^{-1}) *	104 (80-360)
HDL (mg dL^{-1}) *	43.2± 9
LDL (mg dL^{-1}) *	111.9± 32.4
Hypertension (%)	33.9
Smoking (%)	45.8
DM (%)	15.0

LDL cholesterol = Low-density lipoprotein cholesterol; **HDL cholesterol** = High-density lipoprotein cholesterol; **DM** = Diabetes mellitus.
* Mean±SD

Table 2 - Baseline and post tadalafil 5 mg daily treatment IELT, IPSS and IIEF-5 scores of patients.

Variables	Pre-treatment	Post-treatment	p value*
IIEF-5	9.5±3.7	16.1±4.7	<0.001
IPSS	14.1±4.5	10.4±3.8	<0.001
IELT(min)	2.2±1.4	3.4±1.9	<0.001

IIEF-5 = International Index of Erectile Function-5; **IPSS =** International prostate symptom score; **IELT** = intravaginal ejaculation latency time
*p values were derived from the statistical analysis using the independent t-test.

(p=<0.001). The pre- and post-treatment scores of the patients were compared according to the severe, moderate and mild ED groups. In all three groups, a statistically significant difference was found between the pre- and post-treatment values of IPSS variables (p<0.01) and a statistically significant difference was found between the pre- and post-treatment values of IELT variables (p<0.01) (Table-3). However, there was no significant difference between the ED groups in terms of the baseline and post-treatment values of IPSS (p=0.10; p=0.23) or IELT (p=0.83; p=0.48).

Table-2 presents the mean pre- and post-treatment IELT, IIEF-5, and IPSS of the patients. The common side effects were gastrointestinal problems or nausea in 6 patients (10%) and headache in 5 patients (8.3%). In addition, flushing was reported by 3 patients (5%) and muscle and lower back pain by 2 patients (3.3%). Most of the side effects disappeared over time.

Table 3 - Comparison of ED groups in terms of IPSS and IELT scores before and after tadalafil 5 mg daily treatment.

Group		IPSS_PRE	IPSS_POST	p	IELT_PRE (min)	IELT_POST (min)	p
	N	20	20		20	20	
severe ED	Mean	15.70	11.20	0.001**	2.30	3.10	0.001**
	Std. Deviation	3.83	2.97		1.17	1.41	
	N	22	22		22	22	
moderate ED	Mean	13.95	10.64	0.002**	2.09	3.50	0.001**
	Std. Deviation	5.35	4.74		1.48	2.32	
	N	18	18		18	18	
mild ED	Mean	12.47	9.12	0.001**	2.35	3.88	0.001**
	Std. Deviation	3.79	3.04		1.62	2.00	
	N	60	60		60	60	
Total	Mean	14.12	10.39	0.001**	2.24	3.47	0.001**
	Std. Deviation	4.56	3.78		1.41	1.95	
p		0,10	0.23		0.83	0.48	

**p<0.01

DISCUSSION

In this study, the effect of tadalafil 5mg daily treatment on ejaculation time, erectile function and lower urinary tract symptoms was investigated in patients diagnosed with ED. Corona et al. (19) recently conducted a meta-analysis on the relationship between PE and ED, and reported that PE increases the risk of ED approximately fourfold. In addition, this risk was found to be significantly higher in patients with depression and anxiety symptoms, followed by those with diabetes, hypertension and dyslipidemia. The IIEF scores of PE patients and the IELT scores of ED patients were found lower. According to the hypothesis proposed by Jannini et al. (8), PE and ED are part of a vicious cycle in which trying to control ejaculation reduces the instinctive level of stimulation resulting in ED. Similarly, in the effort to have an erection, the patient may try to increase his stimulation, which may result in PE. In order to test this hypothesis, Jannini et al. (8) retrospectively analyzed 184 cases (age range: 18-83), who were referred to the clinic with sexual function problems. The authors found that 29 cases with isolated ED had developed PE before ED. In the same study, 21 cases with isolated PE were found to be accompanied by, or have a history of mild to moderate ED (diagnosed using IIEF). Resulting in low satisfaction with sexual intercourse, PE can create psychological issues, which may lead to the development of ED.

PE can also develop secondarily to the increased stimulation for the creation and maintenance of erection in ED patients or accompanying anxiety (8). In parallel to this hypothesis, it was suggested that there is a higher risk of developing PE-associated ED for cases in which there is a direct correlation between ED and symptoms of anxiety or depression, and for those who do not have a stable sexual partner and experience stressful sexual relationships (19). Waldinger (9) suggested that ED is more commonly seen in patients with acquired PE compared to those with lifelong PE. Lifelong PE reduces sexual stimulation in patients, thus resulting in sexual intercourse accompanied by ED. On the other hand, McMohan et al. (20) used validated diagnostic tests and reported that 33% of the PE patients had been diagnosed with false positive ED. Today, the available PE treatment options include behavior therapy, topical anesthetics, and more predominantly SSRIs. However, studies concerning PDE5 inhibitors have also reported the clinical efficiency of these drugs in the treatment of PE. Studies investigating the therapeutic effects of PDE5 inhibitors alone and in combination with SSRIs have reported the benefits of these inhibitors for PE treatment (14-17). In a well-designed, randomized and double blind study, sildenafil was compared to a placebo (21). The authors reported that sildenafil increased the perception of ejaculatory control and overall sexual satisfaction, and reduced the time between the first and second ejaculation; however, it did not significantly increase IELT. Other studies (22, 23) have demonstrated that the combination of PDE5 inhibitors and SSRIs are more efficient in increasing IELT and overall sexual satisfaction compared to the individual use of these medications. These studies used sildenafil 50mg as the main PDE5 inhibitor.

In a randomized study, Salonia et al. (24) compared the efficacy of sildenafil, various SSRIs and the pause-squeeze technique, and reported that sildenafil increased IELT and sexual satisfaction and reduced anxiety. In addition, sildenafil, clomipramine, paroxetine and the pause-squeeze technique were found to increase IELT by 1 to 15 min, 4 min, 3 min, 4 min and 3 min, respectively in comparison to the baseline values (24). Recently, Ozcan et al. (17) reported a significant increase in IELT of patients with lifelong PE following 5mg daily tadalafil treatment. Although the study had limitations in terms of the small sample size (30 patients) and the short duration of treatment (1 month), it is significant in terms of being the first report on 5mg daily tadalafil treatment. In this study it was reported that IELT increased approximately 2.5 min while in our study increased 1.2 min. Our results showed that tadalafil 5mg daily treatment led to statistically significant improvement in all the measured parameters. Our results are supported by Ozcan et al. (17) who demonstrated that tadalafil 5mg alone could significantly prolong IELT. At the same time, in our study, there was no statistically significant difference between

the ED groups in terms of IELT and IPSS following tadalafil 5mg daily treatment. Mattos et al. (16) study involving effect of tadalafil (20mg) alone and in combination with fluoxetine (90mg) found that the increase in IELT was better in patients who received combined treatment compared with placebo, fluoxetine, or tadalafil alone.

In the treatment of PE, regarding the effect of PDE5 inhibitors, there are several mechanisms involved. All central and peripheral mechanisms are probably important but the particular role that each plays in delaying ejaculation is not known. However, the mechanism that is most speculated to be involved is the reduced sympathetic tone and smooth muscle dilatation. Aversa et al. (25) reported that PDE5 inhibitors display activities through central and peripheral mechanisms. The NO/cGMP signaling pathway is considered to control sexual behavior through a central effect. The possible mechanism of the PDE5 inhibitor action lessens the contracting response of vas deferens (VD), seminal vesicles (SV) and prostate and urethra. This creates a state of peripheral analgesia, which prolongs the duration of the erection and reduces the central sympathetic output (26). The results of these studies demonstrate that PDE5 inhibitors relax VD, SV and smooth muscle tissue in the prostate, and increase the duration of the erection and sexual confidence, resulting in increased overall sexual satisfaction.

Studies have suggested that LUTS cause erectile dysfunction (27) and ejaculatory problems (28). The pathophysiological links between LUTS and ED are not fully understood, and these conditions are suitable to therapy with phosphodiesterase type 5 inhibitors (PDE5-Is). Some studies have determined the role of phosphodiesterase type 5 inhibitors in the treatment of men with LUTS associated with benign prostatic enlargement. Yan et al. (29) conducted a meta-analysis on the use of PDE5 inhibitors in the treatment of LUTS and reported that these inhibitors reduced IPSS by 4.21 points. Similarly, in this study, we found a significant decrease of 4.3 points in IPSS and an increase in the IIEF-5 score after using tadalafil 5mg daily treatment. Oelke et al. study, a post-hoc analysis of four randomized studies in 1477 men, showed that patients treated with tadalafil 5mg once daily versus placebo presented a clinically-meaningful symptom improvement (decrease more than 3 points of total IPSS) (30). Wein et al. (28) study reported that LUTS caused ejaculatory problems. Alpha blockers drugs are very important for the treatment of LUTS. On the other hand, Akın et al. (31) showed that all this alpha-blocker drugs were statistically effective in preventing PE. The authors found that the IPSS score significantly decreased in all groups while there was a statistically significant increase in IELT and a decrease in premature ejaculation profile scores in all alpha-blocker drugs groups in the post-treatment period. In this study, it was observed that tadalafil 5mg daily treatment led to statistically significant improvement in IPSS and IELT. Similarly, Choi et al. (32) reported a significant change using in the LUTS+PE patients after tamsulosin administration according to the results of the premature ejaculation diagnostic tool (PEDT). The positive effect of tamsulosin on PE can be attributed to the decreased contractility of the seminal vesicle or the vas deferens by the drug itself. Furthermore, the improvement of PE could be secondarily affected by the improvement of LUTS. This effect was also demonstrated by Aversa et al. (25), who used PDE5 inhibitors to relax VD, SV and smooth muscle tissues in the prostate.

PE has been found to be related to comorbid disorders such as diabetes. El-Sakka et al. (33) showed that men with diabetes had a high prevalence of PE. Many patients with ED develop PE probably due to the need for intense stimulation or anxiety to initiate and maintain an erection (34). In our study, only 9 patients (15%) had DM and their fasting blood glucose was controlled.

The limitations of our study include a small sample size and the absence of a non-ED control or placebo group. Treatment with PDE5 inhibitors is significant not only in terms of the relationship between ED and PE but also due to improving the erection and reducing the ejaculation problems caused by LUTS. This study is a valuable contribution to the literature in terms of being the first study investigating the effect of tadalafil 5mg daily treatment on ejaculatory time in patients with ED.

Daily 5mg tadalafil treatment is considered to have beneficial effects on ED and PE patients. Therefore, we recommend the use of 5mg tadalafil once daily, specially in those men with PE with erectile dysfunction. Further studies must be conducted with a placebo-controlled larger series and a longer follow-up to contribute to the literature in terms of the effects of daily 5mg tadalafil treatment.

CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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ECLAMC Study: prevalence patterns of hypospadias in South America: multi-national analysis over a 24-year period

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ABSTRACT

Objective: To evaluate prevalence trends of hypospadias in South-America it is essential to perform multicenter and multinational studies with the same methodology. Herein we present systematic data as part of an international multicenter initiative evaluating congenital malformations in South America over a 24-year period.

Materials and Methods: A nested case-control study was conducted using the Latin American Collaborative Study of Congenital Malformations (ECLAMC), between January 1989 and December 2012. Cases were stratified as isolated (IH) and non-isolated hypospadias (NIH). Global prevalence was calculated and discriminated by country. Associations between birth weight and gestational age, and NIH distribution by associated abnormality and severity of hypospadias, were analyzed.

Results: A total of 159 hospitals from six countries participated, reporting surveillance on 4.020.384 newborns. A total of 4.537 hypospadias cases were detected, with a global prevalence of 11.3/10.000 newborns. Trend analyses showed in Chile, Brazil and Uruguay a statistically significant increase in prevalence. Analysis of severity and associated anomalies did not to find an association for distal cases, but did for proximal (RR=1.64 [95% CI=1.33-2.03]).

Conclusion: This is one of only a few Latin American multicenter studies reporting on the epidemiology of hypospadias in South America in the last two decades. Our data adds to evidence suggesting an increase in some countries in the region at different times. There were also variations in prevalence according to severity. This study adds to literature describing associated anomalies at a hospital-based level.

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INTRODUCTION

Despite being one of the most common congenital anomalies of the male external genitalia, after decades of research we still lack knowledge on the exact pathophysiology of hypospadias (1-3). In this regard, multiple authors have identified an increase in prevalence, information generated

from studies in Europe and North America (4-6). Although a global phenomenon is plausible, there is a paucity of information on the trends and impact of this condition in many other parts of the world (7, 8).

To date, there have been few hospital-based studies with regional information including children from other parts of the World, such as Central and South America and Africa (1, 2, 9). To address this, herein we present data gathered in a systematic fashion as part of an international multi-center initiative evaluating congenital malformations in South America. We hypothesized that prevalence patterns should follow similar patterns to those presented in previous publications in other regions around the world. The aim of the present study was to analyze trends and conduct an epidemiologic description over a 24-year period using information from the Latin-American Collaborative Study of Congenital Malformations (ECLAMC) (10).

MATERIALS AND METHODS

Database description

The ECLAMC initiative is a multicenter international collaboration designed to identify associated risk factors and potential causes of congenital anomalies (CA). The data collection methodology has previously been reported (10). For the purpose of the present analyses, we followed a nested case-control design (10), analyzing information forwarded from each participating center to the ECLAMC headquarters. Retrospective review of data from the ECLAMC database encompassed information gathered between January 1989 and December 2012. We focused our evaluation on newborns diagnosed with hypospadias.

Data collection and quality management

Data collection followed a standardized methodology for the entire study period.

Population: Briefly, each participating center conducted daily surveillance of all newborns looking for a detectable CA. For every detected case, the following information was collected: mother's demographic data, prenatal and delivery information, and exposure to medications and toxic substances during pregnancy. Personnel trained specifically in the ECLAMC methodology at each institution conducted these assessments. For every enrolled case, the immediate next same-gender newborn was included as a control, collecting the same information.

Following approval of the study protocol by the ECLAMC board of directors and institutional ethics boards, information about all registered newborns with hypospadias and controls was gathered from the following countries: Argentina, Brazil, Bolivia, Chile, Colombia, Costa Rica, Ecuador, Paraguay, Peru, Uruguay and Venezuela. We excluded information from countries with incomplete registries, defined as those with more than 40% missing information in the database, and from those that failed to provide evidence of a continuous surveillance process over the study's timeframe.

Inclusion criteria

Isolated hypospadias (IH) cases were strictly defined as male newborns with an ectopic urethral meatus located along the ventral aspect of the penis and no other CA. Depending on location, these were further categorized as glanular, coronal, penile and scrotal. (Perineal and penoscrotal were included in this last category) (11). There were 29 megameatus intact prepuce variant cases and for that reason these patients were included in the glanular hypospadias group. Associated scrotal findings were also recorded. Newborns with associated anomalies were separately labeled as non-isolated hypospadias (NIH) cases. Each one of the associated anomalies was described in detail following the ECLAMC protocol for each of the different anomalies.

Statistical analyses

Global prevalence analysis: For each center prevalence rates were registered annually during study period then aggregated and individualized (per country) hypospadias prevalence patterns over time were calculated, testing the null hypothesis of a zero slope curve as evidence of lack of increase over time. Annual rate changes were estimated from the slope of a regression line drawn through the observed values. The signifi-

cance and linearity of the rate trend were tested following the Cochran and Armitage test to assess for gradient in proportions from several independent, quantitatively ordered samples (as reported by Fleiss) (12). This analysis was based on the formula:

Rate=Intercept + slope * year (based on y=b + mx; where m=slope, and b= intercept).

Exclusion of glanular cases prevalence analysis: To confirm the significance of the initial global trend we adjusted by hypospadias severity (exclusion of glanular cases) and performed a second analysis following a similar methodology as described before. We analyzed the population for trends, stratified based on country and hypospadias severity, as previously defined. In addition, this stratification was further evaluated for potential associations with NIH, reported as relative risks.

Secondary analysis adjusted for periods of time and countries: In order to give more support to the analysis, we verified our results and minimized biases by performing a secondary analysis for prevalence trends using a Poisson regression analysis, dividing the results for each country into periods of 5 years and adjusting for the effect of each hospital in the results. This was performed to reduce the effect of hospital registries in the results. The same analysis was performed after excluding all glanular cases for the reason mentioned above.

Associated anomalies analysis: For all NIH cases we analyzed the distribution of each associated abnormality and distribution based on hypospadias severity. In order to standardize assessment, we segregated associated anomalies by affected systems, i.e. genitourinary tract (GUT), gastrointestinal tract (GIT), limbs, facial anomalies (FA), cardiovascular (CV) and nervous system (CNS), abdominal wall (ABD), and others.

Lastly, we evaluated the impact of birth weight and maternal age in cases compared to controls using a Student T test. All analyses were conducted using Excel™.

RESULTS

During the study period, a total of 192 centers in 12 countries supplied data to the centralized information center. After excluding countries with missing or incomplete information the final analysis comprised 159 hospitals from six South American countries.

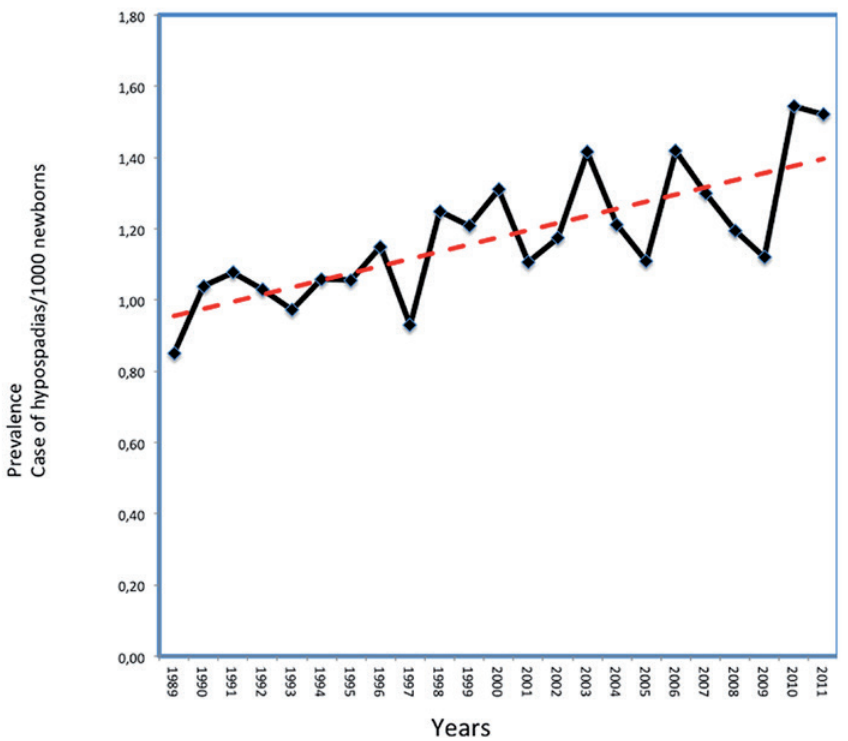
Global population analysis: Between 1989 and 2013, the above-mentioned centers conducted surveillance on 4.020.384 newborns, detecting a total of 4.537 hypospadias, and resulting in a global prevalence of 11.3 per 10.000 newborns. Trend analysis demonstrated a global increase in annual prevalence of 0.2 hypospadias cases per 10.000 newborns per year ($p<0.0001$). This translates into a 3.3% increase over the study period (Figure-1).

The distribution of hypospadias cases by severity is shown in Table-1. A total of 82.2% of the hypospadias cases were isolated, with the remaining 17.8% being associated with other reported anomalies. Although we found an increase in prevalence trends for distal hypospadias, these failed to reach statistical significance (1.3% in 24 years).

Analysis of the severity distribution and presence of associated anomalies during the entire study period revealed no association with distal cases (glanular hypospadias RR=0.93 [95% CI=0.85-1.01], coronal hypospadias RR=0.84 [95% CI=0.76-0.94]) whereas more proximal cases (penile hypospadias RR=1.64 [95% CI=1.33-2.03], scrotal hypospadias RR=2.49 [95% CI 1.80-3.47]) were significantly associated with other congenital anomalies.

When specifically evaluating NIH cases, we identified 809 patients with 1117 associated anomalies. On average there were 1.7 anomalies per NIH patient. The most common associated anomaly was cryptorchidism, representing 15.3% of associated anomalies, followed by minor facial anomalies (7.52%). Distribution analysis showed that after excluding minor facial anomalies, the most commonly affected system was the GUT (23.3%), followed by major FA (20.5%), CV (10%), CNS (8.7%), limbs (8.2%),

Figure 1 - Global prevalence trend of patients diagnosed with hypospadias at birth in 6 different countries in South America. The increasing trend is statistically significant. (p<0.001).



GIT (6.3%), other anomalies (6%), and ABD (3.2%) Table-2. We detected 13 (1.6%) cases of Down's syndrome associated with hypospadias (nine glanular, three coronal and two penile). There were eight (0.7%) cases associated with Edwards` syndrome. VACTERL association was diagnosed in five (0.4%) cases Table-2.

The average birth weight of hypospadias cases was 2914.2+/-621.6 grams, compared with 3251.1+/-753.6 for controls (p<0.001). These results were adjusted for gestational age. The average age of the mother at the time of delivery was 26.2 years old (SD+/-5.6y) for hypospadias cases and 26.3 years old (SD+/-7.4 y) for controls (p=0.27).

Secondary adjusted analysis: Chile, Brazil and Uruguay showed a statistically significant increase in prevalence, while Argentina,

Table 1 - Distribution of hypospadias according to severity and association with other anomalies.

Type of Hypospadias	Total	Isolated	Non-Isolated	RR (95% CI)
	(N - %)[1]	(N)	(N - %)[2]	
Glanular	2189 (48.3%)	1822	376 (16.8%)	0.93(0.85–1.01)
Coronal	1800 (39.7%)	1521	279 (15.5%)	0.84(0.76– 0.94)
Penile	388 (8.6%)	286	102 (26.3%)	1.64 (1.33–2.03)
Scrotal	148 (3.4%)	96	52 (35.1%)	2.49 (1.80–3.47)
Total	**4534 (100%)**	**3725**	**809**	

[1] = Percentage of each type of total; [2] = Percentage of Non-isolated cases of total cases in each type.

Table 2 - Five most common associated anomalies by system and its distribution according to the severity of hypospadias.

Systems	Malformation	Glanular	Coronal	Penile	Scrotal	Total	Percent
Genito-urinary tract	Cryptorchidism	64	58	34	15	171	15.31
(23.8%)	Hydronephrosis	7	9	5	2	23	2.06
	Renal Agenesis	10	5	1	0	16	1.43
	Hydrocele	8	5	0	0	13	1.16
	Inguinal Hernia	7	4	1	1	13	1.16
Facial	Cleft lip/palate	33	31	5	8	77	6.89
(20.5%)	Micrognathia	22	21	7	4	54	4.83
	Low ear implantation	19	18	5	2	44	3.94
	Preauricular pit	20	17	3	0	40	3.58
	Microtia	5	8	1	0	14	1.25
Cardiovascular	Ventricular septal defect	16	9	1	6	32	2.86
(10.0%)	Single umbilical artery	7	10	4	4	25	2.24
	Auricular septal defect	9	6	3	2	20	1.79
	Valvular anomalies	5	2	5	0	12	1.07
	Patent Ductus arteriosus	1	3	3	0	7	0.63
Central Nervous system	Hydrocephalus	32	17	15	9	73	6.54
(8.7%)	Spina bifida	9	3	1	2	15	1.34
	Other	3	4	1	1	9	0.81
Limbs	Polydactyly	19	11	1	1	32	2.86
(8.2%)	Hip Displasia	8	7	1	0	16	1.43
	Clinodactyly	3	6	3	0	12	1.07
	Arthrogryposis	4	2	2	1	9	0.81
	Camptodactyly	3	1	0	0	4	0.36
Gastrointestinal tract	Imperforated Anus	13	12	13	3	41	3.67
(6.4%)	Esophageal atresia	7	5	3	2	17	1.52
	Duodenal atresia	2	3	1	0	6	0.54
	Anal stenosis	2	1	0	0	3	0.27
	Ileal stenosis	0	1	0	0	1	0.09
Abdominal wall defects	Omphalocele	9	10	0	1	20	1.79
(3.0%)	Diaphragmatic anomalies	0	4	1	1	6	0.54
	Rectus abdominus diastasis	1	3	1	1	6	0.54
	Gastroschisis	2	1	0	1	4	0.36
Others	Redundant skin at the neck	3	2	9	9	23	2.06
(6.0%)	Nevus	7	7	0	0	14	1.25
	Hemangioma	9	3	1	0	13	1.16
	Supernumerary nipple	3	6	1	0	10	0.90
	Pterigium colli	3	3	0	1	7	0.63
TOTAL§		**466**	**391**	**163**	**97**	**1117**	

§ Total number of cases includes cases not shown in table.

Colombia and Venezuela did not in the initial analysis. In the secondary analysis with grouping by periods of time, the Poisson regression showed no statistically significant increase or reduction in prevalence. Results per country showed a statistically significant increase in Uruguay and Venezuela in different periods of time during the study period Table-3. Uruguay showed the longest period of significant increase over time. On the other hand, Argentina was the only country with a trend towards reduction since 1992.

After excluding glanular cases, there was no significant increase or reduction in prevalence globally during the study period. Trends by country showed a reduction or increase in prevalence during different periods Table-4. The most significant changes were in Uruguay where an 80% reduction was recorded between 2002 and 2011.

DISCUSSION

The present study provides epidemiological evidence that over the past two decades there has been an increase in hypospadias prevalence in different countries in South America and at different

Table 3 - Results from adjusted regression models evaluating temporal changes in the prevalence of total hypospadias from 1982 to 2011 in six countries of South America.

		Period					
Country [1]		1982 - 1986	1987 - 1991	1992 - 1996	1997- 2001	2002 - 2006	2007 - 2011
ARG	IRR	1.0	0.93	0.75	0.84	0.74	0.60
	95%CI	(Ref.)	(0.74 – 1.16)	(0.62 – 0.92)	(0.71 – 1.00)	(0.58 – 0.95)	(0.37 – 0.98)
	P	-	0.501	0.005	0.055	0.016	0.039
BRZ	IRR	1.0	0.97	1.03	1.10	1.05	0.90
	95%CI	(Ref.)	(0.73 – 1.29	(0.72 – 1.48)	(0.79 – 1.54)	(0.79 – 1.38)	(0.66 – 1.22)
	P	-	0.830	0.871	0.583	0.750	0.489
CHL	IRR	1.0	0.86	0.88	0.76	0.89	0.96
	95%CI	(Ref.)	(0.52 – 1.41)	(0.54 – 1.44)	(0.58 – 1.00)	(0.64 – 1.23)	(0.73 – 1.25)
	P	-	0.545	0.617	0.054	0.476	0.770
COL	IRR	1.0	0.83	1.05	1.63	0.93	0.76
	95%CI	(Ref.)	(0.30 – 2.31)	(0.26 – 4.16)	(1.33 – 2.08)	(0.61 – 1.40)	(0.42 – 1.36)
	P	-	0.798	0.018	0.166	0.403	0.348
URU	IRR	1.0	1.37	1.15	1.55	1.67	1.92
	95%CI	(Ref.)	(0.92 – 2.04)	(0.65 – 2.06)	(1.09 – 2.19)	(0.98 – 2.85)	(1.16 – 3.18)
	P	-	0.120	0.627	0.015	0.058	0.011
VEN	IRR	1.0	1.16	1.67	1.01	1.12	1.58
	95%CI	(Ref.)	(0.88 – 1.52)	(1.30 – 2.14)	(0.74 – 1.37)	(1.04 – 1.19)	(1.49 – 1.67)
	P	-	0.294	<0.001	0.935	<0.001	<0.001
TOTAL	**IRR**	**1.0**	**0.98**	**0.99**	**1.02**	**0.99**	**0.93**
	95%CI	(Ref.)	(0.83 – 1.16)	(0.81 – 1.21)	(0.85 – 1.22)	(0.84 – 1.16)	(0.75 – 1.14)
	P	-	0.847	0.931	0.869	0.893	0.485

[1] Countries: **ARG** = Argentina; **BOL** = Bolivia; **BRZ** = Brazil; **CHL** = Chile; **URU** = Uruguay; **VEN** = Venezuela; **IRR** = prevalence-rate ratios estimated from Poisson regression adjusted by hospital compared to the reference period; **95%CI** = 95% Confidence Interval; P value according the regression model; **ND** = no data coverage.

Table 4 - Number of total births, cases and rates (per 10,000 births) of severe hypospadias by period of time in six countries of South America.

		Period						
Country [1]		1982 - 1986	1987 - 1991	1992 - 1996	1997- 2001	2002 - 2006	2007 - 2011	Total
ARG	Total births (N)	193.692	276.788	311.582	230.747	204.210	99.746	1,316.765
	Cases (N)[2]	98	118	123	113	93	39	584
	Rate[3]	5.1	4.3	3.9	4.9	4.6	3.9	4.4
	95% CI	(4.1 – 6.2)	(3.5 – 5.1)	(3.3 – 4.7)	(4.0 – 5.9)	(3.7 – 5.6)	(2.8 – 5.3)	(4.1 – 4.8)
BRZ	Total births (N)	235.217	204.756	193.227	161.080	185.003	130.900	1,110.183
	Cases (N)[2]	225	162	220	240	284	176	1.307
	Rat[e3]	9.6	7.9	11.4	14.9	14.8	13.2	11.7
	95% CI	(8.4 – 10.9)	(6.7 – 9.2)	(9.9 – 13.0)	(13.1 – 16.9)	(13.1 – 16.7)	(11.3 – 15.3)	(11.0 – 12.3)
CHL	Total births (N)	48.130	85.278	69.042	117.328	149.650	70.318	539.746
	Cases (N)[2]	31	38	45	59	56	20	249
	Rate3	6.4	4.5	6.5	5.0	3.7	2.7	4.6
	95% CI	(4.4 – 9.1)	(3.1 – 6.1)	(4.8 – 8.7)	(3.8 – 6.5)	(2.8 – 4.9)	(1.6 – 4.2)	(4.0 – 5.2)
COL	Total births (N)	11.672	14.762	9.571	2.286	48.308	33.116	119.715
	Cases (N)[2]	12	10	5	4	36	21	88
	Rate[3]	10.3	6.8	5.2	17.5	6.2	6.0	6.8
	95% CI	(5.3 – 18.0)	(3.2 – 12.5)	(1.7 – 12.1)	(4.8 – 44.8)	(4.2 – 8.9)	(3.7 – 9.3)	(5.4 – 8.4)
URU	Total births (N)	52.476	54.050	25.831	59.397	19.573	3.810	215.137
	Cases (N)[2]	23	24	4	21	6	1	79
	Rate[3]	4.3	4.4	1.5	3.5	3.1	2.6	3.7
	95% CI	(2.8 – 6.6)	(2.9 – 6.6)	(0.4 – 3.9)	(2.2 – 5.4)	(1.1 – 6.7)	(0.1 – 14.6)	(2.9 – 4.6)
VEN	Total births (N)	54.036	78.084	84.782	68.686	93.605	28.532	407.725
	Cases (N)[2]	34	33	44	22	45	40	218
	Rate[3]	6.3	4.2	5.2	3.2	4.8	14.0	5.3
	95% CI	(4.4 – 8.8)	(2.9 – 5.9)	(3.8 – 7.0)	(2.0 – 4.8)	(3.5 – 6.4)	(10.0 – 19.1)	(4.7 – 6.1)

[1] Countries: **ARG** = Argentina; **BOL** = Bolivia; **BRZ** = Brazil; **CHL** = Chile; **URU** = Uruguay; **VEN** = Venezuela; **IRR** = prevalence-rate ratios estimated from Poisson regression adjusted by hospital compared to the reference period; **95%CI** = 95% Confidence Interval; P value aording the regression model.

points in time. This finding is consistent with reports from other parts of the World. To our knowledge, our analysis is one of few large-scale studies to specifically focus on the Latin American population, and is particularly valuable given the limited information in our region (13). The global prevalence reported herein (11.3/10.000 newborns) is very similar to other studies worldwide (9, 14, 15). In agreement with data reported by Palouzzi et al., showing an increase in prevalence in the United States during a similar period of time, we also detected an increase in prevalence in South America. A similar trend was recently reported in Sweden (16).

Importantly, the ECLAMC database includes distal hypospadias cases in the analysis (15-17), this provides a more accurate picture of the true prevalence of the condition. Also, the methodology of a standardized physical examination on all newborns for every involved center reduces the probability of over-diagnosis. Surprisingly, in stark contrast with other studies such as the Metropolitan Atlanta Congenital Birth Defects Program (MACDP), we did not detect an increase in more severe hypospadias cases over time in the global analysis, and noticed that

the trends favored higher prevalence at the expense of distal defects. Our detection procedure remained the same throughout the study confirming that these results are not a result of detection bias.

Other reports from developed countries have found an increase in prevalence of hypospadias of 1 to 4%, consistent with our increase of 3.3% (18). We acknowledge that an argument could be made for our findings being subject to artifact triggered by better reporting over time; however, the nature of our methodology dramatically reduced this potential bias. Nonetheless, the secondary analysis excluding glanular cases failed to support the initially detected increase. Specific regions showed an increase or reduction in prevalence. Given the latter there are important characteristics that deserve further evaluation, including the impact of industrialization, environmental pollution with different chemicals, and degree of urbanization in different regions with significant changes in prevalence. Our future studies will evaluate geographical and regional differences according to the secondary analysis results. It is interesting to highlight the different trends detected for different South American countries, raising the possibility of differences in socioeconomics and industrialization as an explanation for the reported trends. This hypothesis is supported by results from other studies, such as that of Li et al., who found that the global increase in prevalence in China was more evident in urban areas (19, 20).

The severity distribution in this report is consistent with previous literature, with a similar breakdown and predominance of distal hypospadias cases. Similarly, we corroborate literature that indicates an association between proximal hypospadias defects and other associated anomalies. For example, Leung et al. established that 20% of patients with hypospadias had other associated anomalies, similar to our results (20). Our data is novel in terms of exploring the degree of this association. For glanular and coronal hypospadias, we found no statistically significant results, whereas for penile and scrotal hypospadias we noted a positive association, with a gradient in favor of anomalies being more prevalent for the most proximal hypospadias defects. Some anomalies share similar molecular mechanisms that may explain their co-occurrence. A literature review on this topic provided little information. For instance, there are no reported associations between cleft lip/palate and hypospadias in the OMIM database, and many other studies have failed to detect links herein described between specific anomalies and proximal hypospadias. Although far from being conclusive and in clear need of further study, the information provided sets the basis for future studies specifically focusing on the presence of multiple anomalies in children with genitourinary congenital defects.

There are important shortcomings and limitations that deserve acknowledgement in the present study. We accept that the methodology employed is sub-optimal, as it did not include the entire population at risk (i.e. all male newborns in each country), leaving proportions of the population out of the analysis. Nevertheless, the surveillance protocol was strictly enforced, allowing us to capture information on a substantial number of babies over a long period of time. In addition, as previously mentioned, our findings may be driven by better detection compared to other surveillance systems, which conceptually can systematically favor good detection. Although plausible, the reported worldwide trends support the possibility of an increase in prevalence that has previously been reported for this large continental region but limited to specific regions for short periods of time.

Hospital-based sources of information may limit the final prevalence trend analysis due to differences in surveillance between centers during the study period. We tried to overcome this limitation with the secondary analysis. Lastly, it could be argued that the number of cases reported in this study could be biased due to detection in referral centers

dealing with high-risk pregnancies. This, however, was not the case, as the included healthcare institutions were busy general hospitals.

Despite these perceived limitations, we propose that our results have value given the standardized way that the data was collected and that the study was conducted during the entire period. All included centers provided uninterrupted surveillance of their patients during their participation in the study, and centers with missing information were excluded to improve the quality of our results. Ultimately, this information supports prevalence patterns described in the literature but the secondary analysis made it difficult to conclude that there has been a clear increase in this previously under-evaluated part of the world.

CONCLUSIONS

Our study is unique given the large geographical region, the study period, and sample size analyzed. We identified an increasing trend in hypospadias prevalence supported by a standardized methodology in specific regions and periods and novel information about associated congenital anomalies. However, subsequent analysis failed to find evidence supporting a global increase, particularly when severe hypospadias were selectively analyzed. Further studies are needed in order to determine whether environmental factors might be involved in specific regions.

ABBREVIATIONS

ABD = Abdominal wall
CNS = Central nervous system
CV = Cardiovascular
ECLAMC = Latin-American Collaborative Study of Congenital Malformations
FA = Facial anomalies
GIT = Gastrointestinal tract
GUT = Genitourinary tract
IH = Isolated hypospadias
NIH = Non-isolated hypospadias

CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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A new material to prevent urethral damage after implantation of artificial devices: an experimental study

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ABSTRACT

Objective: To validate the application of the bacterial cellulose (BC) membrane as a protecting barrier to the urethra.
Materials and Methods: Forty female Wistar rats (four groups of 10): Group 1 (sham), the urethra was dissected as in previous groups and nothing applied around; Group 2, received a 0.7cm strip of the BC applied around the urethra just below the bladder neck; Group 3, received a silicon strip with the same dimensions as in group 2; Group 4, had a combination of 2 and 3 groups being the silicon strip applied over the cellulosic material. Half of the animals in each group were killed at 4 and 8 months. Bladder and urethra were fixed in formalin for histological analysis.
Results: Inflammatory infiltrates were more intense at 4 months at lymphonodes (80% Grade 2), statistically different in the group 2 compared with groups 1 (p=0.0044) and 3 (p=0.0154). At 8 months, all samples were classified as grade 1 indicating a less intense inflammatory reaction in all groups. In group 2, at 8 months, there was a reduction in epithelial thickness (30±1µm) when com-pared to groups 1 (p=0.0001) and 3 (p<0.0001). Angiogenesis was present in groups 2 and 4 and absent in group 3. In BC implant, at 4 and 8 months, it was significant when comparing groups 4 with 1 (p=0.0159).
Conclusion: BC membrane was well integrated to the urethral wall promoting tissue remodeling and strengthening based on morphometric and histological results and may be a future option to prevent urethral damage.

ARTICLE INFO

Keywords:
Urinary Incontinence; Urethra; ethyl-2-hydroxyethylcellulose [Supplementary Concept]; Polysaccharides, Bacterial; Biocompatible Materials

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INTRODUCTION

Urinary incontinence (UI) represents a urological problem of growing prevalence, affecting about 2.5 to 40% of individuals, and in addition to determining a high economic cost to the health institutions, reveals itself as a devastating phenomenon, implying physical and psychological

misfortunes that affect the quality of life and self-esteem of the patient, contributing to social isolation from family, friends and sexual partner (1-3).

This is a symptom of various causes, and may be linked to bladder dysfunction (hyperactivity), weakness of the urethral rabdoesfincter (post prostatectomy radical injury or female UI) (4). The weakness of the external urinary sphincter usually comes from surgical trauma, neurological conditions and congenital anomalies (5).

Alternative treatments, conservative measures are provided (water restriction, use of absorbent, penile clamp, exercise and pelvic floor biofeedback, condom-catheter and pharmacological treatment) or more invasive approaches (implantation of sub-urethral sling or artificial urinary sphincter and the injection of periurethral bulking agents) (6, 7).

The problem associated with the anti-incontinence devices, especially artificial sphincters, is that to produce urethral occlusive force to achieve urinary continence, chronically occluded urethral wall, undergoes structural weakening, contributing to occurrence of atrophy and/or erosion (3). The urethral atrophy occurs by the decrease in the diameter of the urethra, leading to the insidious return of urinary incontinence. Erosion results in the migration of a component of anti-incontinence device into urethral lumen (8, 9) which is clinically expressed by perineal discomfort, vaginal discharge, hematuria, dysuria and urinary infection (10).

There is a growing interest in the search for alternatives that could reduce erosion rates and urethral atrophy after the implantation of these devices.

The use of biomaterials around urethra to act as protective barriers have been stimulated and currently used in clinical practice. In this regard, bacterial cellulose (BC), a biomaterial, has demonstrated efficacy as a driver cell and inductor in healing process (11, 12). Studies have proven its safety and efficacy in experimental and clinical models (11-13).

The objective of this study was to validate the application of bacterial cellulose membrane as urethral strengthening wrap, in an animal model in order to substantiate its clinical application as a protecting barrier to the urethra following the implantation of anti-incontinence devices based in morphometric and histological aspects of BC membrane integration.

MATERIALS AND METHODS

Animal model and experimental design

Wistar rats (n=40), weighing from 205g to 320g (250.12±17.26g), were used in the present study. Animals were kept in an appropriate environment with temperature and humidity control, day-night cycle artificially established for periods of 12 hours with free access to drinking and feed ad libitum. This study followed the principles governing the Code of Experimental Ethics and Laws for Protection of Animals, according to the standards in Brazil, receiving full approval from the Ethics Committee on Animal Experimentation of the Center for Biological Sciences, UFPE according to the process No. 23076.020552/2012-06.

Animals (n=40) were divided into four groups, with 10 animals per group, and named as follows: Group 1 (G1)=Sham; Group 2 (G2)=Bacterial Cellulose; Group 3 (G3)=Silicon; Grupo-4 (G4)=Bacterial Cellulose + Silicon. Half of the animals in each group were sacrificed at 4 and 8 months in order to evaluate short and long term outcomes.

Surgical Procedure

The animals were identified and weighed prior to the surgical procedure. The anesthetic procedure was performed according to the standard operating procedures of the Nucleus of Experimental Surgery.

Animals were attached to the operating Table in the supine position. The abdominal antisepsis was performed with chlorhexidine solution. An abdominal midline incision of ±3cm was done above the pubic symphysis. Access to the abdominal cavity occurred by sequential dissection of the anatomical planes. The bladder and urethra were then exposed. With the use of scissors, a small suburethral space (±5mm) was created, just below the bladder neck to give passage to silicon tape and/or BC membrane. The control

animals had the same procedure without placement of any material.

The silicon tapes and BC membrane were standardized in 3mm width and 7mm in length. Thicknesses were respectively 0.05mm for silicon and 0.54mm for BC. The membrane was wrapped around the urethra, and left in place. There was no need to fix it with suture since it is auto-adhesive. Contrariwise, silicon tapes after wrapped around the urethra were anchored with 5-0 Vycril. The abdominal wall was closed in two planes with 4-0 chromic catgut. Antibiotics were not used.

In the postoperative period, the animals were kept in an animal facility, in the same conditions. Five rats per group were killed four months after surgery. The second half (20 rats) had the same procedure after eight months. The killing process was done by intra peritoneal injection of sodium thiopental followed by intracardiac lethal dose of barbiturate. After the longitudinal access to the abdominal cavity, the bladder and urethra were removed en bloc.

Summary of bacterial cellulose membrane

The BC membrane used in our study is a polysaccharide obtained by bacterial synthesis from sugarcane molasses in a process developed at the Experimental sugarcane Station of the Federal Rural University of Pernambuco (UFRPE), involved in the study. This material was supplied packed in isopropyl alcohol and then sterilized with gamma rays at the Metrology Laboratory of the Department of Nuclear Energy (UFPE), following pre-established concepts of surgical sterilization. Silicon membranes were donated sterile and in the pre-established dimensions by Medicone© Innovation for Health Ltd. (Cachoeirinha, RS, Brazil).

Histological Analysis

The study material was sent to the Pathology Department which conducted the process of preparation of the slides. After fixed in formalin, cross section of the urethra was done just below the bladder neck. This segment was embedded in ethanol in increasing concentrations, diaphanized by xylene and impregnated with liquid paraffin. Fragments included for analysis were oriented in such a way that cross sections could be obtained perpendicular to the major axis of the urethra. The slides were stained with Hematoxylin-Eosin (HE) and Massom's trichrome.

The evaluation of the slides and the capture of the images was done with the bright field microscope and immunofluorescence Axio Imager. M2m/Zeiss, connected to the digital camera AxioCam HRc/Zeiss, responsible for transferring images to a computer. The capture of the images was done through the ZEN-2012/Zeiss software.

Measuring the intensity of the inflammatory reaction

All the measurements were performed by a blinded observer and the slides were randomly selected. Microscopic evaluation aimed to register the presence of neutrophils, lymphoplasmocytes, multinucleated giant cells (MNGC) and granulomatous infiltrate from the HE staining, in a semi-quantitative analysis. The intensity of the inflammatory reaction was graded as absent, mild, moderate and severe. This graduation was based on the following criteria [15]: 0-absent, with less than 5% of the examined area; 1-Lightweight, reaction involving between 5-25% of the examined area; 2-moderate, compromising between 25-70%; 3-Severe reaction, involving more than 70% of the area analyzed.

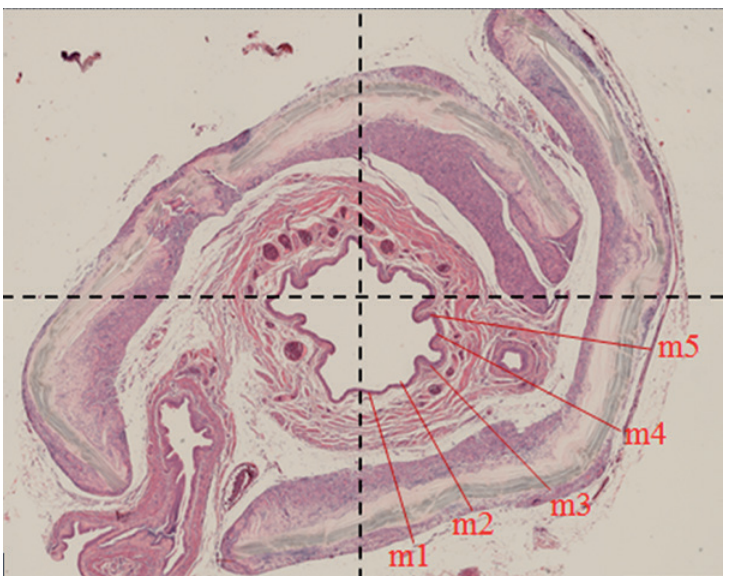
Measuring Urethral Wall Height

To perform the measurements of the urethral wall, the area was divided in four quadrants to obtain the average of 20 measurements for each animal, making up 5 measurements in each of them (Figure-1). The images were captured with an increase of 5x in slides stained by HE. The measurements were made through the program Image J45 (National Institute of Health, Bethesda, MD, USA). For the different groups, the measurement of the thickness of the urethral wall was made starting from the lamina propria from the area in close contact with the urothelium, until the last outer muscle layer.

Density of Blood Vessels

The density of blood vessels was made according to a method previously described for the quantification of microvessel density and the

Figure 1 - The urethral wall was measured in the lamina propria to the outer limit of the muscular layer according with the image. The area was divided in four quadrants to obtain the average of 20 measurements for each animal, making up 5 measurements in each of them.



development of in vivo implants (14). Thus, the bladder neck wall cuts images were stained with HE and captured at 400x magnification and loaded into the image J software version 1.45. A contiguous area of 10.000µm² implant was then drawn using the image J and all the vessels in the region bounded, in its light containing red blood cells, were counted.

The density of blood vessels was determined by dividing the number of vessels by the implant area and the results expressed as number of vessels/mm². In samples from normal regions of the urethral wall, the vessel density was assessed in the lamina propria, defined as the loose connective tissue between the urothelial basal membrane and the inner portion of the muscle layer.

Deposition of Collagen

Collagen intensity analysis was semi-quantitative and based on the same criteria described for analysis of the inflammatory response. The Masson method for staining provided an analysis of the concentration of collagen fibers present in the implant area and periurethral tissue.

Statistical analysis

Statistical analysis was done by using Graph Pad Prism 5.0® software (Graph Pad Software Inc. USA). The values of the above study parameters were statistically evaluated for verification and confirmation of conditions such as adhesiveness and integration of the cellulosic membrane urethral wall, compared with silicon tape.

Parametric continuous variables (height of urothelium and urethral wall) were compared using the t test. For non-parametric (density of blood vessels) the Mann-Whitney test was applied. Scores (adhesiveness and integration, collagen deposition and answers inflammatory)

were compared using the chi-square Pearson test. Statistical significance was set at $p \leq 0.05$. The statistical tests were performed using the GraphPad Prism 5.0 Program® (GraphPad Software Inc., USA).

RESULTS

After the killing process, it was observed during the dissections that there was greater adherence to adjacent tissues including epiploic migration in G3. The animals in G1 showed the lowest adherence. Removed parts were stored in 10% buffered formalin before being sent to the pathology department.

The urethral epithelium responded similarly to the presence of both materials when applied alone, at the 4-months analysis. In the group of eight months, there was a reduction of epithelium height in G2 (30±1µm) and increase in G3 (51±2µm) when compared to G1 group (45±1µm). However, in G4 it was observed reduction in epithelium height when compared to the group followed for 4 months (24±1) and 8 months (33±3) (Table-1).

Vasculogenesis in the implant with BC was similar between 4 and 8 months (4.44±0.57µm^2 and 4.93±1.32, respectively), with guidance from the peripheral region toward the central region (centripetal) of the remaining material when compared to the group receiving the silicon (4 months: 0.53±0.34µm^2, 8 months: 1.60±0.55µm^2) (Table-2).

Inflammatory infiltrates became more strongly present from the periphery towards the central portion of the implant in the BC group (80% of the samples with moderate inflammation, at 4 months), with lymph nodes, without having, however, the confluence of the same. At 8 months the inflammatory response was mild (100% of the samples graduated as 1) (Supplementary Table-1, Figure-2).

In the tissue insertion in the urethra/silicon (G3), inflammatory infiltrates were dispersed (33%) and absent in 67% of samples at 4 months and absent all samples at 8 months (Supplementary Table-1, Figure-2).

In animals receiving BC remaining material was observed in the central area of the implant and in the more peripheral regions, especially periurethral, with moderate formation of CGMN (at 4 months) and intense (after 8 months). Small and medium vessels were also observed in this area. In the groups that received silicon, it was observed a fibrous capsule with the presence of CGMN only at 4 months (Figure-3).

In G4 group, it was observed intense CGMN formation between these materials. Histologically, it was seen increased collagen deposition (thin mature collagen fibers) in the group with BC when compared to the group receiving silicon (Supplementary Table-2).

There was intense presence of fibroblasts, although not quantified, especially at the region

Table 1 - Urethral Wall and Urothelium Height.

Wall Height (mm)	Sham		Bacterial Cellulose		Silicone		BC + Sil.	
Follow-up	4 months	8 months	4 months	8 months	4 months	8 months	4 months	8 months
Urethral	0.40±0.07	0.51±0.15	0.51±0.08	0.53±0.10 [b]	0.58±0.12	0.41±0.10	0.50±0.14	0.37±0.11 [d,f]
Urothelium	0.041±0.003	0.045±0.001	0.041±0.003	0.030±0.001 [a,b]	0.034±0.002	0.051±0.002	0.024±0.001 [d,e,f]	0.033±0.003 [d,e,f]

Values expressed as Mean±SD. Student's t test significant if $p \leq 0.05$, to [a]BC≠Sham; [b]BC≠Silicone; [c]Silicone≠Sham; [d]BC≠BC+Sil.; [e]Silicone≠BC+Sil; [f]BC+Sil.≠Sham.
NA=Not applicable, **BC**=Bacterial Cellulose; **Sil.**=Silicone. Urethral wall p-value [b]=0.0249; [d]=0.0020; [f]=0.0414.
Urothelium height p-value at 4 months: [b]=0.058; [d]=0.0009; [e]=0.0103; [f]=0.0037 and at 8 months: [a]=0.0001; [b]<0.0001; [d]=0.3446; [e]=0.0020; [f]=0.0108.

Table 2 - Density of Blood Vessels in the implant area.

Density (μm^2)	Sham		Bacterial Cellulose		Silicone		BC + Sil.			
							4 months		8 months	
Follow-up	4 months	8 months	4 months	8 months	4 months	8 months	BC	Sil.	BC	Sil.
Vasculogenesis	1.90±0.36	2.27±0.43	4.44±0.57[a,b]	4.93±0.13[b]	0.53±0.34	1.60±0.55[c]	5.76±0.12[f]	0.68±0.37 [d,e,f]	2.48±0.10[f]	1.04±0.43 [d,e,f]

Values expressed as Mean±SD. Mann Whitney test, significant if p≤0.05, to [a]BC≠Sham; [b]BC≠Silicone; [c]Silicone≠Sham; [d]BC≠BC+Sil.; [e]Silicone≠BC+Sil; [f]BC+Sil.≠Sham. **NA**=Not applicable. BC: Bacterial Cellulose; **Sil.:** Silicone. Vasculogenesis p-value at 4 months: [a]0.0159; [b]0.0357; [f]0.0159 for BC; [d]0.0079; [e]0.0357; [f]0.0032 for Sil. and at 8 months: [b]0.0571; [c]0.0286; [f]0.0159 for BC and [d]0.0357; [e]0.0159; [f]0.0317 for Sil.

Supplementary Table 1 - The intensity of the inflammatory reaction.

Scores (%)	Sham		BC		Sil		BC + Sil.			
							4 months		8 months	
Follow-up (months)	4	8	4	8	4	8	BC	Sil.	BC	Sil.
0	100	100	0	0	67	100	0	25	0	100
1	0	0	20	100	33	0	25	0	20	0
2	0	0	80	0	0	0	75	0	80	0
3	0	0	0	0	0	0	0	0	0	0
p *value*			a0.0044 b0.0154	a0.0143 b0.0455			f0.0076	f<0.0001	f0.0078	f<0.0001

Values expressed as percentage (%). Chi-square test, significant if $p \leq 0.05$, to [a]BC≠Sham; [b]BC≠Silicone; [c]Silicone≠Sham; [d]BC≠BC+Sil.; [e]Silicone≠BC+Sil; [f]BC+Sil.≠Sham. **NA**=Not applicable. **BC**= Bacterial Cellulose; **Sil.**= Silicone.

adjacent to the remaining BC membrane and light close to the fibrous capsule involving the silicon implant (Figure-4).

DISCUSSION

Biomaterial is any natural or synthetic substance with the capacity to integrate into the tissue receiver, given a particular therapeutic purpose. Research community is engaged in the search for the ideal model. Among many requirements, it should be non-carcinogenic, easy acquisition and low cost and not associated with immunogenic reaction, qualifying as safe structure to several clinical applications (15). Collagen is the most common used matrix in medical practice, specially because it has proven adequate biocompatibility (16, 17).

In the present study, we used a cellulosic matrix, that has already been evaluated in biomechanical testing and biocompatibility as well as in cytotoxicity assays as a urethral reinforcing

Figure 2 - Photomicrography of the implant-tissue interface. A) Bacterial Cellulose group and B) Silicone group after 8 months. (∆) BC) (∆) Area where the silicon implant had occupied; (→) Blood Vessels; (→) vascular congestion; (→) inflammatory cells. Staining with hematoxylin and eosin. Note: Silicone implants did not resist the histological processing, for that reason the area appears as an empty space.

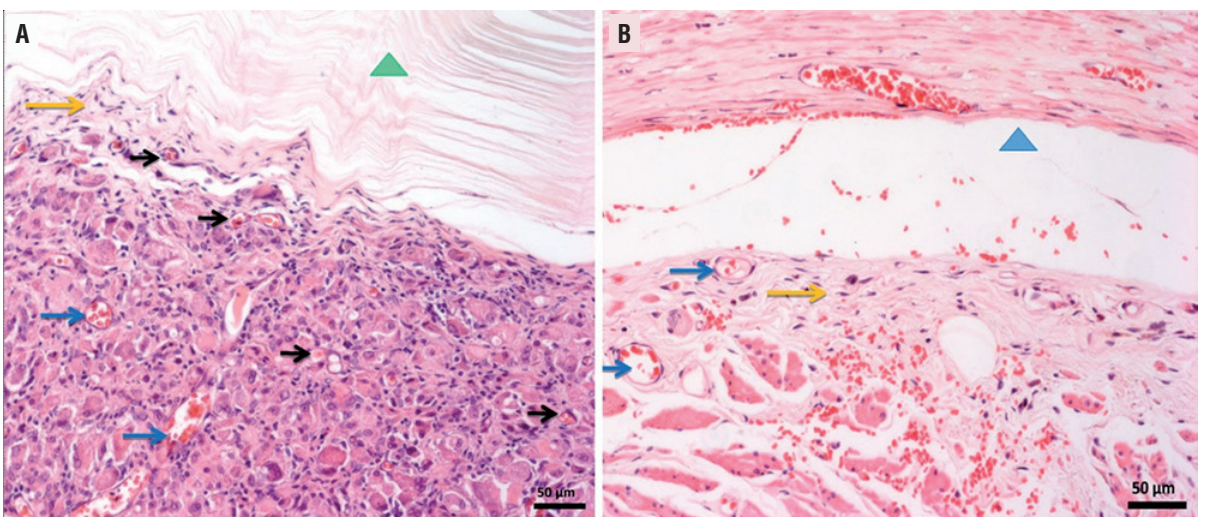
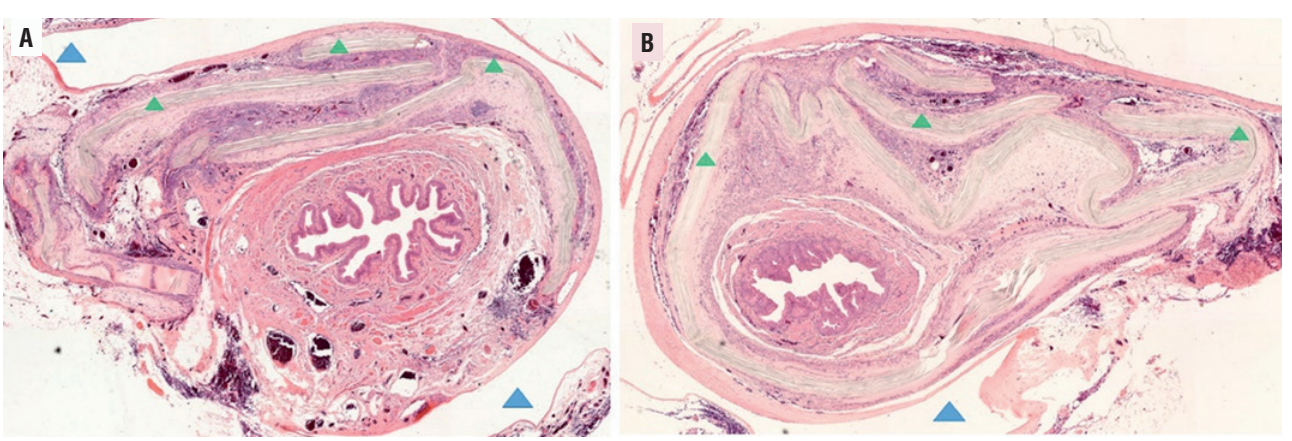


Figure 3 - Rats urethra photomicrograph cross section showing bacterial cellulose plus silicon implant after 4 months (A) and after 8 months (B). (∆) Area where the silicon implant had occupied; (∆) bacterial cellulose implants. Staining with hematoxylin and eosin (10X). Note: Silicone implants did not resist the histological processing, for that reason the area appears as an empty space



wrap in rats by evaluating its integration and remodeling to the host tissue (13, 18, 19). One of the main questions that we consider of interest in this evaluation is the change in the urethral wall layers thickness. We found that at 4 months, statistically similar behavior concerning urethral thickness when comparing BC (G2) and silicon (G3). This trend changes at 8 months, when there has been an increase of this measurement in the G2 when compared to the silicon group (p=0.0249). The results in G1 are supported in the literature in an article in which the author, employing porcine retail of small intestinal submucosa (SIS) in urethral fistula repair induced in rabbits, produced similar results, however without detailing the steps in this process and its effects on the urethral layers (20). The urethral wall, when measured from the lamina propria to the outer li-

Supplementary Table 2. Deposition of the Collagen in Periurethral area.

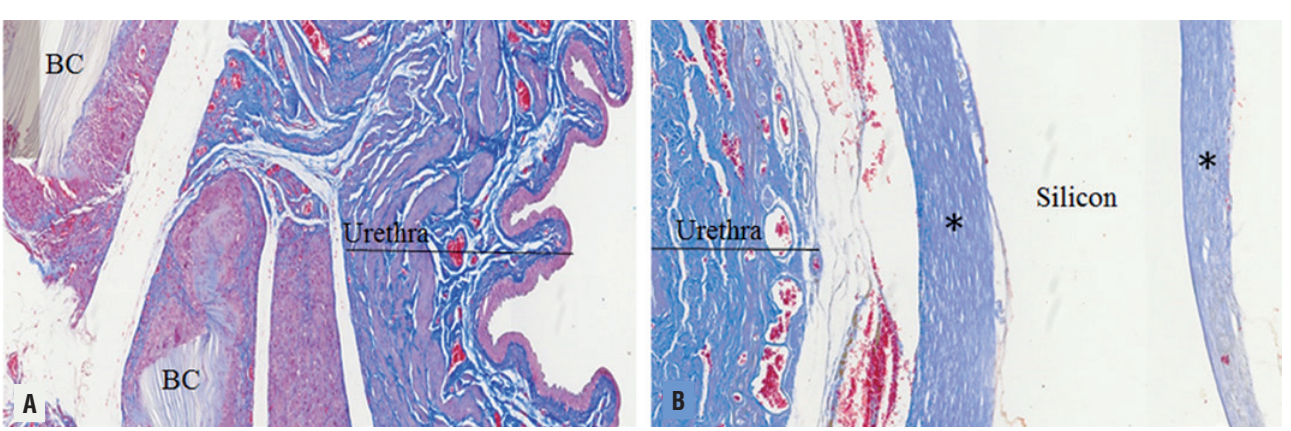
Scores (%)	Sham		BC		Sil		BC + Sil.			
							4 months		8 months	
Follow-up (months)	4	8	4	8	4	8	BC	Sil.	BC	Sil.
0	NA	NA	0	0	0	0	0	0	100	0
1			100	0	0	0	100	0	0	0
2			0	100	0	0	0	0	0	0
3			0	0	100	100	0	100	0	100
***p** value*			[a]0.0044 [b]0,0154	[a]0.0143 [b]0,0455			[f]0.0076	[f]<0.0001	[f]0.0078	[f]<0.0001

Values expressed as percentage (%). Chi-square test, significant if p≤0.05, to [a]BC≠Sham; [b]BC≠Silicone; [c]Silicone≠Sham; [d]BC≠BC+Sil.; [e]Silicone≠BC+Sil; [f]BC+Sil.≠Sham. **NA**=Not applicable. **BC:** Bacterial Cellulose; **Sil:** Silicone.

mit of the muscular layer, showed very different pattern. The later results (8 months) showed that there is significant structural gain in the isolated BC group when compared to the other groups that received the implant.

In G4 implant group when compared to the Sham group, it was found significant reduction in the layers thickness. This was the group where this phenomenon was more evident. On the other hand, the inflammatory reaction has declined significantly changing from moderate to light, in both opportunities of observation. In the BC group, the presence of CGMN and residual cellulosic material could be seen at eight months. In addition, at this time, presence of blood vessels of small and medium-sized implant in the region was evident. The G3 group showed lower inflammatory reaction than the G2, but a fibrous capsule formation was found at 4 months. The double implant group shows intense CGMN infiltration between silicon and urethra. It is well known that silicon is reactive, inducing foreign body reaction

Figure 4 - Photomicrography of collagen deposition in bacterial cellulose implants (A) and silicone implants (B), both after 8 months. Masson's Trichrome Staining Protocol for Collagen Fibers (20X). *Thick collagen fibers. Note: Silicone implants did not resist the histological processing, for that reason the area appears as an empty space



and encapsulation. However, this behavior has been seen when the material is implanted in host tissue intimacy, such as in slings (20-22).

Vasculogenesis was significantly present in the BC group when compared to groups where the silicon was used in both phases of the study. This was also observed in G1. At the same time, the presence of collagen at the implant area with the BC, ranged from mild to moderate, with the presence of mature collagen fibers, while identifying infiltrated unquantified fibroblasts around the residual cellulose. This was different from that observed in the groups, which received silicon alone, where there was intense collagen deposition, at different periods of measurement. It is well known that the biocompatibility of a material is validated by the degree of inflammatory reaction of vascularized tissue at the implant area (23).

This answer can range from inconsistent with the presence of fibrotic capsule formed and no new vessels, to consistent, with integration of mature collagen, vasculogenesis and inflammatory reaction that does not compromise the integration of the material to the host (20, 21).

The process of biocompatibility is induced through the recruitment of inflammatory cells in the implant area, which in turn contributes to the onset of neovascularization and consequent nutritional intake, necessary for the survival and transformation of the implanted matrix (23-25). It is worth to emphasize that in the inflammatory process itself cellularity is gradually replaced by fibroblasts and, therefore, collagen deposition, serves as a platform basis for the appearance of a new tissue structure (24).

In our experiment, BC induced the appearance of fibroblasts at the implant area, which was in equilibrium with collagen deposition, without tendency to encapsulation. This phenomenon was observed with the use of silicon. The literature on this subject is scarce. However, clinical study results suggest that silicon in direct contact with the urethral tissue increases the risk of erosion, regardless of the pressure applied on the area (25).

In other studies with the BC, it has been found intense inflammatory response in the early phase of the incorporating process, which decreases with time, as more collagen is incorporated without evidence of encapsulation. This phenomenon is interpreted by the authors as a sign that endorses the condition of biocompatibility of the material (11, 12).

Analyzing the different phases of BC incorporation to the host tissue our final impression is that the level of collagen deposition parameters, vasculogenesis and structural increase in urethral wall thickness lead to the belief that this may represent new perspective for longer survival of artificial implants in urology and other areas. Since the BC membrane is a natural product obtained from renewable source with low cost, may be considered as a future new material to be used in urology area.

CONCLUSIONS

The BC was well integrated into the tissue receptor, contributing to its architecture remodeling and strengthening based on the morphometric and histological aspects of BC membrane integration. We can therefore accept that this new material may be a future option to prevent urethral damage.

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CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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The effect of tadalafil therapy on kidney damage caused by sepsis in a polymicrobial septic model induced in rats: a biochemical and histopathological study

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ABSTRACT

Introduction: Sepsis is an inflammatory reaction to bacteria involving the whole body and is a significant cause of mortality and economic costs. The purpose of this research was to determine whether tadalafil exhibits a preventive effect on sepsis in a septic model induced in rats with cecal ligation and puncture (CLP).
Materials and Methods: Rats were randomly separated into groups, 10 rats in each: (i) a sham (control) group, (ii) an untreated sepsis group, (iii) a sepsis group treated with 5mg/kg tadalafil and (iv) a sepsis group treated with 10mg/kg tadalafil. A polymicrobial sepsis model was induced in rats using CLP. Rats were sacrificed after 16h, and blood and kidney tissues were collected for biochemical and histopathological study.
Results: Levels of the inflammatory parameter IL-6 decreased significantly in the sepsis groups receiving tadalafil in comparison with the untreated sepsis group ($p<0.05$). In terms of histopathology, inflammation scores investigated in kidney tissues decreased significantly in the sepsis groups receiving tadalafil compared to the untreated sepsis group ($p<0.05$). In addition, levels of creatinine and cystatin C measured in septic rats receiving tadalafil were lower by a clear degree than in septic rats ($p<0.05$).
Conclusion: In this study, tadalafil exhibited a preventive effect for sepsis-related damage by suppressing inflammation in serum and kidney tissue of septic rats in a polymicrobial sepsis model induced with CLP.

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Keywords:
Acute Kidney Injury; Tadalafil; Kidney Failure, Chronic; Renal Insufficiency

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INTRODUCTION

Sepsis, defined as a systemic inflammatory reaction to bacterial agents, continues to be a significant cause of mortality and economic burdens (1). Although the cause is not fully understood, according to the most commonly accepted view, an immune response is thought to be initiated in association with various endotoxins, pro-inflammatory cytokines and mediators resulting from monocyte activity against bacteria (2). Injury that may occur due to sepsis is associated with the severity of the response. When the inflammatory response initiated is prolonged or extended, several pro-inflammatory cytokines and mediators, and particularly free oxygen radicals (ROS), may become involved and cause organ losses and death (3). Impairment of the oxidant-antioxidant

balance resulting from an increase in oxidants is responsible for the development of these injuries. MPO and MDA are often used as oxidant parameters and SOD and CAT activity as antioxidant parameters in studies. Ritter et al. reported that CAT activity is related to sepsis mortality (4).

Despite advances in technology and medicine, the prevalence of sepsis and the mortality it causes are increasing. Sepsis is responsible for approximately 30% of hospital deaths in America, and is the 11th most common cause of death (5). Extensive research is therefore being performed across the World aimed at the treatment of sepsis. Several drugs have been tested for therapeutic purposes in sepsis models.

Tadalafil which has a long-lasting effect inhibits phosphodiesterase enzyme-5 (PDE5) which hydrolyzes cGMP. Therefore, inhibition of PDE5 with tadalafil increases the level of both cGMP and nitric oxide in medium (6). PDE5 enzyme inhibition has been shown to increase local levels of cAMP and cGMP, which cause expansion in the vascular system (7). Additionally, PDE5 inhibitors cause the activation of the enzymes endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS), which are involved in the synthesis of nitric oxide (NO). cAMP and cGMP that have important roles in several intracellular events such as inflammation are significant second messengers (8). Various studies concerning PDE5 inhibitors have reported different effects. These include intracellular NO production inhibiting platelet aggregation (9), improved renal blood flow in a model of partial ureteral obstruction induced in rats (10) and protection against tubular apoptosis (11).

Tadalafil increases levels of cGMP/NO, which is involved in several physiological processes. It can reduce inflammation in the vascular system and kidney tissue by reducing inflammatory response occurring during the course of sepsis. Our hypothesis was that tadalafil would protect against sepsis by suppressing inhibition in the vascular system and kidney tissue in a polymicrobial sepsis model.

This study investigated whether tadalafil would protect against sepsis-related injury in the kidney and vascular system in a polymicrobial sepsis model.

MATERIALS AND METHODS

Animals

Forty male albino Wistar rats with initial weights of 225-250gr were used in this study. Following approval from Ondokuz Mayıs University Faculty of Medicine local ethical committee, the animals were taken from the Samsun Experimental Animals Research and Application Center (EARAC). Rats were housed in steel cages, with a maximum 5 animals to a cage, throughout the experiment. They were allowed ad libitum access to food and water. The room in which they were housed was adjusted to a 12h light, 12h dark cycle. In addition, a split air conditioner was used to endure that the room temperature did not exceed 22±1^{o}C. (Temperature was measured with a thermometer and humidity with a hygrometer). Ventilation was provided by a room aspirator.

Experimental plan

Rats were randomly separated into groups, 10 rats in each. Groups were established as follows; Group 1 sham (control; n: 10), Group 2: untreated sepsis (cecal ligation and puncture (CLP)) group, Group 3: sepsis group receiving 5mg/kg tadalafil (CLP+TAD 5mg; n: 10) and Group 4: sepsis group receiving 10mg/kg tadalafil (CLP+TAD 10mg; n: 10). Each group was placed into separate cages following this procedure.

Sepsis model

The CLP polymicrobial sepsis model was established by ligating the distal part of the rat cecum and two-hole was punctured in the cecum. Ketamine hydrochloride (i.m. 60mg/kg) and xylazine (i.m. 10mg/kg) were used for pre-surgical anesthesia. Once the surgical area had been cleaned, an abdominal incision was made. The cecum was isolated and ligated distally to the ileocecal valve with 3/0 silk. The part of the cecum located distally to the ligation was pierced in two places using a 22 gauge needle and then replaced in the cavity. Then, the surgical site was closed properly with an absorbable suture.

Once the wound had been closed, 1% lidocaine was injected into the incision line for analgesia. In the sham group, laparotomy only was

performed, and the cecum was merely raised and then replaced, with no piercing. The procedure was concluded with anatomical closure. All rats were given subcutaneous 2mL/100g saline solution for fluid solution during surgery and for 6h post-surgically. Once the procedure was completed, tadalafil suspended in saline solution was administered by gavage in doses of 5 or 10mg/kg. Taking account of variations in blood flow occurring during sepsis and the related variations in stomach and intestinal absorption, high tadalafil doses of 5 and 10mg/kg were used. These doses have been previously used in the literature and are known to be used reliably in the literature. The same amount of saline was given to the sham and CLP groups, which did not receive tadalafil. Access to food, but not water, was prevented for 16 after surgery. On the 16th hour postoperatively, rats were sacrificed by exsanguination under anesthesia and blood and tissue specimens were collected. Studies have shown that depending on the size of the incision opened in the intestines to induce sepsis, frequently rats die of sepsis within 12-20 hours of incision. In studies, it is known that hyperdynamic, hyperinsulinemic, high blood lactate levels and hypermetabolic situations occur about 10 hours after CLP. Without treatment in this period, the majority die. Studies by Hubbard et al. showed that nearly 16 hours after CLP rats were hypoglycemic, hypoinsulinemic, had hypodynamic blood circulation and increased blood lactate levels. As a result, it was necessary to provide treatment within this period and to administer a higher dose than the classic dose. Thus the mortality rates may be reduced. The kidneys were quickly extracted and washed in iced saline. Half of each kidney was then stored at-80°C for biochemical investigation, while the other half was placed in 10% formalin solution for histopathological study (12-15).

Biochemical analyses in tissue and serum

After being kept at room temperature for 30 min, blood specimens placed into gel-containing tubes were centrifuged at 3000g for 15 min. Serum samples separated from the centrifuged blood were stored as aliquots with left kidney tissue at-80^{o} C until analysis.

Creatinine assay

Creatinine measurements in serum were performed using Abbott brand commercial creatinine (Creatinine Lot No: 78067UN14) kits on an Abbott Architect C8000 autoanalyzer in the Ordu University Faculty of Medicine biochemistry laboratory.

Cystatin-C measurement

Cystatin-C measurements in serum specimens were performed using a rat-specific ELISA kit (Boster, EK1109, Lot No: 6551072708). Specimens were investigated with 1:50 dilution. Results were read at a 450nm wavelength on an ELISA reader (Biotek, ELX 800).

Malondialdehyde assay

Tissue MDA measurement was performed using rat-specific ELISA kits (Bluegen: E02M0023, Lot No: 20140825). Prior to homogenization, tissues were washed in PBS buffer (0.02mol/L, pH 7.0-7.2) to remove all blood. Homogenization was then performed (100mg tissue/1mL PBS). The tissue homogenates were frozen and thawed twice and then centrifuges at 1500g for 15 min. The kit prospectus was applied with 1:10 dilution in the supernatants. The results were read at a 450nm wavelength. These were standardized with the mg protein level in the homogenate. MDA levels in serum specimens were measured using the same kit without dilution.

Superoxide dismutase activity measurement

SOD activity in rat kidney tissue and serum was measured using spectrophotometry (CAYMAN; 706002). Blood was removed by washing the tissue with PBS, and specimens were then placed in homogenization buffer (20mM HEPES, 1mM EGTA, 210mM mannitol, 70mM sucrose; ph 7.2). The homogenates obtained were centrifuged at 1550g for 5 min (4^{o} C). These were used for SOD measurement following 1:250 dilution. Results were read at a 450nm wavelength. Rat serum specimens were studied using the same method with 1:50 dilution.

Myeloperoxidase activity measurement

MPO measurement in rat kidney tissue and serum was performed using competitive enzyme

immunoassay ELISA kits (Bluegene; E02M0032, Lot No: 20140825). Prior to homogenization, tissues were washed in PBS buffer (0.02mol/L, pH 7.0-7.2) to remove all blood. Homogenization was then performed (100mg tissue/1mL PBS). The tissue homogenates were frozen and thawed twice and then centrifuged at 1500g for 15 min. The supernatant was diluted 1:100 and the results read at 450nm on an ELISA reader. Serum specimens were studied with 1:10 dilution.

Catalase activity measurement

CAT activity was measured using spectrophotometry with a commercial kit (Cayman; 707002). Tissues placed in homogenization tissue (50mM potassium phosphate, 1mM EDTA, pH 7.0) were homogenized in ice (5mL buffer/1gram tissue) and then centrifuged at 10.000g for 15 min. Enzyme activity was measured by diluting the supernatants obtained 250 times. Results were read at a 540nm wavelength. Rat sera were studied without dilution.

Interleukin-6 measurement in serum

IL-6 assay in rat serum was performed using a rat-specific IL-6 ELISA kit (Boster; EK0412, Lot No: 1331047708). Results were read at a 450nm wavelength.

Serum procalcitonin measurement

Serum PCT measurements were performed on a VidasBioMerieux device using Vidas Brahms PCT (Lot: 1003569530) kits.

Tissue protein measurement

Protein assay in homogenates prepared as described above was performed spectrophotometrically using the Bradford method (Thermoscientific; 23200). Measurements in specimens diluted 1:20 were performed at 595nm, and the protein level in a specimen was determined based on a standard.

Histopathologic evaluation of kidney tissue

The kidney tissues were fixed with buffered 4% paraformaldehyde and embedded in paraffin. The paraffin-embedded kidney specimens were cut into 5µm sections and processed for anti-Macrophage antibody (MAC387, sc-66204, Santa Cruz Biotechnology, Inc.) immunohistochemistry and Masson trichrome staining.

Masson's Trichrome Stain Procedure

The slices were processed for assessment of the structural alterations in the kidney tissue (tubule damage, vacuolization, tubular dilation and cast structure, infiltration, interstitial changes and renal corpuscle morphology) with Masson's trichrome staining. The deparaffinized sections were treated with Weigert's iron hematoxylin, hydrochloric acid, ponceau acid fuchsin, phosphomolybdic acid, phosphotungstic acid and aniline blue solutions. The sections were washed in distilled water and rinsed in glacial acetic acid solution to take off excessive the aniline blue. Finally, the sections were processed with ethanol and xylene, followed with mounting media (16).

Mac387 Antibody Staining

The sections were stained with Staining System (sc-2017, Santa Cruz Biotechnology, Inc.) for the Mac387 (1:200 dilution) antibody. The paraffin sections were deparaffinized in xylene. The sections were treated with hydrogen peroxide solution for remove the endogen peroxidase activity. The sections were dehydrated with alcohol series, processed with the normal serum. The sections were treated by the primer antibody on overnight at+4°C. The sections were treated by the secondary antibody and then with enzyme reagent. The sections were visible using AminoethylCarbazole (AEC) as chromogen and mounted for analysis. The negative controls of sections processed without the primary antibody (16). Eventually, the stained sections were photographed with a camera (Leica DFC295) put on a microscope (Leica DM2500) and saved as Tagged Image File Format (TIFF).

Inflammation-based score for kidneys

The sections were acquired systematically and sampled randomly. They were scored depending on the quantity of inflammation in the kidney as follows: 0: no cells; 1: a few cells; 2: many cells and 3: numerous cells in the kidney.

The sham group kidneys exhibited normal-appearing glomeruli, tubules and interstitium when stained with Masson's trichrome. The kidney injury in the untreated sepsis group was significantly greater compared with the sham group and the 5 and 10mg/kg tadalafil+sepsis groups (P<0.05). In the untreated sepsis group, glomerulus deformities, tubular injury and inflammatory cell infiltration in the glomeruli and interstitium were observed. Tubular injury, defined as tubular dilatation, vacuolization, lumen enlargement, epithelial flattening and cell sloughing, was also seen in the untreated sepsis group. Tubular dilatation, vacuolization and epithelial flattening decreased and no inflammatory cell infiltration was observed in the glomeruli or interstitium in the 5 and 10mg/kg tadalafil+sepsis groups (Figure-1). Mac387 immunoreactivity scores in the untreated sepsis group were significantly higher compared with those in the sham, 5 and 10mg/kg tadalafil+sepsis groups (P<0.05). Mac387 immunoreactivity scores in the 5 and 10mg/kg tadalafil + sepsis groups were not significantly different from those in the sham group. There was also no significant difference between immunoreactivity scores in the 5 and 10mg/kg tadalafil + sepsis groups (Figure-2).

Statistical analysis

The data were firstly analyzed using Levene's test and the Shapiro-Wilk test for equality of variance and for normality assumption, respectively. Normally distributed continuous data (CAT, SOD, MPO, MDA, cystatin C and creatinine) were expressed as sample size, mean with standard deviation and secondly analyzed by using one-way ANOVA and the Tukey test. Finally, when homoscedasticity and normality were not confirmed by the tests (P<0.05) for IL-6, the Kruskal-Wallis H test and Steel-Dwass multiple comparisons were used to determine the differences among the four groups. Non-normally distributed data were expressed as sample size, mean, standard deviation, median values, interquartile range (IQR), minimum and maximum values. If the p-value is under 0.05, results are considered statistically significant.

RESULTS

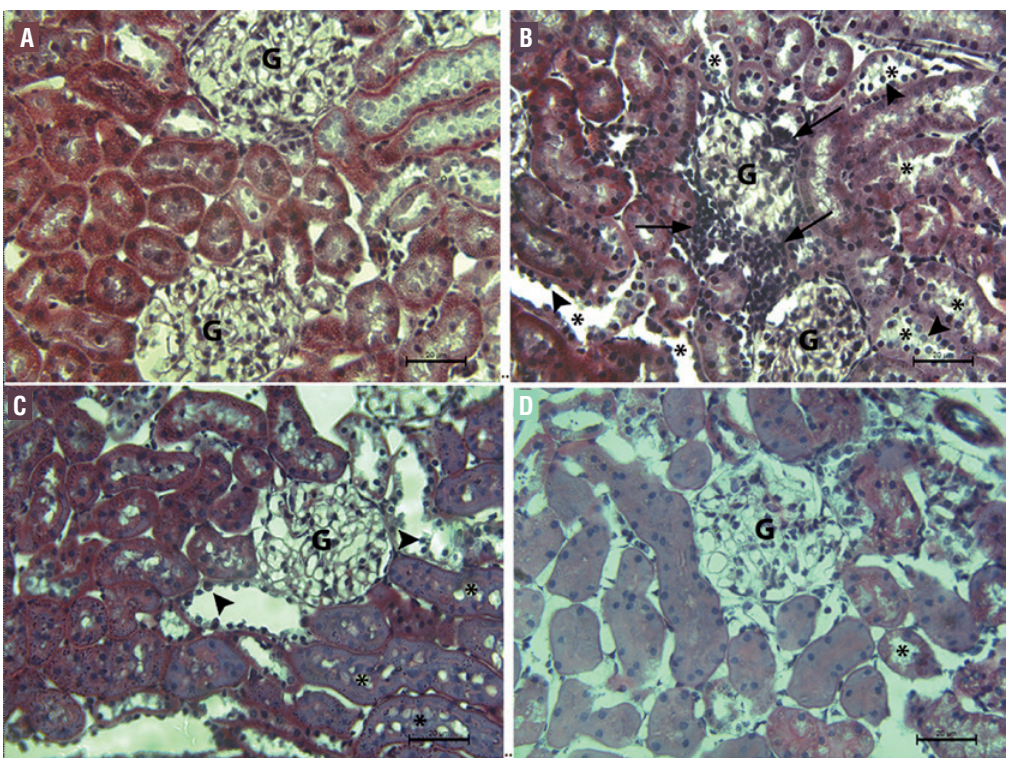
Oxidant and antioxidant activity were measured in serum and kidney tissue in order to determine the level of injury occurring during the septic process. MPO and MDA activities were measured as oxidant markers and SOD and CAT activities as antioxidant markers. SOD, CAT, MPO and MDA measurement results from kidney tissue are shown in Table-1 and results from serum are given in Table-2.

IL-6 levels used as a marker of inflammation were measured in serum. These were significantly lower in the groups receiving 5 and 10mg/kg tadalafil than in the untreated sepsis group (p<0.05) (Table-3). Creatinine and cystatin C levels were measured in serum in order to evaluate kidney functions. Creatinine and cystatin C levels measured in serum were significantly higher in the untreated sepsis group compared to the sham group (P<0.001) (Table-4). In addition, cystatin C and creatinine levels were significantly lower in the groups receiving 5 and 10mg/kg tadalafil than in the untreated sepsis group (p<0.001). There was no difference in effect between 5 and 10mg/kg doses.

Histopathological results

The sham group kidneys exhibited normal-appearing glomeruli, tubules and interstitium when stained with Masson's trichrome. The kidney injury in the untreated sepsis group was significantly greater compared with the sham group and the 5 and 10mg/kg tadalafil+sepsis groups (P<0.05). In the untreated sepsis group, glomerulus deformities, tubular injury and inflammatory cell infiltration in the glomeruli and interstitium were observed. Tubular injury, defined as tubular dilatation, vacuolization, lumen enlargement, epithelial flattening and cell sloughing, was also seen in the untreated sepsis group (Table-5). Tubular dilatation, vacuolization and epithelial flattening decreased and no inflammatory cell infiltration was observed in the glomeruli or interstitium in the 5 and 10mg/kg tadalafil+sepsis groups (Figure-1). Mac387 immunoreactivity scores in the untreated sepsis group were significantly higher compared with those in the sham,

Figure 1 (A-D) - Representative photomicrographs of Masson's trichrome staining of the renal cortex in the sham group (a) and sepsis group (b) 5mg/kg (c) and 10mg/kg tadalafil+sepsis groups (d). Arrows indicate areas of inflammatory cell infiltration.



Arrow heads indicate flattened tubular epithelial cells in dilated tubules. * indicate tubular dilatation and vacuolization. **G**, glomerule

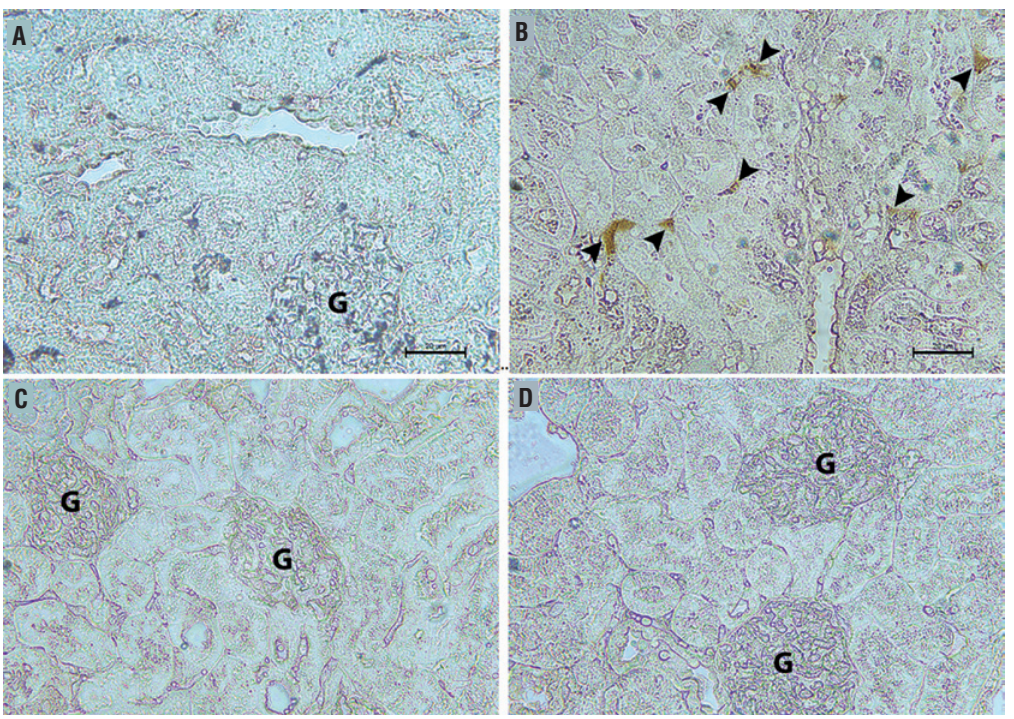
5 and 10mg/kg tadalafil+sepsis groups (P<0.05). Mac387 immunoreactivity scores in the 5 and 10mg/kg tadalafil+sepsis groups were not significantly different from those in the sham group. There was also no significant difference between immunoreactivity scores in the 5 and 10mg/kg tadalafil+sepsis groups (Figure-2).

DISCUSSION

Both biochemical and histopathological analysis in serum and kidney tissue revealed that tadalafil exhibits a protective effect against kidney injury in sepsis. This protective effect may derive from suppression of oxidative stress and inflammation involved in tissue injury caused by polymicrobial sepsis. Its anti-inflammatory effect was also proved by a decrease in inflammatory scores in kidney tissue at histopathological analysis. Tadalafil may exhibit this effect by increasing the levels of cGMP and NO that decrease in association with cytokines and mediators occurring during the septic process.

There is a known association between severity and duration of inflammatory response against a bacterial agent and the resulting tissue injury (17). The bacterium encounters macrophages where it enters the body, and these seek to eliminate it. If the bacterium cannot be quickly eliminated, the inflammatory process is converted into a systemic response through pro-inflammatory mediators and cytokines such as tumor necrosis factor (TNF-α), interleukin-1 (IL-1) and IL-6 released by the macrophages (18). Neutralization of mediators released from macrophages in peritonitis induced in a rat model has been shown to prolong survival and reduce organ loss (19). In our study, levels of the pro-inflammatory mediator IL-6 de-

Figure 2 (A-D) - Representative photo micrographs of Mac387 immuno histochemistry of the renal cortex in the sham group (a) and sepsis group (b) 5mg/kg (c) and 10mg/kg tadalafil+sepsis groups (d).(a) and sepsis group (b) 5mg/kg (c) and 10mg/kg tadalafil+sepsis groups (d). Arrows indicate areas of inflammatory cell infiltration.



Arrow heads indicate Mac387 positivecells. **G** = glomerule

Table 1 - Effect of tadalafil on CAT, SOD, MPO and MDA levels identified in renal tissue with sepsis.

	Sham group	Sepsis group	Sepsis+TAD (5mg)	Sepsis+TAD (10mg)
n	10	10	10	10
CAT (nmol/dk/ugprot)	1.93±0.61	1.64±0.37	2.22±1[a*]	1.98±0.48[a*]
SOD (U/mg protein)	2.69±2.29	1.64±0.24	1.98±0.7[a*]	2.67±2.7[a*]
MPO (ug/mg protein)	54.48±35.64	57.08±40.54	38.89±24.55[a*]	34.91±21.08[a*]
MDA (ng/mg protein)	0.42±0.07	0.45±0.08	0.43±0.13[a*]	0.44±0.07[a*]

Values are mean±standard deviation in each group.

n = number of rats; [a] = compared with sepsis group; *p>0.05.

creased in the groups receiving tadalafil compared to the untreated sepsis group. In addition, inflammatory scores in kidney tissue decreased at pathological analysis. This shows that tadalafil suppresses the inflammatory process. Kermarrec et al. reported that nitric oxide suppressed inflammation in a model of endotoxic lung injury induced in rats, and this was compatible with our own data (20). Tadalafil may exhibit this anti-inflammatory effect by increasing cGMP/NO levels.

The production of oxygen radicals with pro-inflammatory effects such as endothelial injury, accumulation of oxidants and the formation of chemotactic factors increase during sepsis (21). Other significant effects include the inactivation of the enzyme guanylyl cyclase (sGC), which plays

Table 2 - Effect of tadalafil on CAT, SOD, MPO and MDA levels in rat serum with sepsis.

	Sham group	Sepsis group	Sepsis+TAD (5mg)	Sepsis+TAD (10mg)
T	10	10	10	10
CAT (umol/dk/mL)	0.54±0.28	0.51±0.28	0.90±0.65[a*]	0.73±0.38[a*]
SOD (U/mL)	2.78±2.78	1.53±0.26	1.65±0.35[a*]	1.57±0.42[a*]
MPO (ng/mL)	16.5±9.05	16.8±13.5	14.43±7.31[a*]	14.02±9.84[a*]
MDA (ng/mL)	3.42±0.99	3.54±0.79	3.16±0.58[a*]	3.29±0.74[a*]

Values are mean±standard deviation in each group.
[a] = compared with sepsis group; *p>0.05.

Table 3 - Variations in IL-6 levels between the groups.

Groups	n	Mean	SD	Median	IQR	Min	Max	χ^2value	P-value
Sham group	10	4.46	1.95	4.03[c]	2.64	1.86	8.68	33.71	<0.001
Sepsis group	10	27826.67	33993.74	2469.14[a]	66886.76	1053.5	70207.5		
Sepsis+TAD (5mg)	10	53.5	32.79	40.9[b]	43.74	17.98	114.5		
Sepsis+TAD (10mg)	10	40.2	32.25	31.45[b]	29.42	114.5	107.8		

a,b,c = indicate differences between the groups (p<0.01).

Table 4 - Effect of tadalafil on cystatin C and creatinine levels in rats serum with sepsis.

	Sham group	Sepsis group	Sepsis+TAD (5mg)	Sepsis+TAD (10mg)
n	10	10	10	10
Cystatin C (ng/mL)	511.17±57.72	988.27±288.44	527.58±92.851[a*]	575.31±41.931[a*]
Creatinine (mg/dL)	0.48±0.06	0.56±0.14	0.51±0.03[a***]	0.50±0.02[a**]

Values are mean±standard deviation in each group.
[a] = compared with sepsis group; *p<0.001, **p<0.01,***p<0.05.

Table 5 - Histopathological variations in renal tissue.

	Sham group	Sepsis group	Sepsis+TAD (5mg)	Sepsis+TAD (10mg)
n	6	6	6	6
Mean inflammation score	0	3	1[a*]	1[a*]
Tubular degeneration	0	3	2[a*]	2[a*]

n = number of rats; [a] = Compared with CLP group; *p<0.05

an important role in the synthesis of NO and thus a decrease in cGMP/NO levels (22). If sepsis cannot be halted, the inflammatory process becomes increasingly severe and results in organ dysfunction (23). The kidney is the principle vital organ injured. Sepsis is the underlying cause in approximately 50% of cases developing acute kidney failure, and approximately 70% of these cases are fatal. Protection of the kidneys in sepsis is reported to be of vital importance in terms of survival (24).

The protective effect of tadalafil in septic rats in this study, and particularly the protective effect in terms of kidney functions, was shown by creatinine and cystatin C levels measured in serum and at histopathological analysis in renal tissue. Özbek et al. reported, in agreement with our results, that tadalafil exhibited a protective effect against kidney damage (25). This may be attributed to the cGMP/NO level that decreases during sepsis being protected by tadalafil. One previous study showed that NO levels in kidney tissue decrease in renal injury (26). In the light of all these findings, cGMP/NO has a clear suppressing effect on the inflammatory process. The decrease in IL-6 levels measured in the groups receiving tadalafil compared to the untreated sepsis group also corroborates this. Muzaffar (27) and Vignozzi et al. (28) also reported the anti-inflammatory effects of PDE5 inhibitors.

Another system that is compromised during the inflammatory process is the coagulation system. This has been shown in studies involving septic animals and humans (29). One of the most commonly seen coagulation disorders, disseminated intravascular coagulation (DIC), is particularly observed in cases of severe sepsis and has been shown to be an independent risk factor for mortality in these patients (30). Yoshimoto et al. reported that intracellular NO production inhibited aggregation and caused antithrombotic effects (31). This may constitute another of the beneficial effects of tadalafil in sepsis. In association with this, we think that one of the beneficial effects of tadalafil in sepsis is that it improves coagulation disorders impaired in sepsis. The fact that no evaluation of coagulation was performed in this study represents one of its limitations.

Very few studies have investigated the use of PDE5 inhibitors in sepsis. In one such study, Cadirci et al. investigated the effect of sildenafil in a sepsis model induced with CLP through biochemical analysis in serum and through histopathological analysis in lung and kidney tissue. At the end of the study they reported that sildenafil reduced inflammation in both kidney tissue and serum and had a protective effect against sepsis (14). Our findings in terms of renal outcomes were similar. In contrast to that study, however, we determined no difference between the untreated sepsis group and the sepsis groups receiving tadalafil in terms of antioxidant and oxidant parameters measured in serum and kidney tissue.

There are various limitations to this study. One is that coagulation parameters were not investigated. Another is that cGMP and NO levels in serum and kidney tissue were not established.

CONCLUSIONS

In conclusion, this study shows that tadalafil has a protective effect in the kidney and vascular system in sepsis through improvement in antioxidant and oxidant parameters investigated in both serum and kidney tissue. A protective effect on kidney functions was determined both through the measurement of creatinine and cystatin C levels in serum and through pathological investigation in kidney tissue. For the protective effect against sepsis, 5mg/kg dose of tadalafil was found to be sufficient. There was no difference in effect between 5 and 10mg/kg doses. We think that these findings are important for various clinical conditions, such as sepsis, and now need to be supported by further studies. If the beneficial effects identified in animals can also be shown in humans, then tadalafil may represent a new dawn in the treatment of sepsis and erection therapy.

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CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Effects of nitric oxide inhibitors in mice with bladder outlet obstruction

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ABSTRACT

Purpose: To investigate the lower urinary tract changes in mice treated with L-NAME, a non-selective competitive inhibitor of nitric oxide synthase (NOS), or aminoguanidine, a competitive inhibitor of inducible nitric oxide synthase (iNOS), after 5 weeks of partial bladder outlet obstruction (BOO), in order to evaluate the role of constitutive and non-constitutive NOS in the pathogenesis of this experimental condition.
Materials and Methods: C57BL6 male mice were partially obstructed and randomly allocated into 6 groups: Sham, Sham + L-NAME, Sham + aminoguanidine, BOO, BOO + L-NAME and BOO + aminoguanidine. After 5 weeks, bladder weight was obtained and cystometry and tissue bath contractile studies were performed.
Results: BOO animals showed increase of non-voiding contractions (NVC) and bladder capacity, and also less contractile response to Carbachol and Electric Field Stimulation. Inhibition of NOS isoforms improved bladder capacity and compliance in BOO animals. L-NAME caused more NVC, prevented bladder weight gain and leaded to augmented contractile responses at muscarinic and electric stimulation. Aminoguanidine diminished NVC, but did not avoid bladder weight gain in BOO animals and did not improve contractile responses.
Conclusion: It can be hypothesized that chronic inhibition of three NOS isoforms in BOO animals leaded to worsening of bladder function, while selective inhibition of iNOS did not improve responses, what suggests that, in BOO animals, alterations are related to constitutive NOS.

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INTRODUCTION

Nitric oxide (NO) is synthesized from its precursor L-arginine via NO synthases (NOS), which exist in three isoforms: neuronal (nNOS), endothelial (eNOS) and inducible (iNOS). The first ones are constitutively expressed and produce small quantities of NO and the last one is induced by cytokines, infection or other stimuli and produces large amounts of NO. Mice obstructed for 5 weeks exhibit morphologic and functional disorders and these changes were attributed to enhanced expression of iNOS early after obstruction, which would be responsible for improving oxygenation during obstruction-induced ischemia (1). Although NO can be produced by several sources, including endothelial cells, nerves, smooth muscle and urothelium, studies demonstrated that major sites of NO

release were urothelium and afferent nerves (2).

Treatment of BOO rats with aminoguanidine, a competitive inhibitor of iNOS, has shown good results, as decreases in iNOS ameliorated functional and fibrotic changes in the bladder (3, 4). The same consequences have been observed in iNOS knockout mice (1, 4). Treatment with L--NAME, a non-selective competitive inhibitor of NOS, inhibited generation of nitrotyrosine, which is produced by nitrogen reactive species and, as consequence, improved bladder contraction (5). However, another study showed that a feeding diet rich in L-arginine was beneficial for rabbits with 2 weeks of severe BOO (6).

In the current study, we investigated lower urinary tract changes in mice treated with L-NAME or aminoguanidine after 5 weeks of BOO, since these drugs represent non-selective and selective NOS inhibitors, respectively.

MATERIALS AND METHODS

Animals and Experimental Groups

The experimental protocols were approved by the Ethical Principles in Animal Research adopted by the Brazilian College for Animal Experimentation (COBEA, No 2030-1). Male C57BL6 mice (25-30g), 8-9 weeks old, were used and randomly allocated into six experimental groups: Sham (Sham-operated), Sham + L-NAME (Sham that received L-NAME), Sham + aminoguanidine (Sham that received aminoguanidine), BOO (bladder outlet obstruction), BOO + L-NAME (BOO that received L-NAME) and BOO + aminoguanidine (BOO that received aminoguanidine). Doses of L--NAME (150mg/Kg) and aminoguanidine (20mg/Kg) were chosen according to previous study (7). All animals were placed into individual cages with food ad libitum and received drugs given in the drinking water immediately after surgery for a period of 5 weeks, when all in vitro and in vivo studies were performed.

Surgical Procedures

Animals were anesthetized by intraperitoneal injection of ketamine (2mg/Kg) and xylazine (30mg/Kg) and placed in the supine position. A lower midline abdominal incision was made and, after exposure of the bladder and proximal urethra, partial BOO was created by tying a 6-0 nylon suture around the urethra. A 0.6mm diameter tubing was used as a guide to prevent total urethral occlusion. In Sham group, identification of bladder and proximal urethra was done, with no further surgical manipulation. Both abdomen muscles and skin were closed with a 6-0 nylon suture.

In vivo and in vitro bladder functional assessment

Bladder weights

Bladders were carefully withdrawn and weighted. Also, mice were weighted for normalization of values and the ratio bladder weight (mg) to body weight (g) was obtained. This parameter was used to verify if BOO animals exhibited bladder weight increase, showing that pathological alterations due to this condition, imposed by surgery, has occurred.

Filling Cystometry

Animals were anesthetized by intraperitoneal injection of urethane (1.2g/Kg) and a lower midline incision was made to expose the bladder. A 25-gauge butterfly cannula was inserted into the bladder dome and was connected via a 3-way stopcock to a pressure transducer (AD Instruments, Sydney-NSW, Australia) and infusion pump (Harvard Apparatus, Holliston, MA). After bladder emptying, saline solution at room temperature was infused at a rate of 0.6mL/h until detrusor equilibration and then, counted for 30 minutes after the end of the first micturition cycle. Pressure registers were obtained by a Powerlab 4/30 data acquisition (6.0 system, AD Instruments, Sydney--NSW, Australia). Maximum detrusor pressure was defined as the peak of intravesical pressure during a micturition. Bladder capacity was determined as the total infused volume immediately before the first micturition. Compliance was measured as bladder capacity divided by the maximum detrusor pressure minus baseline pressure. Non-voiding contractions (NVC) were defined as increases in intravesical pressure (>4mmHg) not associated with release of urine through urethra. Micturition

frequency was measured considering the number of micturition cycles within 30 minutes. Threshold pressure was defined as the pressure immediately before micturition (8). Bladders of mice used for cystometry were not used in the other experiments.

Tissue bath

Animals were killed by inhalation of CO_2. Bladders were rapidly dissected, removed, weighted and put immediately in a physiologic saline solution of Krebs-Henseleit (NaCl 117mM, $NaHCO_3$ 25mM, $C_6H_{12}O_6$ 11mM, KCl 4.7mM, $NaHPO_4$ 1.2mM, $MgSO_4 7H_2O$ 1.2mM and $CaCl_2 2H_2O$ 2.5mM, pH 7.4). Two longitudinal muscle strips with intact urothelium were obtained from the bladder body, measuring 2 x 2 x 10mm and were mounted in 10mL organ baths containing Krebs-Henseleit solution at 37°C bubbled with a gas mixture of 95% O_2 and 5% CO_2. Changes in isometric force were recorded using a computer Software (PowerLab v.7.0 system, AD Instruments, Sydney-NSW, Australia). The resting tension was adjusted to 5mN at the beginning of the experiments, the equilibration period was 60 minutes and the bathing medium was changed every 15 minutes.

Cumulative concentration-response curves to the full muscarinic agonist Carbachol (1ηM to 30μM) were constructed using one-half log unit. Nonlinear regression analysis to determine the pEC_{50} was carried out using Graph Pad Prism (Graph Pad Software, Inc., San Diego, CA, USA) with the constraint that F=0. All concentration–response data were evaluated for a fit to a logistics function in the form: $E = E_{max} / ([1 + (10c/10x)n] + F)$, where E is the maximum response produced by agonists; c is the logarithm of the EC_{50}, the concentration of drug that produces a half-maximal response; x is the logarithm of the concentration of the drug; the exponential term, n, is a curve-fitting parameter that defines the slope of the concentration–response line, and F is the response observed in the absence of added drug. Data were normalized to the wet weight of the respective urinary bladder strips, and the values of E_{max} were represented in mN/mg.

Bladder strips were stimulated with Electric Field Stimulation (EFS at 2, 4, 8 and 16Hz), 50V, during 0.2ms and an interval of 2 minutes between pulses. Responses were recorded and maximal tensions compared, expressed by mN/mg.

Statistical analysis

The values recorded were mean±SEM, and the statistical significances were determined by One-Way ANOVA followed by the Tukey test. $P<0.05$ was considered significant. InStat© was used for statistical analysis.

Drugs

No-Nitro-L-arginine methyl ester hydrochloride (L-NAME), urethane, Carbachol and aminoguanidine hemisulfate were obtained from Sigma Chem. Co. (St Louis, MO).

RESULTS

Bladder weights

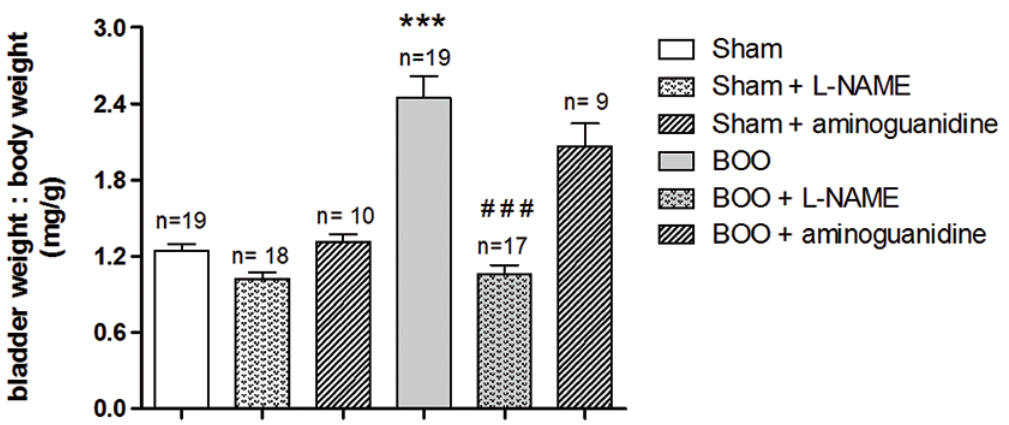
Bladder outlet obstruction (BOO) animals showed increased bladder weight to body weight ratio ($P<0.001$), almost 2-fold when compared to Sham animals. Oral treatment with L-NAME in BOO animals decreased bladder weight to body weight ($P<0.001$). Treatment with aminoguanidine had no significant effect on this parameter (Figure-1).

Filling Cystometry

A total of 9 Sham, 6 Sham + L-NAME, 6 Sham + aminoguanidine, 13 BOO, 6 BOO + L-NAME and 7 BOO + aminoguanidine were evaluated. Figure-2 represents cystometric profiles obtained after 5 weeks of surgical procedures for all experimental groups.

BOO animals showed more NVC and higher compliance than Sham. Treatment with L-NAME increased non-voiding contractions and diminished bladder capacity and compliance in BOO animals. BOO mice treated with aminoguanidine showed less NVC and lower bladder capacity. Although a tendency of increase in micturition frequency caused by L-NAME and aminoguanidine in BOO mice was observed, there were no statistical differences for this parameter, for maximum detrusor pressure or threshold pressure. Sham mice treated with aminoguanidine showed lower bladder capacity (Figure-3 and Table-1).

Figure 1 - Bladder weight to body weight ratio in Sham and BOO mice treated or not with L-NAME or aminoguanidine for 5 weeks.



***P<0.001 versus Sham. # # # P<0.001 versus BOO.

Figure 2 - Representative Cystometrograms in Sham (A), Sham + L-NAME (B), Sham+aminoguanidine (C), BOO - Bladder outlet obstruction (D), BOO + L-NAME (E) and BOO + aminoguanidine (F) groups. Arrows indicate micturition peaks.

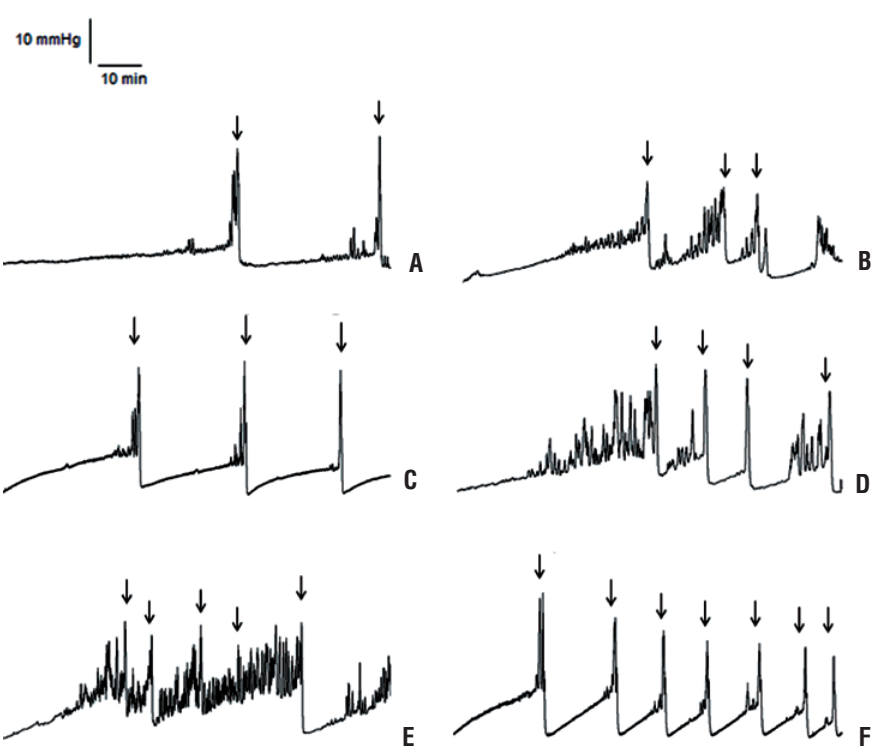
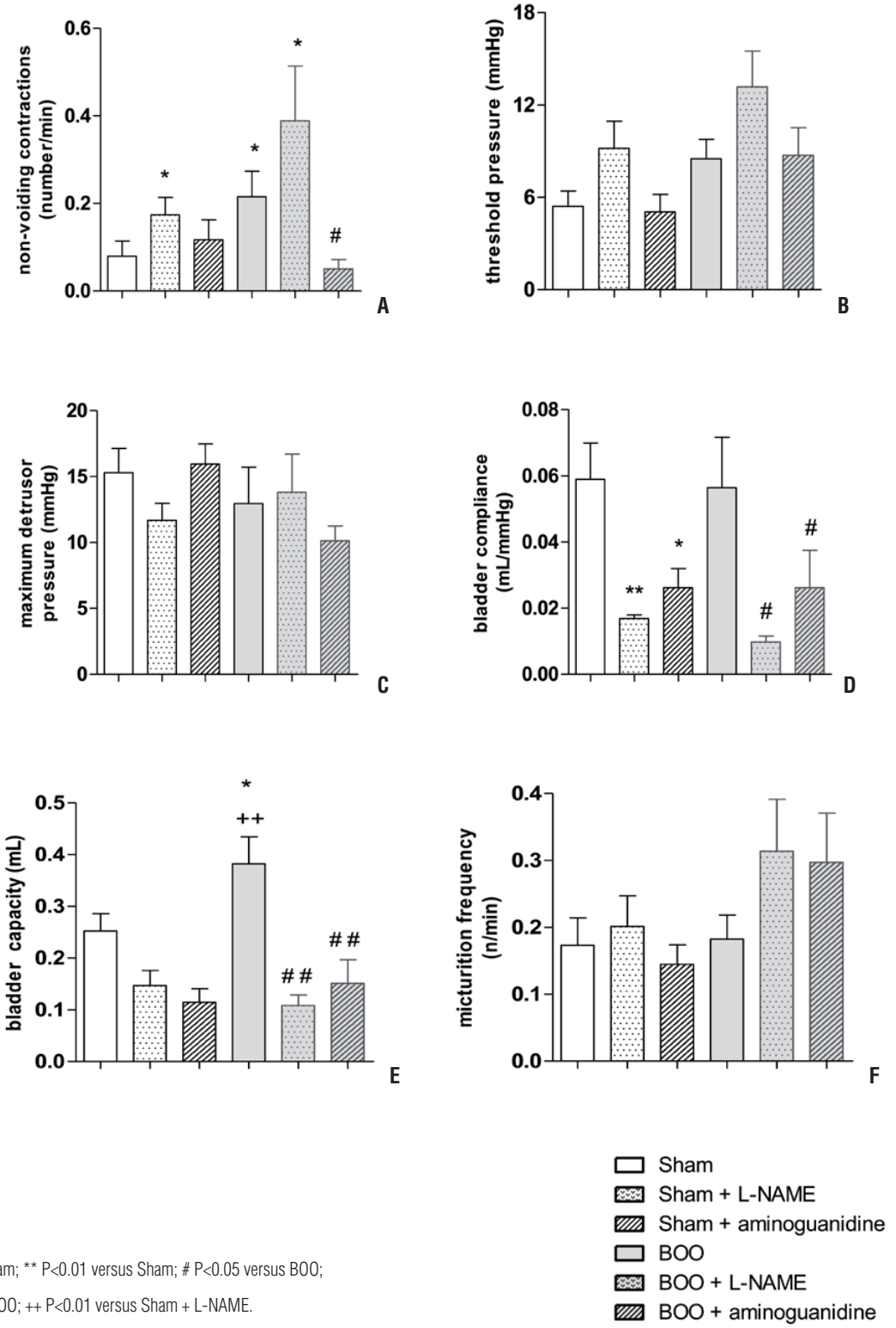


Figure 3 - Cystometric parameters in Sham and BOO mice treated or not with L-NAME or aminoguanidine for 5 weeks: non-voiding contractions (A), threshold pressure (B), maximum detrusor pressure (C), compliance (D), bladder capacity (E) and micturition frequency (F).



* P<0.05 versus Sham; ** P<0.01 versus Sham; # P<0.05 versus BOO;

P<0.01 versus BOO; ++ P<0.01 versus Sham + L-NAME.

Table 1 - Cystometric parameters evaluated for each experimental group. NVC: non-voiding contractions.

Experimental Group	NVC (n/min)	Micturition frequency (n/min)	Threshold pressure (mmHg)	Maximum detrusor pressure (mmHg)	Bladder capacity (mL)	Compliance (mL/mmHg)
Sham (n=9)	0.079 ± 0.034	0.173 ± 0.041	5.406 ± 0.985	15.288 ± 1.859	0.252 ± 0.034	0.059 ± 0.011
Sham + L-NAME (n=6)	0.174 ± 0.039	0.201 ± 0.046	9.168 ± 1.753	11.673 ± 1.306	0.147 ± 0.029	0.017 ± 0.001 **
Sham + aminoguanidine (n=6)	0.117 ± 0.045	0.145 ± 0.029	5.045 ± 1.146	15.933 ± 1.542	0.115 ± 0.026	0.026 ± 0.006 *
BOO (n=13)	0.215 ± 0.059 *	0.183 ± 0.036	8.496 ± 1.294	12.965 ± 2.741	0.382 ± 0.052 * ++	0.056 ± 0.015
BOO + L-NAME (n=6)	0.388 ± 0.125 *	0.314 ± 0.078	13.155 ± 2.329	13.808 ± 2.905	0.108 ± 0.021 ##	0.001 ± 0.001 ##
BOO + aminoguanidine (n=7)	0.050 ± 0.021 #	0.297 ± 0.074	8.717 ± 1.785	10.133 ± 1.114	0.151 ± 0.046 ##	0.026 ± 0.011 ##

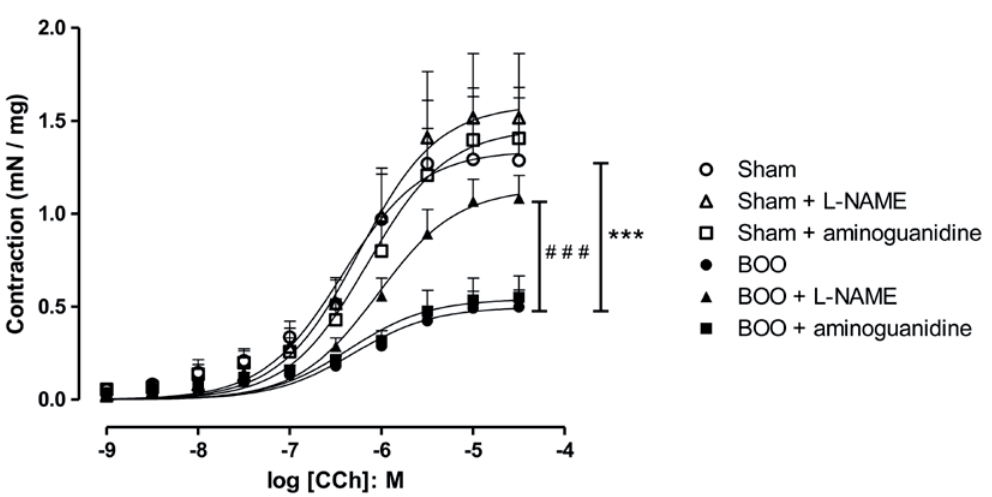
* P<0.05 versus Sham; # P<0.05 versus BOO; # # P<0.01 versus BOO; ++ P<0.01 versus Sham + L-NAME.

Tissue bath

Cumulative addition of the muscarinic agonist Carbachol (1ηM to 30μM) to bladder preparations produced concentration-dependent contractions (Figure-4 and Table-2). Sham + L-NAME mice showed higher responses compared to Sham. BOO and BOO + aminoguanidine groups exhibited a significant decrease in maximal responses to this agonist. The pEC_{50} values for Carbachol did not change significantly for any group.

EFS induced a frequency-dependent increase in the amplitude of contractions in isolated bladder smooth muscle. BOO animals showed lower contraction when compared to Sham animals at all frequencies evaluated. L-NAME BOO treated animals showed decreased contraction at 2 and 4Hz compared to Sham + L-NAME and increased contraction compared to BOO mice at 8 and 16Hz. There was no difference for aminoguanidine treated animals (Figure-5 and Table-3).

Figure 4 - Bladder smooth muscle contractions to Carbachol in Sham and BOO mice treated or not with L-NAME or aminoguanidine for 5 weeks.



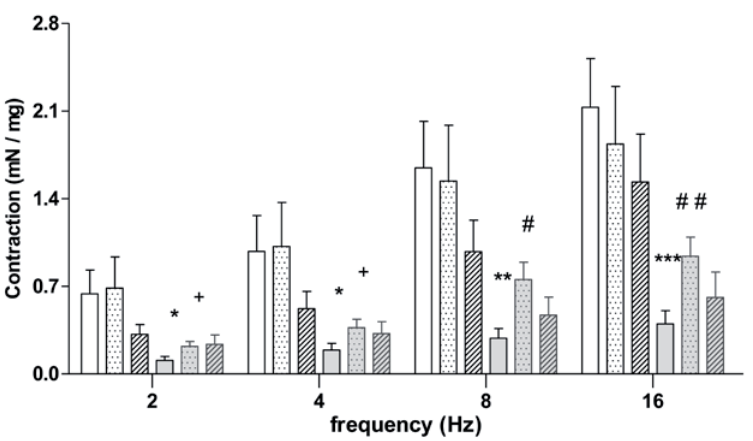
*** P<0.001 versus Sham. # # # P<0.001 versus BOO.

Table 2 - Maximal responses (E_{max}) and Potency (pEC_{50}) to Carbachol (Cch) in bladder smooth muscle. Data were obtained in Sham and BOO animals, treated or not with L-NAME or aminoguanidine.

Experimental group	Emax (mN/mg)	pEC50
Sham (n=9)	1.29 ± 0.13	6.43 ± 0.21
Sham + L-NAME (n=3)	3.06 ± 0.40***	6.38 ± 0.17
Sham + aminoguanidine (n=4)	1.40 ± 0.27	6.15 ± 0.16
BOO (n=10)	0.49 ± 0.09*	6.30 ± 0.17
BOO + L-NAME (n=6)	1.08 ± 0.12 +++#	6.04 ± 0.10
BOO + aminoguanidine (n=3)	0.55 ± 0.11*	6.35 ± 0.17

* P<0.05 versus Sham; *** P<0.001 versus Sham; # P<0.05 versus BOO. +++ P<0.001 versus Sham + L-NAME.

Figure 5 - Contractile responses to electrical-field stimulation (EFS; 2-16 Hz) in bladders from Sham and BOO mice, treated or not with L-NAME or aminoguanidine for 5 weeks.



* P<0.05 versus Sham; ** P<0.01 versus Sham; *** P<0.001 versus Sham; # P<0.05 versus BOO; # # P<0.01 versus BOO; # # # P<0.001 versus BOO; + P< 0.05 versus Sham + L-NAME.

DISCUSSION

Despite the high prevalence of benign prostatic hyperplasia among men, the mechanisms responsible for voiding dysfunction induced by bladder outlet obstruction (BOO) are not well understood. Research and development of appropriate therapies include the use of animal models in order to understand the physiological control of urinary continence, as well as pathophysiological conditions involved in bladder dysfunction (9).

Several studies used rats (3, 4), mice (1, 10, 11), rabbits (12), guinea pigs (13) and pigs (14) to mimic BOO, whose structural and physiological changes of the bladder wall are similar to those observed in men suffering from BPH (9).

We used an established animal model of BOO. We found that BOO mice showed increased

Table 3 - Contraction (mN) produced by electric stimulation in isolated bladder smooth muscle.

Experimental Group	2 Hz	4 Hz	8 Hz	16 Hz
Sham (n=9)	0.639 ± 0.190	0.977 ± 0.288	1.645 ± 0.372	2.129 ± 0.388
Sham + L-NAME (n=5)	0.686 ± 0.249	1.018 ± 0.352	1.539 ± 0.445	1.836 ± 0.459
Sham + aminoguanidine (n=4)	0.316 ± 0.077	0.520 ± 0.138	0.976 ± 0.252	1.532 ± 0.382
BOO (n=10)	0.108 ± 0.031 *	0.191 ± 0.052 *	0.285 ± 0.077 **	0.398 ± 0.106 ***
BOO + L-NAME (n=8)	0.220 ± 0.038 +	0.369 ± 0.066 +	0.754 ± 0.139 #	0.939 ± 0.153 # #
BOO + aminoguanidine (n=3)	0.236 ± 0.076	0.322 ± 0.095	0.469 ± 0.143	0.610 ± 0.203

* P<0.05 versus Sham; ** P<0.01 versus Sham; *** P<0.001 versus Sham; # P<0.05 versus BOO; # # P<0.01 versus BOO; + P<0.05 versus Sham + L-NAME.

bladder weight to body weight ratio, almost 2-fold when compared to Sham animals (2.45±0.17 and 1.25±0.05, respectively), according to 2.5-fold observed in a study with female mice (10). In fact, mice 1 week after obstruction already have a rapid increase in bladder mass, which worsened 3 and 5 weeks after the surgical procedure (11). Similarly, rats and rabbits have increased blood flow after 24 hours of obstruction, which could be the first stimulus for hypertrophy (12, 15). This event occurs due to stretch of the bladder wall components, leading to thickening of epithelium, muscle layer and serosa (16) and increase in synthesis and deposition of collagen (17). After initial bladder function compensation, blood flow tends to diminish (18) and, as the process becomes chronic, more hypoxic-reperfusion areas can be observed in muscular layer (19). The increased wall thickness and wall tension result in cyclical ischemia-reperfusion during and subsequent to each voiding contraction (19, 20) and cause progressive deterioration of bladder function (17). The oxidative stress increases the production of reactive oxygen species (ROS), leading to enhanced malondialdehyde (MDA) and diminished superoxide dismutase (SOD). Free radicals originating from ischemia-reperfusion injury are one of primary etiologies in obstructed bladder dysfunction (21). A 4-week BOO female mice study revealed increased edema and lymphocytic infiltrate in the lamina propria, and hypoxia was seen in the urothelium, lamina propria and detrusor (5). Six-week BOO male investigation showed increase in muscular hypertrophy and fibrosis (22).

Treatment with the non-selective NOS inhibitor L-NAME showed decrease in bladder weight. In rats treated for 4 weeks with the same drug, morphometric studies showed increased thickness of trigone smooth muscle without affecting detrusor smooth muscle (DSM) thickness (23). Moreover, although early administration of L-NAME enhances ischemic damage at the beginning of the obstructive process, it inhibits the generation of nitrotyrosine and results in preservation of nerve density, reducing initial free radical damage associated with BOO (5). On the other hand, at chronic obstruction, L-NAME also prevents increases in blood flow and ROS generation from the compromised mitochondria, but nitrotyrosine production enhances and leads to worsen in bladder function (24).

In contrast, aminoguanidine treatment did not induce significant reduction of bladder weight in BOO mice, which is in contrast to another study (3), when 2 weeks BOO iNOS knock out and aminoguanidine treated rats presented less fibrosis. In both 3 and 6 week obstructed rats, eNOS activity decreases when compared to Sham animals, but nevertheless is higher than iNOS activity (21). Hypertrophied rat obstructed bladders exhibited weak nNOS expression after 3 and 6 weeks (3). In our study, this indicate that inhibition of iNOS alone did not affected bladder gain mass, unlike inhibition of the three isoforms of NOS had significant results.

Urodynamic characteristics normally correlate with bladder weight, but detrusor overac-

tivity in BOO animals may develop without an increase in bladder weight, suggesting that major disturbances caused by BOO may lie in the afferent signaling pathway (25). Some authors propose that NO may be involved in the regulation of the threshold for bladder afferent firing (26). Moreover, the effects of urothelial NO may not be mediated by a direct action on smooth muscle, because DSM lack soluble guanylate cyclase (GC), an important component in NO-mediated relaxation (27, 28).

Although maximum detrusor pressure and capacity did not reveal significant differences between BOO and Sham animals, the first parameter tended to increase, following compliance, that was higher in BOO mice. This is in agreement with studies with BOO rats (4, 29-31), but is different from what has previously been demonstrated in the obstructed mouse bladder (9, 11). Moreover, BOO mice had more NVC than Sham, similarly to another studies with mice and rats (4, 11, 31). In the unstable bladder, alterations of the smooth muscle can be a consequence of the "patchy denervation" of the detrusor (32), mediated by oxidative stress, since reduced amount of glutathione, the most abundant non-protein thiol with antioxidant capacity, was observed in BOO bladders (33). Predominance of protrusions junctions and ultra-close cell abutments in BOO (34) lead to more sensibility and lose of synchronism, what features detrusor overactivity (3).

L-NAME BOO treated animals had more NVCs than BOO, suggesting worsening of bladder function. The same result was found in chronically L-NAME treated rats (35) and was due to DSM super sensitivity to muscarinic agonists via increases in the levels of [^{3}H] inositol phosphate (IP_3), accompanied by reduction of β_3-adrenoceptor-mediated DSM relaxations (23). Aminoguanidine treatment decreased NVC in BOO mice, as shown in BOO rats treated with the same drug, due to attenuation in fibrosis. Studies using iNOS knockout mice instead of aminoguanidine treatment present concordance of results, in which NO contributes to ischemic injury and inhibition of iNOS is of therapeutic benefit (4).

Although with no significant difference in micturition frequency for L-NAME and aminoguanidine BOO treated groups, tendency of increase in this parameter was according to the smaller bladder capacity observed for these animals.

In vitro muscle physiology studies showed that BOO strips were able to generate less tension in response to cholinergic stimulation and EFS. Other authors have already demonstrated that bladder function maintains stable until 3 weeks post obstruction, but after 5 weeks there is impairment in detrusor function (1, 11). Unstable human bladders frequently show patchy denervation of the muscle bundles. Some muscle fascicles may be completely denervated, while neighboring bundles appear normal. Other regions may show intermediate innervation. The areas of reduced innervation become infiltrated with connective tissue (36). Guinea-pig obstructed bladders also present denervation, diminished response to EFS and muscarinic agonists (33).

Aminoguanidine treated animals had no role in bladder contraction evaluated by tissue bath experiments. However, L-NAME leaded to higher contraction in BOO mice. Obstructed rabbits treated with L-NAME presented higher bladder contraction to Carbachol and EFS 3 and 7 days post obstruction, in addition to less generation of nitrotyrosine and, consequently, reduction in membrane damage. This could account for the augmentation of contractile function and higher nerve densities observed for these animals (5). Another research with BOO rabbits treated with L-NAME showed reduced apoptosis due to ischemia-reperfusion and had significantly increase in contractile responses compared with non-treated animals (37). In rats chronically treated with L--NAME, it was observed that DSM relaxations mediated by β_3-adrenoceptors reduced, what suggests that prolonged NO deficiency leads to an overactive bladder (23).

CONCLUSIONS

L-NAME resulted in less bladder mass gain. The higher contractile responses and the increment in NVC suggests that the overactivity already presented by the BOO animals became worse. Although aminoguanidine treatment had decreased NVC, it leaded to reduced bladder capacity, did not

prevent the increase in bladder weight and was not effective in ameliorating contractile responses. Taking the results together, it can be hypothesized that chronic inhibition of three NOS isoforms in BOO animals leaded to worsening of bladder function, while selective inhibition of iNOS did not improve responses, what suggests that, in BOO animals, alterations are related to constitutive NOS.

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CONFLICT OF INTEREST

None declared.

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Intraparenchymal hematoma as a late complication of retrograde intrarenal surgery

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ABSTRACT

A 34 year-old woman was admitted to our hospital with left flank pain. A non-contrast enhanced computerized tomography (NCCT) revealed a 1.5x2cm left proximal ureter stone. Patient was scheduled for ureterorenoscopy (URS) and stone removal. She was submitted to retrograde intrarenal surgery (RIRS). At the postoperative 1st day, the patient began to suffer from left flank pain. A NCCT was taken, which revealed a subcapsular hematoma and perirenal fluid. The patient was managed conservatively with intravenous fluid, antibiotic and non-steroidal anti-inflammatory drug therapy and was discharged at the postoperative 6th day. Two weeks after the discharge the patient was admitted to emergency department with severe left flank pain, palpitation and malaise. KUB (kidney-ureter-bladder) radiography showed double-J stent (DJS) to be repositioned to the proximal ureter. Patient was evaluated with contrast enhanced CT which revealed an 8cm intraparenchymal hematoma/abscess in the middle part of the kidney. A percutaneous drainage catheter was inserted into the collection. The percutaneous drainage catheter and the DJS were removed at the 10th day of second hospitalization. RIRS surgery is an effective and feasible choice for renal stones with high success and acceptable complication rates. However, clinician should be alert to possible complications.

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INTRODUCTION

Retrograde intrarenal surgery (RIRS) refers to the retrograde type of operation for upper urinary tract pathologies by means of an ureteroscope. Since the introduction of first flexible ureteroscopic procedures in 1960's there have been many refinements in ureteroscopes in terms of image quality, durability and deflection capability (1). With the addition of working channel and irrigation systems to the ureteroscopes and the introduction of holmium: yttrium aluminium garnet (YAG) laser systems, flexible ureteroscopy (FU) has emerged as a choice for both diagnosis and treatment of urinary lithiasis and urothelial malignancies of the upper urinary tract (1, 2).

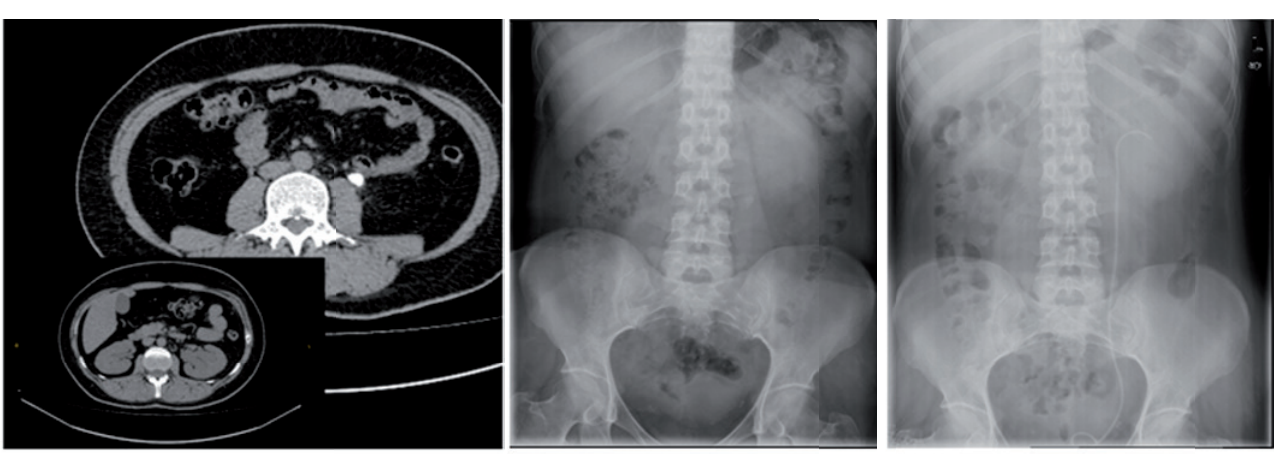
RIRS is stated as an appropriate alternative to extracorporeal shock wave lithotripsy (ESWL) and percutaneous nephrolithotomy (PNL) for proximal ureter and pyelocaliceal stones smaller than 2cm particularly in obese patients and patients with pyelocaliceal diverticula and infundibular stenosis (2).

We present here a case of intraparenchymal renal hematoma, which occurred three weeks after RIRS.

CASE REPORT

A 34 year-old woman was admitted to our hospital with left flank pain. A 1.5x2cm left sided opacity was apparent on KUB (kidney-ureter-bladder) radiograph at L3-L4 vertebra level (Figure--1a). The non-contrast enhanced computerized tomography (NCCT) revealed a 1.5x2cm left proximal ureter stone accompanying with grade-2 hydronephrosis (Figure-1b). Patient was scheduled for ureterorenoscopy (URS) and stone removal. The patient had undergone operation within 5 days. During the ureteroscopy with semi-rigid ureterorenoscope the stone migrated into the kidney so we decided to perform a RIRS with flexible ureterorenoscope. A 12F ureteral access sheet (UAS) was passed over a 0.038mm guide-wire up to the ureteropelvic (UPJ) under the guidance of C-arm scope. Then a 270-micron laser fiber was used to fragment the stone in the upper calyx of the kidney. Laser lithotripsy was carried out in 110 minutes with energy of 1.2 joules and a frequency of 8 hertz. Irrigation pressure pump was used to maintain clear vision. Stone fragments larger than 2mm were basketed out with nitinol NGage™ type basket then a 6F 22cm Double-J stent (DJS) was inserted into the ureter. The operation was terminated by insertion of an indwelling urethral catheter. The urethral catheter was removed at postoperative 1st day. Postoperative KUB radiograph demonstrated the DJS to be in the renal pelvis (Figure-1c). At the postoperative 1st day, the patient began to suffer from left flank pain. Whereupon a NCCT was taken which revealed a subcapsular hematoma and perirenal fluid (Figure-2a). Blood pressure and hemoglobin-hematocrit levels were found in normal ranges without a significant alteration compared to preoperative levels, 125/80mmHg and 12.4g/dL-38%, respectively. The patient was managed conservatively with intravenous fluid, antibiotic and non-steroidal anti--inflammatory drug therapy. Repeated ultrasonography examination showed the resolution of the renal hematoma and the patient was discharged at the postoperative 6th day. Two weeks after the discharge the patient was admitted to emergency department with severe left flank pain, palpitation and malaise. Blood pressure was 110/60mmHg and body temperature was 37°C. Hemoglobin and hematocrit levels were found diminished compared to postoperative levels, 10.7mg/dL and 32%, respectively. Urine culture was negative. KUB radiography showed upper end of the DJS to be fell down from the renal pelvis and migrated to the proximal ureter (Figure-2b). Patient was evaluated with contrast enhanced CT which revealed an 8cm intraparenychemal hematoma/abscess in the middle part of the kidney (Figure-2c). Intravenous antibiotic, analgesic and fluid therapies were given. A percutaneous drainage catheter was inserted into the

Figure 1 - Preoperative KUB radiograph and non-contrast enhanced abdomen CT and postoperative KUB Radiograph.

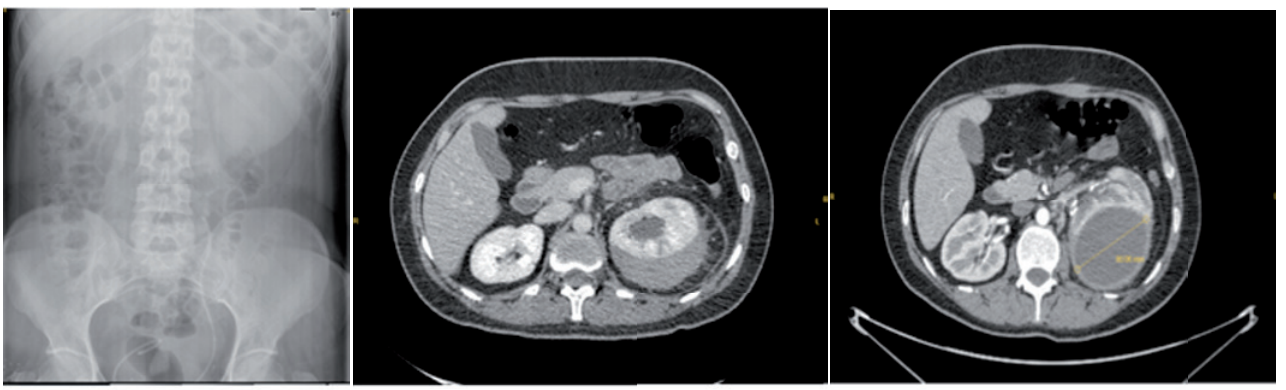


A. Preoperative NCCT. Stone in left proximal ureter and hydronephrosis are apparent.

B. Preoperative KUB radiograph. Opacity is apparent on the left side at L3-L4 vertebra level

C. Postoperative KUB radiograph. Upper part of the DJS is seen in renal pelvis localization

Figure 2 - Postoperative KUB radiograph and contrast-enhanced abdomen tomographies.



A. KUB radiography 3 weeks after the operation. Upper part of the DJS is seen in the mid ureter

B. Contrast enhanced CT at postoperative first day. A subcapsular hematoma is seen in the left kidney.

C. Contrast enhanced CT 3 weeks after the operation. A 8 cm intraparenchymal hematoma is apparent in the left kidney.

collection. Output from the drain was 150mL of hemorrhagic fluid at the first day. Culture of this fluid was also negative. Ultrasonography examination on the 7th day confirmed 75-80% shrinkage of the hematoma by the volume with irregular borders. Upon no output from the drain at the following days, the percutaneous drainage catheter and the DJS were removed at the 10th day of hospitalization. The patient had no need of blood transfusion and was discharged at the same day of catheter and DJS removal.

DISCUSSION AND FUTURE PERSPECTIVES

The main complications of RIRS consist of: fever, flank pain, urinary infection, transient hematuria, acute urinary retention, ureteral and pelvicalyceal abrasion, stone street, subcapsular hematoma, fornix rupture, extravasation, urinoma, ureter avulsion, bleeding requiring transfusion and sepsis (2, 3). Reported complication rates vary between 0% and 25% in different studies (1, 2, 4, 5).

There is no consensus on the use of UAS (1, 6) but it might lessen complication rates by prevention of ureteral injury at repeated FU accesses, maintaining adequate irrigation, decreasing intrarenal pressure and facilitating stone removal (1, 4, 6). On the other hand, it may cause transient ureteral ischemia and ureteral stricture as a late term complication (4, 6).

Physiologic intrarenal pressure is approximately 10mmHg while the threshold for pyelovenous and pyelolymphatic backflow is 30-45mmHg (6, 7). It has been shown that high intrarenal pressure might be a risk factor for septic complications (8).

Hemorrhage can occur as a result of UAS/laser injury or renal calyceal avulsion. Moreover, parenchymal and forniceal rupture because of high intrarenal pressure during the procedure may cause hemorrhage (9, 10). Subcapsular and intrarenal hematomas are rare complications with a few cases in the literature (2). To the best of our knowledge, intraparenchymal hematoma occurring 3 weeks after RIRS following subcapsular hematoma is the first case in the literature. In our case, intraparenchymal hematoma may have arisen due to increased intrarenal pressure after repositioning of DJS.

RIRS surgery is an effective and feasible choice for renal stones with high success and acceptable complication rates. Complications are usually minor that can be treated conservatively. However, clinicians should be alert to possible complications. Shorter operation time, lower stone burden, decrease intrarenal pressure and use of

UAS during the procedure may result in reduction of complication rates.

CONFLICT OF INTEREST

None declared.

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RADIOLOGY PAGE

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Carcinoma prostate masquerading as a hemorrhagic pelvic cyst

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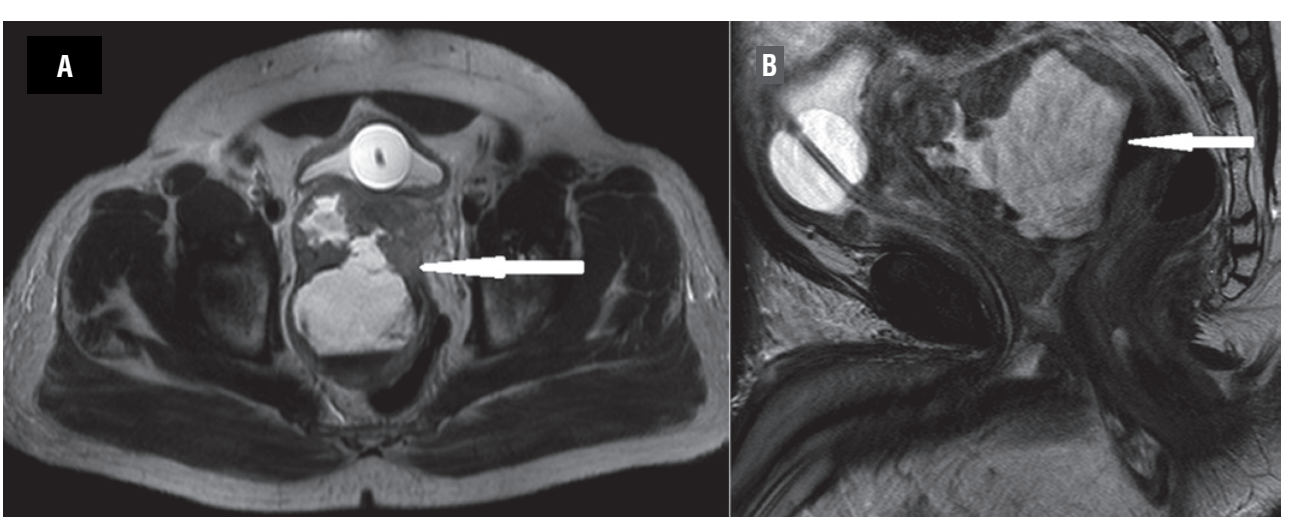
CASE

A 53 year-old man, with untreated lower urinary tract symptoms for two years was catheterized for acute retention of urine. He had an enlarged, boggy non tender prostate.

The transrectal ultrasound (TRUS) revealed a fluid filled cystic mass arising from the prostate and 200cc of hemorrhagic fluid was aspirated. There was no growth on culture of fluid and Xpert® MTB/RIF along with three acid fast bacillus smears was negative for tuberculosis. Cytology was not performed on the aspirated fluid. Magnetic resonance imaging revealed prostate volume of 52cc and a 94x77mm cystic and solid lesion (Figure-1) with seminal vesicle and rectal infiltration. There was abnormal signal intensity with T2-weighted hypointensity of the peripheral zone of the prostate. Close to the apex of the prostate, the cystic lesion merged with the right side of the base of the prostate. Above mentioned area near the apex showed restricted diffusion. His prostate specific antigen (PSA) was 910.0ng/mL. TRUS guided biopsy of the cyst wall revealed adenocarcinoma of prostate (Gleason grade: 4+4=8). The Technetium-99m methylene diphosphonate scan showed no evidence of osseous metastasis.

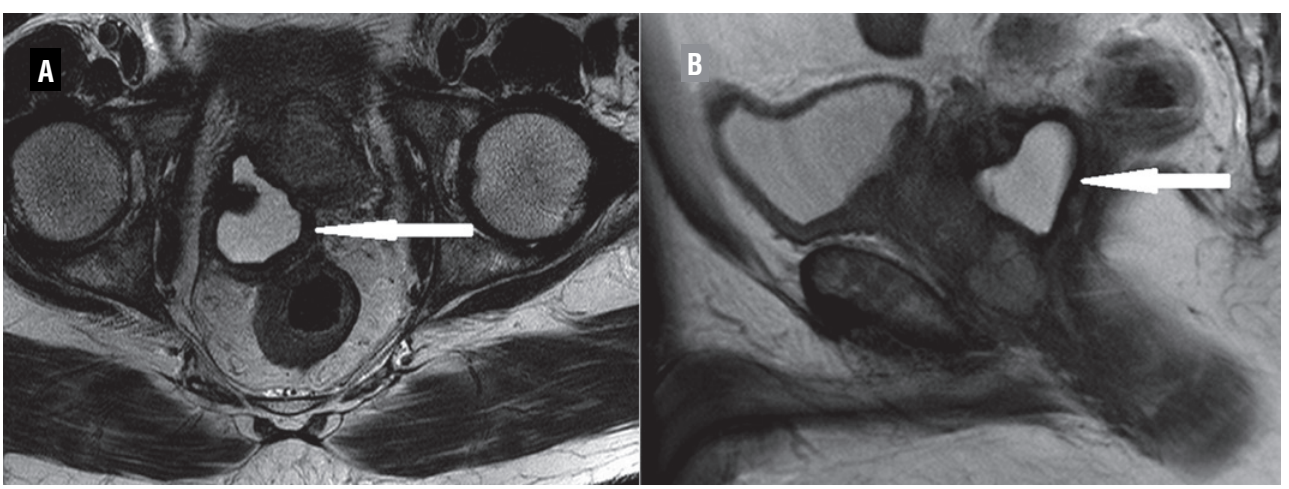
Figure 1 - (A and B): Hemorrhagic cyst (arrow) at initial presentation.



Based on the locally advanced nature of the disease, he was initiated on neoadjuvant Leuprolide acetate 22.5mg subcutaneously (luteinizing hormone releasing hormone analog) and he voided successfully without a catheter on follow-up. At three months, his PSA was 21.9ng/mL with marked reduction in the size of the cystic lesion (50x40mm) (Figure-2). He received radiotherapy (Intensity Modulated Radiation Therapy technique with image guidance) after six months of androgen deprivation. A total dose of 79.2Gy was delivered in 44 fractions and his nadir PSA was 0.04ng/mL after 18 months of follow-up. Leuprolide is being continued for three years.

Hemorrhagic pelvic cyst remains a rare presentation of carcinoma prostate (1-5). With a raised PSA, a high index of suspicion should be maintained with a low threshold for a TRUS guided biopsy.

Figure 2 - (A and B): Reduction in size of cyst (arrow) after androgen deprivation therapy.



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VIDEO SECTION

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Nutcracker Syndrome: laparoscopic external stenting of the renal vein ("the shield technique")

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ABSTRACT

Nutcracker syndrome refers to the complex of clinical symptoms caused by the compression of the left renal vein (LRV) between the abdominal aorta and the superior mesenteric artery, leading to stenosis of the aortomesenteric portion of the LRV and dilatation of the distal portion. Hematuria, proteinuria, flank pain, varicocele and pelvic congestion may occur, occurring more frequently in young adults. Conservative management, might be the option whenever it is possible.
When surgical treatment is required, classically open surgery have been performed, with major surgeries as LRV transposition or bypass techniques. The main caveats regards the fact that these are large and risky surgeries. Endovascular surgery with venous stent placement has gained some space as it is minimally invasive alternative. However, venous stents are associated with a high number of trombotic complications and in many cases requirement of life-long anticoagulants. External stenting of the LRV with this "shield technique" is a minimally invasive alternative, with good medium term results. We herein demonstrate our second experience with the technique of this surgery in a patient with 12 months of follow up and excellent results.

CONFLICT OF INTEREST

None declared.

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VIDEO SECTION

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Robotic pyelolithotomy in a congenital pelvic kidney: side docking and robotic prostatectomy port – site approach

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ABSTRACT

Introduction and Objectives: Ectopic pelvic kidneys with renal stones are challenging to treat. We report our experience in managing a case of ectopic pelvic kidney with a pelvic stone by robotic pyelolithotomy after failure of flexible ureteroscopy.
Materials and Methods: A 46-year old male with 2 months history of vague lower abdominal pain was found to have on Computed Tomography scan a left ectopic pelvic kidney with a 12mm stone in an anomalous renal pelvis. Flexible ureteroscopy failed to reach the stone twice and a 4.7 French ureteric stent was placed.
Results: Side docking was utilized with the patient in supine Trendelenburg position. Port placements were similar to robotic assisted laparoscopic prostatectomy. Docking time was 35 minutes and console time was 150 minutes. Multiple attempts failed to follow the course of the ureter to the renal pelvis. Subsequently the renal pelvis was directly opened through the mesocolon and a flexible cystoscope was used to basket the stone out. Estimated Blood Loss was <100ml. The patient was discharged 2 days postoperatively.
Conclusion: Robotic pyelolithotomy is safe and feasible for management of ectopic pelvic kidneys with pelvic stones. The use of flexible cystoscopy helped in localizing and extracting the stone in our case. Detailed understanding of patient's anatomy helps in the success of this procedure.

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VIDEO SECTION

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Laparoscopic Pyeloplasty in children with Horseshoe Kidney

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ABSTRACT

Introduction: Horseshoe kidney occurs in 1 per 400-800 live births and are more frequently observed in males (M:F 2:1). Ureteropelvic junction obstruction (UPJO) is commonly associated with horseshoe kidneys. The variable blood supply, presence of the isthmus and high insertion of the ureter contribute to this problem.
Case report: An asymptomatic 6 year-old boy presented with antenatal hydronephrosis. Ultrasonography and CT scan demonstrated left UPJO associated with a horseshoe kidney.
DMSA showed 33% of function on the left side. DTPA showed a flat curve and lack of washout. A left dismembered laparoscopic pyeloplasty was performed after identification of crossing vessels and abnormal implantation of the ureter. After one year, the child is asymptomatic. DTPA demonstrated a good washout curve.
Results: Our cohort consisted of six patients, five males and one female, with a mean age of 6 years (range 6m-17 years) and a mean follow-up of 3 years. Ureteropelvic junction obstruction was more common on the left side. Symptoms appeared only in 34% of the cases.
Mean operative time was 198 minutes (range 120-270 minutes). Crossing vessels were common (observed in 50% patients). High implantation of ureter was seen in 67% patients and intrinsic obstruction in 83%. Surgical difficulties were found in two cases. Hospital stay was 4.3 days (3 to 6 days), with only one patient having a mild complication (pyelonephritis). All cases had clinical and radiologic improvement.
Conclusion: Laparoscopic pyeloplasty is safe and feasible in children with UPJO in horseshoe kidneys, with good results and minimal morbidity.

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