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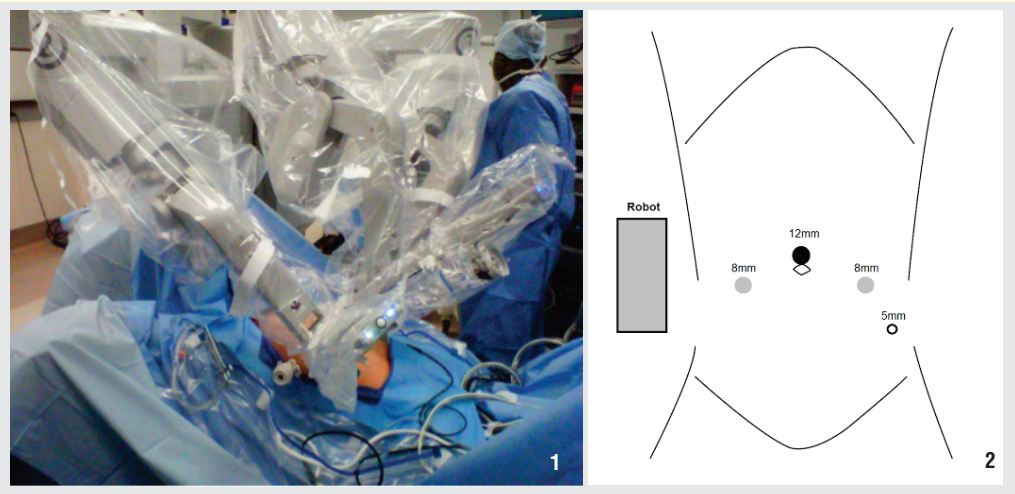

Figure 1 - daVinci robotic Si surgical platform port placement.

Figure 2 - Side docked position. (Page 1155)

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This is the last 2015 issue of the 41th year of publication of the International Brazilian Journal of Urology. During this year, we have published more than 200 articles, and the number of submissions has increased continuously, requiring an intensive work of our reviewers.

In this number, we publish a controversy in the difference of opinion section (pages. 1043-1045): Experts (Dr. Vieira from Brazil and Drs. Chilles and Schlegel from US) discuss the pros and cons of vasectomy reversion as the first step in a vasectomized man who wants to father again in a new marriage.

In page 1049 a review article confirms the effectiveness of the use of alpha-1 blocker drugs in the medical expulsive therapy for ureteral calculus in children. The drugs increased the probability of calculus expulsion, independent of its size (cut –off 5mm), however it was included only three satisfactory studies in this review, showing the need for more investigation in this area.

There are few studies regarding adjuvant chemotherapy for locally advanced upper tract urothelial carcinoma. The group from Seoul, Korea has shown no benefits with cisplatin based therapy after nephroureterectomy in a retrospective cohort of 72 patients (page 1067).

Recently, it was demonstrated that the cessation of preventive aspirin use is not necessary in the majority of surgeries, except in prostatic and brain procedures. The group from New Delhi, India, concluded in almost 700 patients that aspirin cessation is not necessary during the realization of transrectal prostatic biopsies (12 cores, page 1096).

Married status seems to be a protective factor for oncologic patients, as already demonstrated for prostate cancer, penile cancer and other prevalent neoplasms. The groups from Georgia University and Roswell Park (US) concluded that married patients presented better survival rates also in adrenocortical carcinoma. Single, widowed or divorced patients presented a 25-30% higher risk of mortality in this usually aggressive tumor (page 1108).

In page 1126, the Washington University group demonstrated that hemorrhagic cystitis in stem cell transplant patients presents higher mortality when continuous bladder irrigation is required.

In neuro-urology, an Egyptian group compared the results of intra-

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vesical injection of 100 versus 200 units of botulin toxin A in the treatment of neurogenic bladder; and there is an experimental study from Toulouse and London researchers that investigated for the first time in humans the immunohistochemical expression of SPK-1 (a bladder wall modulator) in the vesical wall of patients with neurogenic bladder dysfunctions (page 1141).

Villar's group from Federal University of Pernambuco reported satisfactory tissular interaction of a new material, the cellulose exopolysaccharide (CEC) inserted as pubovaginal sling in rats.

To reinforce the debate regarding open versus laparoscopic nephrectomy, a group from Ankara (page 1202) has shown that pulmonary function was less affected in patients who underwent laparoscopic rather than open surgeries.

Video section presents a technique associating resection of the urethral plate and an elongated ventral buccal graft for patients with extensive obliterans urethral stenosis, resulting in 81% of success in 36 Serbian patients.

The group from Cleveland Clinic investigated prostatic biopsies in patients under testosterone reposition. Additionally, we have robotic articles from US and Italy, prognostic evaluation of localized prostatic cancer patients from Sao Paulo University, an Iranian investigation of interleukin 8 as a serum marker for children with febrile urinary tract infections, Chinese experimental studies etc...

We wish a happy and productive new year for all worldwide Int Braz J Urol contributors, friends and relatives.

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The pathophysiology of Peyronie's disease: beyond the Smith's space

Peyronie's disease (PD) is a benign condition clinically characterized by penile nodules and fibrosis of the tunica albuginea (1). The result is penile bending and erectile dysfunction in some patients. Until now urologists can only offer treatments in the late phase of the disease, after the installation of fibrosis. On the other hand, an intriguing course of inflammation precludes the fibrosis of the tunica. In PD this phase is not well studied. Understanding the initial phase, the induction of the inflammation, and the pathway until fibrosis could lead to the development of a treatment that prevents the fibrotic phase in PD.

What is the etiology of PD? It is accepted that penile trauma is an inductor of PD, and the splitting of internal and external tunica layers could accumulate extravascular blood, creating a milieu to inflammation and aberrant wound healing (delamination hypothesis) (2). Moreover, the entrapment of inflammation could perpetuate the process and increase fibrosis (3). TGF beta 1 and myofibroblasts are important steps in PD pathophysiology (4). However, this hypothetical model has not been thoroughly explained.

It is very difficult to study the pathological anatomy of the early steps of PD due to ethical issues, thus we have to retrieve information from former studies. In 1966, Smith (5) revised the histology of 26 cases of PD and compared with 30 cases of autopsies. In the normal anatomy, a space of "vascular, loose, areolar connective tissue sleeve" without elastic fibers separates the corpus cavernosum from the tunica albuginea (Smith's space). In patients with PD, data regarding the onset of the disease was obtained in 21 patients, while 13 patients had less than 6 months of symptoms. The histology varied in function of time of symptom onset; less than 3 months of symptoms presented with inflammatory cellular infiltrate, while longer lesions more fibrosis and ossification. The inflammatory process with lymphocytes and plasma cells in early lesions was located in the areolar connective tissue of Smith's space. The inflammation was perivascular, occasionally with endothelial proliferation and perivascular fibrosis. More advanced cases demonstrated fibrosis of the connective tissue in the Smith's space, advancing on the cavernous tissue and destroying the smooth muscle bundles in the intercavernous septum. Ossification was also located in the Smith's space.

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Recently, new discoveries regarding the initiation of the inflammatory process in other tissues paved the way to understand the pathophysiology of PD in the early phase. Based upon this body of evidence we can build a theoretical model to explain in part the pathophysiology of PD.

The first step is the vascular inflammation in the Smith's space or intersinusoidal space. After trauma or other stimuli, endothelial pro-inflammatory modification occurs. Post-capillary venous endothelial cells are more prone to this reaction. The "sterile" inflammatory response is initiated mostly by exposure of innate immune cells, primarily macrophages, dendritic cells and neutrophils, or damage-associated molecular patterns (DAMPs) (6). This results in the secretion of inflammatory cytokines IL-1β, IL-6 and TNF-α, and many different chemokines. Several molecules can act as DAMPs including hyaluronan, heparin sulfate, heat shock proteins, and ATP. The vascular endothelium expresses chemoattractants and adhesion molecules in response to these proinflammatory stimuli. Neutrophils in circulation are recruited due to action of E-selectin, P-selectin and integrins (VCAM-1, ICAM-1 and 2) and migrate to the site of tissue damage under the influence of CD99, PECAM-1, VE-Cadherin and ICAM-1 (6). Another important step to complement this process of inflammation is platelet aggregation and local coagulation (7). Trauma can damage the endothelium and expose subendothelial molecules that promote platelet aggregation via P-selectin. Immunothrombosis in veins of the subtunical space could amplify the process of inflammation. Interestingly, uncontrolled coagulation is implied in inflammation and fibrosis in liver, heart, kidney, and lungs (8). Those processes could recur several times, contributing for phases of recrudescence and resolution of inflammation. Also, it is possible that in some areas inflammation and fibrosis are in different phases, leading to the conclusion that PD is in continuing remodeling.

As discussed before, the Smith's space and the surrounding corpus cavernosum are ideal sites for the development of PD under this model. The fibrosis advancing the intersinusoidal space could also be explained. It is very unlikely that PD occurs only inside the tunica albuginea and septum as in the delamination model.

The importance of the proposed model is that we can envision several experiments to test the endothelium, the immune system and the hemostasis as contributors to the inflammation in the development of PD. Genetic variations and differences in the expression of molecules under the optics of this model could identify a cohort of predisposed population for PD. If we could control the inflammation or even avoid it, a prophylaxis of PD would be possible.

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DIFFERENCE OF OPINION

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Vasectomy Reversal must be the first step for a man who had a vasectomy and wants a children from a new marriage?

Opinion: Yes

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Keywords: Vasovasostomy; Sterilization Reversal; Infertility; Azoospermia; Fertility

Decision on which is the best treatment for a problem that has more than one way to be dealt with goes much further from exclusively looking to the final results. Decision must include which are the final results you are looking for, how many variables are present in both options, money expenditure to reach the result and finally, how much resources are available for it.

The beginning of 90's brought consolidation of vasectomy reversal as the treatment for man who had a vasectomy and want to become a father again. With the paper publishing with published paper from Belker and cols. that established time from vasectomy as a main variable for patency rate and pregnancy after surgery (1).

In 1992 the first pregnancy obtained from an injection of a single sperm into a oocyte was published by Palerno et al. (Intracitoplasmic Sperm Injection – ICSI) (2) and two years later Shrivrastav and cols. published the technique for sperm retrieval from epididymis by a percutaneous puncture (Percutaenous Epididymal Sperm Aspiration – PESA) (3), introducing a second option for man with vasectomy to become a father and new variables that may alter the results. ICSI/PESA introduced variables that come from the female partner, mainly age, and laboratory variables as the number of metaphase oocytes manipulated, fertilization rate and embryo classification (4-7).

The final objective of infertile couples seeking for treatment is having a healthy baby and not only getting pregnant, so, papers objecting the comparison between vasectomy reversal and ICSI with sperm retrieval (SR) must also look for live birth rate. Silber and cols., in 1995 published the results of 72 cycles of Microsurgical Sperm Aspiration (MESA) and ICSI showing a 42% ongoing pregnancy rate. Since then the literature shows pregnancy rates ranging from 28 to 42% and live birth from 27 to 32% (7-13). Data from Society for Assisted Reproductive Technology (SART) shows that live birth rate for male factor was 34.5 % in 2003 and raised to 37.3% in 2008 and the use of ICSI for male factor raised from 84% to 87%, but those numbers are for all male factors (14).

Lee and cols. published a literature review comparing vasectomy reversal and

sperm retrieval (SR) /ICSI and showed a data compilation of reversal results with patency rate range from 60 to 90% and pregnancy rate range from 26 to 84% with median patency rate of 86% and pregnancy rate of 56%. Excluding the best and worst results from the analysis the median patency rate was 81% and pregnancy rate 44% (15).

The pregnancy and live birth rates are very similar and the analysis of prognostic variables may help taking a decision towards the most suitable. Belker and cols. showed that time from vasectomy is the prognostic factor for patency rate (1). Four papers studied time from vasectomy as a prognostic factor for pregnancy and live birth after SR/ICSI for man with vasectomy (9-12). Borges et al. and Abdelmassih results showed that time since vasectomy was a prognostic factor for pregnancy and Bromage et al. and Nicopoullos et al. found just the opposite, but all agreed that female partner age was an important prognostic factor. Friedler et al. studied the prognostic factors for ICSI with sperm retrieval in obstructive azoospermia and concluded that the female age and number of metaphase oocytes manipulated were the best indicators for pregnancy and live birth. So female age is important and correlates with pregnancy and live birth for SR/ICSI. Butwhat about the role of female age as a prognostic factor for pregnancy and live birth after vasectomy reversal?

Hsieh et al., using a decision analysis model to choose between SR/ICSI and vasectomy reversal concluded that female age was more important than time since vasectomy for pregnancy rate and live birth after a vasectomy reversal and the correlation is also true for SR/ICSI, so younger female will perform better in both scenarios leaving us without one best option (16).

The last two aspects for decision making is the cost-effectiveness, a concept of how much resources do we spend to achieve a certain result. In our case live birth is one and the other one is how much money the couple intends to spend to have a child called "willing to pay" (WTP) (16, 17). Those two concepts are addressed in cost-effectiveness studies which are very few in the literature. Search on PUBMED (("Vasovasostomy/economics"[Mesh] or "Vasovasostomy/statistics and numerical data"[Mesh])) AND "Cost-Benefit Analysis"[Mesh] resulted in 12 papers. Three were excluded from the analysis: one in German, one animal model study and one which analyzed vasectomy as a reversible method. After reading the other 10, we excluded two more for not address the subject.

Garceau et al. in a systematic review of assisted reproductive technology, elected 59 papers addressing economics evaluations, cost studies and economic benefits among 2.547 papers searched on the literature. The authors extracted the economic data, converted to UK pounds and adjusted inflation with the objective of analyzes cost for assisted reproductive technology, including cost per delivery after vasectomy reversal and SR/ICSI. The authors found a median cost per delivery of £ 42.163,50 for SR/ICSI and £ 16.134,00 for a vasectomy reversal. Despite the small sample from one of the papers, 25% lost follow up from another and the median (50-80%) of quality requirements from a great number of papers, they concluded on behalf of the superiority of vasectomy reversal (17). SR/ICSI would be more cost-effective if the pregnancy rate was higher or the couples would have more money to spend (WTP). Hsieh and cols. with a simulation decision model using the available data on the literature and working with diversified scenarios of female age and vasectomy reversal patency rates, concluded that IVF is more effective for pregnancy rate over 60% and vasectomy reversal has an advantage until US$ 65.000 of WTP (16). The most recent published reviews also confirmed vasectomy reversal as a first line treatment for vasectomy (14, 18).

Some facts must be discussed as advantage and disadvantage for both treatments. Lee and cols. as Roob & Sandlow and Shridharani & Sandlow considered direct and indirect costs of IFV that inflates IVF cost explaining the advantage for vasovasostomy. The need for contraception after a successful vasectomy reversal may lead the couples to SR/ICSI but might also be counter pointed by the higher chance for multiple births after IFV. Specialized centers have better results either for IVF and reversal. Limitations of cost-effectiveness studies due to different fares and regional coverage for ART treatment in US may bias the option for one or other treatment. Studies concentrated on US and developed countries may not be generalized for other countries.We do not have cost-effectiveness studies in Brazil analyzing SR/ICSI and vasectomy reversal (14-16, 18).

Our center has both options for man with vasectomy, and the decision of which treatment will be done, is ultimately taken by the couple after knowing the results and analyzing costs. From 1994 until 2012 four hundred and fifty SR/ICSI cycles were done for man with vasectomy to treat 332 couples, 318 for the first time. The pregnancy rate per cycle was 31%(141/450) and the live birth per cycle was 22% (10/450). Cumulative pregnancy rate and live birth rate was 42% (141/332) and 30 %(102/332) respectively. Couples were submitted up to seven cycles and all the live births occurred until the fourth cycle, just one pregnancy after four cycles and none live birth after four cycles done (19). Since 2008 we did 166 vasectomy reversals, 147 data available for semen analysis results with 83% patency rate (123/147). A hundred and two patients could be contacted by phone for late follow up and amongst that, 40 healthy babies were born, live birth rate 39% (40/102).

All these factors can lead us to conclude to either side but common sense and until now, published results still reaffirm reversal as a primary treatment for vasectomy.

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DIFFERENCE OF OPINION

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Vasectomy Reversal must be the first step for a man who had a vasectomy and wants a children from a new marriage?

Opinion: No

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Keywords: Vasovasostomy; Sterilization Reversal; Infertility; Azoospermia; Fertility

The evaluation of any couple with infertility for any reason, including known post-vasectomy obstructive azoospermia, involves simultaneous evaluation of the female for her fertility potential with male evaluation. The goal of vasectomy reversal is to allow a couple to have a healthy child. In this overview, we will clarify that sperm retrieval with IVF is the most effective means for a couple to achieve their goal of having at least one healthy child. The limiting factor for natural conception and live birth after vasectomy is not typically the male partner's obstructive azoospermia, however, it is directly related to the female partner's fertility. The Practice Committee of the American Society for Reproductive Medicine clearly state in their guideline for management of obstructive azoospermia using reconstruction: "Before vasectomy reversal is performed to restore fertility, evaluation of the female partner's reproductive potential is prudent and recommended..." (1).

Women have a decline in reproductive potential that becomes significant by age 32-35 with a subsequent rapid decline (2). Age is not the only reason for impaired female fertility, and there is no age below which normal female reproductive potential can be guaranteed. Hormonal and congenital abnormalities as well as endometriosis are a few examples of common issues that greatly decrease the chance a couple has for a successful natural conception; all can be issues for women of any age. The observation that men are less likely to have a pregnancy with a new partner after vasectomy reversal than with same female partners, emphasize the role that the female plays in success rates of vasectomy reversal (3). These increased risks underscore the importance of the female evaluation because her fertility potential will frame the conversation regarding the risks, benefits, and likely outcomes of all reproductive possibilities, including natural conception.

Vasectomy reversal is an option for couples interested in fertility after vasectomy reversal. Vasectomy reversal has been reported to have some cost-benefits related to use of ART (4, 5). However, the "costs" associated with ART are overestimated by low pregnancy rates in historical published literature and excessive frequency of multiple gestations. Original cost-effectiveness studies with vasectomy reversal assumed a pregnancy

rate nearly half the current pregnancy rate achieved with surgically retrieved sperm. In addition, the frequency of multiple gestations has been dramatically decreased by single embryo transfer and other contemporary enhancements in IVF. These changes dramatically improve the cost--effectiveness of sperm retrieval and IVF. Both treatment with both vasectomy reversal as well as sperm retrieval and IVF mandates that the male partner will undergo a procedure, the extent of the procedure with vasectomy reversal is far more extensive, requiring hours of anesthesia, typically general anesthesia, with its associated risks. The question at hand is not whether vasectomy reversal is a procedure that should be offered to couples. The question is whether it should be the first step for these couples. Every advantage that a vasectomy reversal provides couples relies on the female partner having normal fertility potential; therefore, a vasectomy reversal should only be performed after female reproductive capacity is confirmed.

There are, however, additional factors that must be considered prior to vasectomy reversal. The availability of a trained microsurgeon must certainly be taken into consideration. Without a microsurgeon skilled in both vasovasostomy as well as vasoepididymostomy available to perform the procedure, vasectomy reversal should not be undertaken and couples may be better served with sperm retrieval and IVF. Furthermore, men who have any evidence of a decline in fertility and spermatogenesis may be poor candidates for vasectomy reversal. For example, men with small, soft testes or an elevated FSH may have compromised sperm production, and additional evaluation including endocrine work-up is needed (6). The sperm production may be so impaired that the couple would require ART even if sperm returned to the ejaculate, causing a delay in achieving a pregnancy – but still requiring ART. Progressive decline in sperm production becomes more common as men age – and the population of men requiring vasectomy reversal tend to be older. Vasectomy reversal is unnecessarily invasive in subfertile males because sperm can be acquired through testicular or epididymal aspiration for the ART procedure which would have been required anyway. Furthermore, these same subfertile couples will appreciate not requiring additional contraception if the vasectomy can be maintained during and after ART. Men who had pelvic, inguinal, or prostate surgery after their vasectomy may also not be candidates for reversal because of the possibility of additional damage to the ejaculatory system which would make repair at the site of the vasectomy fruitless.

The desire for multiple children is an oft-cited reason for vasectomy reversal as a primary treatment for obstructive azoospermia. Again, however, the female partner's fertility will dictate whether this is a realistic possibility or not. Because it can take up to two years for sperm to return to the ejaculate after vasectomy reversal, at least this much time must be factored into the equation that determines a couple's likelihood of pregnancy through natural conception (7). The not inconsequential number of couples who fail their initial attempt at vasectomy reversal and require a reoperation will also have more years of waiting added to their reproductive timelines, as will the couples who have secondary stricture of the anastomosis who then require revision. Not all female partners will have the reproductive staying power to tolerate this multi-year delay, and thus a thorough understanding of her fertility potential is warranted prior to undertaking the vasectomy reversal process.

The definition of success after vasectomy reversal must be carefully evaluated when counseling patients regarding outcomes. Understandably, most authors advocate for "patency" to be the marker of success, and this number is often inappropriately quoted to patients as the "success rate" of vasectomy reversal. Although return of sperm to the ejaculate is clear evidence that the obstruction has been eliminated, and using this definition, vasectomy reversal can have impressive success rates of 44-97% (8, 9). Closer examination of the published literature and actual definition of success, however, is warranted. Couples do not request vasectomy reversal because they simply desire a return of sperm to the ejaculate; the ultimate goal is actually to have a healthy child. With this outcome in mind, vasectomy reversal is not nearly as "successful" upon re-examination of the literature as the patency rates may suggest.

Information regarding pregnancy and live birth rates from natural conception is often lacking in vasectomy outcomes papers, posing a challenge to adequately inform and counsel the couple. For example, a recent study quoted a 98% patency success rate after 1331 vasectomy reversals performed by expert microsurgeons, yet they report only 410 events of natural conception, yielding a rate of conception per vasectomy reversal of only 30.8% (410/1331); live birth rate was not addressed (10). Other studies, taken together, indicate that the rate of documented pregnancies after vasectomy reversal was only 26%, even when couples were followed for 3 years after reversal (11, 12). While many factors unrelated to the technical success of a vasectomy reversal will clearly inform this particular outcome, the drastic difference between patency rate and natural conception rate should prompt thoughtful consideration of what defines success after a vasectomy reversal.

One must realistically consider and compare these data. Vasectomy reversal has a pregnancy rate of 26% after 3 years. IVF has a pregnancy rate of 44% in each cycle, with cycles repeatable within 2 months. The cumulative pregnancy rate within 6 months will easily be more than 90%. With this frank comparison of outocome data, why even consider surgical vasectomy reversal?

Although the urge to satisfy a couple's request for a vasectomy reversal is understandable, blindly performing the procedure first without the appropriate evaluation or consideration of the option of sperm retrieval/IVF is not appropriate. For the male fertility expert counseling a couple interested in fertility after vasectomy, sperm retrieval with IVF is the preferred option. Even if vasectomy reversal is initially attempted, the vast majority of couples will require IVF.

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REVIEW ARTICLE

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The use of alpha-1 adrenergic blockers in children with distal ureterolithiasis: a systematic review and meta-analysis

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ABSTRACT

Introduction: Urinary lithiasis is the main urologic cause of emergency treatment in adult patient. In the past years, the incidence in children population has increased. However, literature about the use of alpha-1 adrenergic blockers in pediatric population with distal ureterolithiasis is still scarce. The drug acts by decreasing ureter contractions, especially in the distal portion, facilitating calculus expulsion.
Objective: This review has the objective to evaluate the use of alpha-1 adrenergic blockers as medical expulsive treatment in children with distal ureterolithiasis.
Evidence Acquisition: An electronic literature search was performed using the MEDLINE, COCHRANE, and LILACS databases. We further searched manually the references of the primary studies. Searches were concluded on October 4th, 2014. Articles were selected, independently and in pairs, by the respective titles and summaries. Any divergence was resolved by consensus.
Evidence Synthesis: Alpha-1 adrenergic antagonists increased the probability of calculus expulsion by 27% (NNT=4). Calculi smaller than 5mm, increased by 33% (NNT=3). Larger than 5mm, increased by 34% (NNT=3).
Conclusion: Alpha-1 adrenergic blocker use is related with a greater incidence of expulsion of ureteral calculi, smaller or greater than 5mm, and fewer episodes of pain when compared to ibuprofen. However it is necessary larger samples to enhance the power analysis of the expulsion of ureteral calculi larger than 5mm and the episodes of pain.
Patient Summary: This review analyzed the outcome of alpha adrenergic antagonist in children with ureteral calculi. We conclude that it is the best medicine for use, since it helps the expulsion of the stone.

ARTICLE INFO

Key words:
Ureterolithiasis; Adrenergic alpha-1 Receptor Antagonists; Child; Review Literature as Topic

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INTRODUCTION

Urinary lithiasis is the main urologic cause of emergency treatment in adult patients (1). It can occur at any age, including children.

In the United States, during the 1950s, the disease was the cause for hospitalization in one out of every 7600 pediatric patients; in the 1990s, in one out of every 1000, and between 2002 and 2007, in one out of every 685 (2, 3).

The reason for this increased incidence is not clear. There are controversial theories that associate these numbers to eating and life habits.

Typical in the pediatric patient, ureterolithiasis consists of a calcium oxalate calculus, found in 55% of cases in the distal ureter (4). The clinical picture consists of general symptoms, such as unspecific pain in the abdomen, flanks, or pelvis (5). Additionally, 90% of the cases manifest with macroscopic or microscopic hematuria, and may progress with acute urinary tract infections and urinary retention.

The prevalence of cases increased with areas that are hot, arid, and have dry climate. Therefore, the locations most affected are United States, British Isles, Scandinavian countries, Central Europe, Mediterranean countries, Turkey, Pakistan, north of India, parts of the Himalayan Peninsula, China, north of Australia (6, 7).

Treatment is determined by the size of the calculus and clinical picture. Those smaller than 5mm are generally eliminated without intervention, whereas the largest stones are commonly treated by extracorporeal lithotripsy, ureteroscopy, and percutaneous nephrolithotomy (6, 8). The presence of urinary infection is an indication for surgical intervention, regardless of the size of the calculus.

In cases where there is no associated urinary infection and pain is not intense or is controlled with analgesics, a "wait-and-see" approach may be taken, expecting the spontaneous elimination of the calculus.

In the adult patient, there are various conservative treatments to treat calculi smaller than 12mm, such as the use of calcium blockers, non-steroidal anti-inflammatory agents, or alpha-1 adrenergic blockers, which is the better approach (9). The blocker acts by decreasing ureter contractions, especially in the distal portion, facilitating calculus expulsion. However, in the pediatric population, literature is still scarce (3, 10). There are papers with high strength of evidence, but with few cases.

OBJECTIVE

This review has the objective to evaluate the use of alpha-1 adrenergic blockers as medical expulsive treatment in children with distal ureterolithiasis.

EVIDENCE ACQUISITION

Identification and selection of studies

An electronic literature search was performed using the MEDLINE, COCHRANE, and LILACS databases.

The MEDLINE research was made through PubMed using the combination of the terms (Ureteral Calculi OR ureteral stone) AND (Adrenergic alpha-Antagonists) AND (Child* OR Adolescent). At LILACS, the following search strategy was used: strategy (alpha adrenergic antagonist) AND filters (Therapy and Children and Adolescent). At COCHRANE database, the strategy was (Adrenergic alpha antagonists) AND (Child). We further searched manually through the references of the primary studies. The searches were concluded on October 4th, 2014.

The articles were selected, independently and in pairs, by reading the respective titles and summaries. Any divergence was resolved by consensus.

Inclusion and exclusion criteria

The inclusion criteria used consisted of the following: randomized clinical trials comparing the use of an alpha-1 adrenergic antagonist to standard analgesia in children with distal ureterolithiasis.

The exclusion criteria covered non-randomized clinical trials, cohort and case-control studies, patients with proximal ureterolithiasis and papers about adult population.

Outcomes analyzed

The outcomes analyzed were calculus expulsion, pain episodes (as necessity of analgesia and hospitalizations), expulsion of calculi smaller than 5mm and expulsion of calculi larger than 5mm.

Methodological Quality

The methodological quality of the primary studies was evaluated by the GRADE system proposed by the Grades of Recommendation, Assess-

ment, Development and Evaluation group (11). The system offers several advantages in comparison to other evidence grading systems. One important advantage is to separate the quality evaluation of the evidence from the strength of recommendation evidence.

Statistical Analysis

The meta-analysis was performed with the Cochrane Review Manager 5.2 program (12). Data were evaluated by intention-to-treat.

The evaluation of the dichotomic variables was performed by the difference in absolute risk adopting a 95% confidence interval. When there was a statistically significant difference between the groups, the number needed to treat (NNT) or the number needed to cause harm (NNH) was calculated.

The continuous variables were evaluated by the difference in means. Studies that did not show data in terms of means and their respective standard deviations were not included in the analyses.

The power of analysis was calculated using the program Open Epi 3.03 (13). It was considered statistically relevant power greater than 80%.

Heterogeneity and sensitivity analysis

Inconsistencies among the clinical studies were estimated using the chi-squared heterogeneity test and quantified using I^2. A value above 50% was considered substantial. Studies that generated heterogeneity were represented by funnel plots.

EVIDENCE SYNTHESIS

Selection of studies

A total of 28 articles (MEDLINE=23; COCHRANE=3; and LILACS=2) were retrieved by electronic searches. In the manual search no articles were found in addition to those previously selected. Three articles were found both in MEDLINE and in COCHRANE and one article was found both in MEDLINE and in LILACS; three were excluded by the title, seven by the reading of the abstract because they were not in English or not about distal ureterolithiasis. Ten other articles were excluded after full reading the papers: one for being a cohort, two for being a review and seven for not dealing with pediatric patients. Thus, three randomized clinical trials were preselected and included in this review (Figure-1).

The three studies included patients randomized into two groups, totaling up 145 patients; 76 were in the intervention group (alpha-1 adrenergic antagonist) and 69 in the control group (ibuprofen).

Methodological quality evaluation performed by GRADE is represented on Table-1.

Description of the studies included

The study by Aydogdu et al. (10) consisted of a prospective study and included 39 patients with distal ureterolithiasis. These patients were randomized into two groups, 19 in the group of alpha-1 adrenergic antagonists and 20 in the ibuprofen group. The rate of calculus expulsion, mean time for expulsion, and adverse events of alpha-1 adrenergic antagonists are the outcomes evaluated.

In the study by Erturhan et al. (15), 45 patients with distal ureterolithiasis were randomized; 24 in the alpha-1 adrenergic antagonist group and 21 in the ibuprofen group. The outcomes analyzed were the rate of calculus expulsion and the mean time of expulsion. Median number of pain episodes was 1 (interquartile range 1-1) in the alpha-1 adrenergic antagonist group and 1 (interquartile range 1-2) in the ibuprofen group (p=0.023).

The study by Mokhless et al. (17) is a randomized prospective study, carried out between 2007 and 2010, which analyzed 61 patients, 33 in the alpha-1 adrenergic antagonist group and 28 in the ibuprofen group. The rate of calculus expulsion, mean time of expulsion, the need for analgesia, and possible adverse effects of the drugs were evaluated. Number of pain episodes was 1.4 ± 1.2 (Mean$\pm$SD) in the alpha-1 adrenergic antagonist group and 2.2 ± 1.4 in the ibuprofen group ($p<0.02$) (Table-2).

Analysis of Calculus Expulsion

The three primary studies analyzed the outcome of calculus expulsion. The incidence of ureteral calculus expulsion was 81.58% in the al-

Figure 1 - Prisma 2009 Flow Diagram (15).

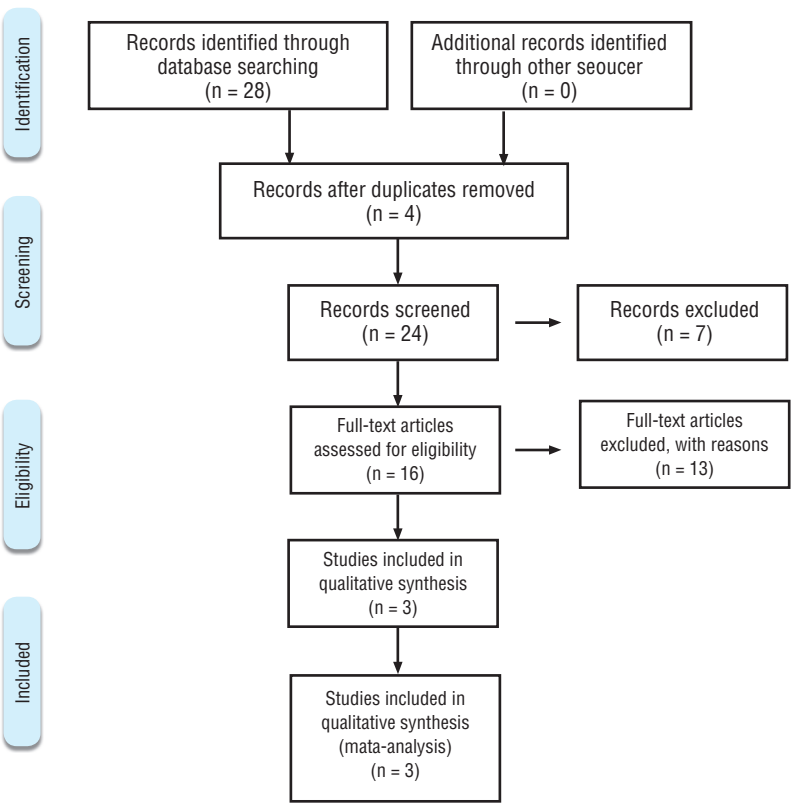


Table 1 - Methodological evaluation by GRADE.

PARAMETERS	AYDOGDU 2009	MOKHLESS 2012	ERTURHAN 2013
Whas the study randomized	Y	Y	Y
Was the allocation of patients to the groups confidential?	Y	Y	Y
Were the patients analyzed in the groups to which they were randomized (was the analysis by intention-to-treat)?	Y	Y	Y
Were the patients analyzed in the groups to wich they were known prognostic factors?	Y	Y	Y
Was the study blind?	ND	ND	ND
Except for the experimental intervention, were the groups treated equally?	Y	Y	Y
Were the losses significant?	N	N	N
Did study have a precision estimate for the effects of treatment?	Y	N	N
Are the study patients similar to those of interest?	Y	Y	Y
Are the outcomes of the study clinically relevant?	Y	Y	Y
Were the potential conflicts of interest declared?	N	N	N

Legend: Y: Yes, N: No, ND: Not Described.

Table 2 - Description of the included studies.

Author	Number of Patients AA1A	Number of Patients SA	Age of Children (Years)		Treatment		Stone Passage Mesured by
			AA1A+SA	SA	**AA1A+SA**	**SA**	
Aydogdu (10)	19	20	6.2±2.4	5.1±2.2	**Doxazosin** 0.03mg/Kg/day **Ibuprofen** 10mg/Kg 2x/day	**Ibuprofen** 10mg/Kg 2-4x/ day	Urinary filtration
Erturhan (15)	24	21	6.0±3.5	7.2±3.5	**Doxazosin** 0.03mg/Kg/day **Ibuprofen** 10mg/Kg 2-4x/day	**Ibuprofen** 10mg/Kg 2-4x/ day	X-Ray KUB and US and NCCT*
Mokhless (16)	33	28	7.3.0±4.2	7.1±3.2	**Tamsulosin** >4years:0.4mg <4years:0.1mg **Ibuprofen** ND	**Placebo** ND **Ibuprofen** ND	Urinary filtration

Legend: AA1A: alpha-1 adrenergic antagonist; SA: standard analgesia; ND: not described; x/d: Times per day; X-Ray KUB: radiography of the kidneys, ureters, and bladder; US: ultrasonography; NCCT: non-contrast-enhanced spiral computed tomography; *: In case of any suspicion.

pha-1 adrenergic antagonist group (62 out of 76 patients) and 55.07% in the ibuprofen group (38 of 69 patients). The alpha-1 adrenergic antagonists increased the probability of calculus expulsion by 27% (95% CI 0.13 to 0.41; p=0.0002 and I^2=13%), needing to treat 4 patients to achieve this benefit (NNT=4) (Figure-2). Power was 93.4%.

Analysis relative to pain episodes

Two primary studies analyzed the pain episode outcome. The difference in the mean between the groups was 0.54 (95% CI 0.00 to 1.08; p=0.05 and I^2=46%). The alpha-1 adrenergic antagonists decreased the mean of pain episodes (Figure-3). Power was 61.46%.

Analysis of the Expulsion of Calculi Smaller than 5mm

Two primary studies analyzed the outcome of expulsion of calculi smaller than 5mm. The incidence of ureteral calculus expulsion was 96.15% in the alpha-1 adrenergic antagonist group (25 out of 26 patients) and 61.54% in the ibuprofen group (16 out of 26 patients). The alpha-1 adrenergic an-

Figure 2 - Meta-analysis of the incidence of ureteral calculus expulsion.

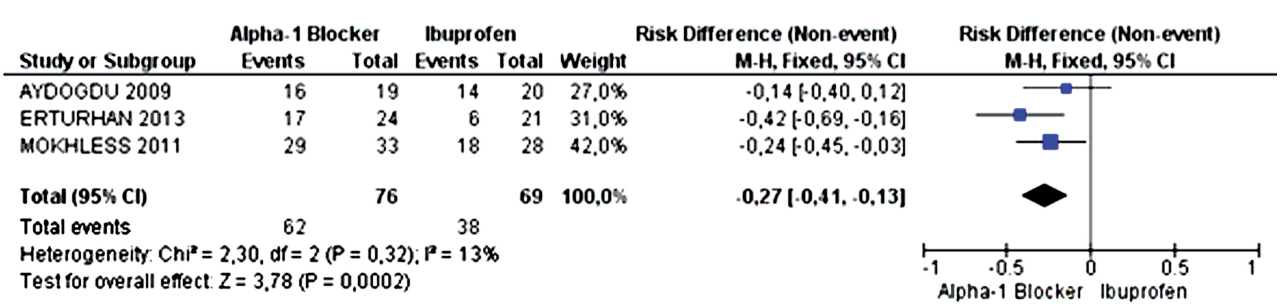
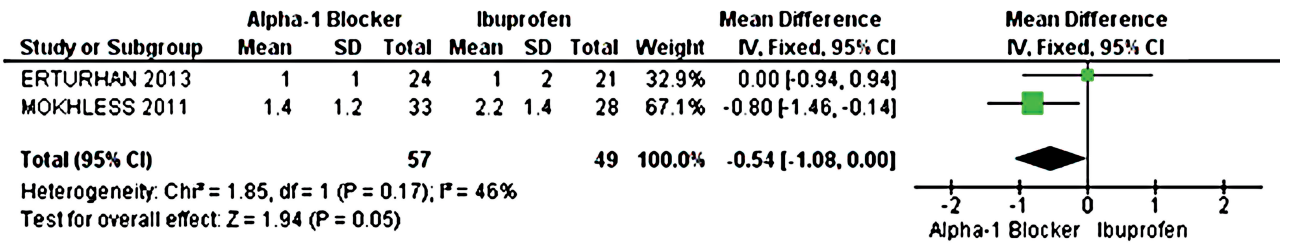


Figure 3 - Meta-analysis of the difference in means of pain episodes.



tagonists increased the probability of expulsion of calculi smaller than 5mm by 33% (95% CI 0.13 to 0.52; p=0.001) and I^2=79%), with 3 patients needing treatment to achieve this benefit (NNT=3) (Figure-4). The funnel-plot of this outcome is represented in Figure-5. Power was 88.69%.

Analysis of Expulsion of Calculi Larger than 5mm

Two primary studies analyzed the outcome of expulsion of calculi larger than 5mm. The incidence of ureteral calculus expulsion was 67.74% in the alpha-1 adrenergic antagonist group (21 out of 31 patients) and 36.36% in the ibuprofen group (8 out of 22 patients). The alpha-1 adrenergic antagonists increased the probability of calculus expulsion by 34% (95% CI 0.10 to 0.57 p=0.005 and I^2=0%), with 3 patients needing treatment in order to achieve this benefit (NNT=3) (Figure-6). Power was 62.45%.

DISCUSSION

Symptomatic ureterolithiasis represents the most frequent urological patients in emergency services (1). Over the last two decades, with the development of extracorporeal lithotripsy associated with the progress of endourology and the appearance of progressively less rigid or even flexible endoscopes, there has been an advance in the treatment of ureterolithiasis (10). Nevertheless, despite such procedures being extremely effective, with success rates between 98.5% and 100%, (15) it is imperative to evaluate the high cost and risk of complications, such as perforation, avulsion, and ureteral narrowing reported in about 3 to 5% of the procedures (1, 10). Thus, the pharmacological treatment seeking the spontaneous expulsion of stones is amply preferred as the first choice of treatment (1).

Even so, the spontaneous expulsion of distal ureteral calculi depends on various factors, including size, number and location, smooth muscle spasm, and ureteral edema (11, 16). In this context, prior studies demonstrated that the inhibition of alpha-1 receptors located primarily in the distal ureteral smooth muscle reduces intra-ureteral pressure and increases peristalsis, therefore favoring calculus elimination (10). Additionally, some studies clearly concluded that the use of alpha-1

Figure 4 - Meta-analysis of the incidence of ureteral calculi expulsion smaller than 5mm.

Figure 5 - Funnel-plot of the outcome that presented heterogeneity above than 50%.

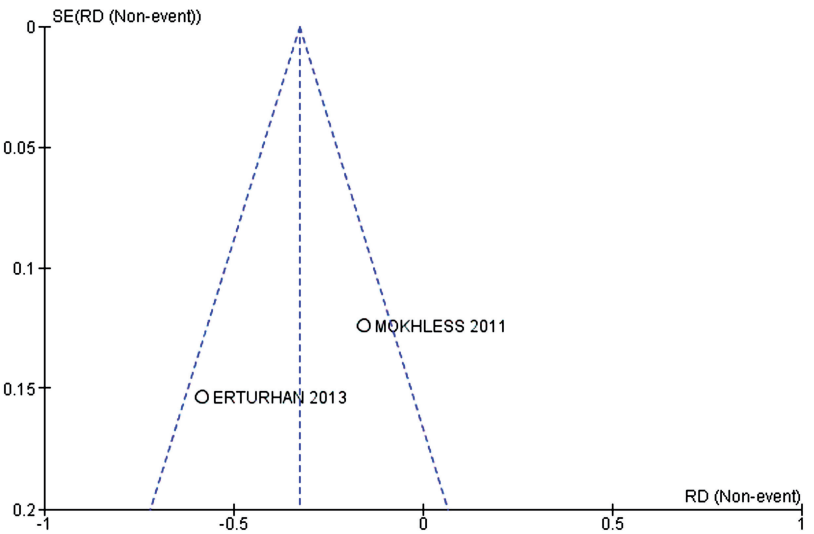


Figure 6 - Meta-analysis of the incidence of expulsion of ureteral calculi larger than 5mm.

adrenergic inhibitors could also be beneficial for residual fragments after extracorporeal shock lithotripsy (15). Increasing the level of evidence of the result of isolated studies, two meta-analyses identified clinical benefit in the use of alpha--blockers in adult patients with ureteral calculi by showing that the use of alpha-blockers compared to placebo increased the probability of calculus expulsion by 52% and 44%, respectively (1).

And finally, despite ureteral calculi having been amply studied in adults, to date the same benefits of alpha blockers have not been confirmed in the pediatric population based on the meta--analysis of randomized clinical trials (10).

The systematic review with meta-analysis is a type of study with scientific precision that selects the best evidence available in medical literature and demonstrates the methodological quality of the primary studies, which is a fundamental condition for attaining precise conclusions on the effect of interventions. To avoid distortions, it was decided to only include results with clinical and statistical homogeneity.

The search strategy showed that there are few controlled and randomized clinical trials available that compare alpha-1 adrenergic antagonists and ibuprofen in the treatment of distal ureterolithiasis in children.

A possible source of bias may be the difference between the processes of randomization of the studies included. However, the quality of the allocation process was considered adequate in all studies. All patients analyzed met the defined eligibility criteria. In the statistical analysis, calculation

of the size of the sample and the analysis as per intention-to-treat were used. A common limitation of the analysis of the outcomes was the variety of alpha-1 adrenergic antagonists and their dosages.

The last source of limitation or bias would be the difference of methods for measuring calculi expulsion (Table-2). The study by Erturhan et al. (15) measured the expulsion rate through imaging studies (X-ray, ultrasound and non-contrast CT), method with higher accuracy. The two other studies, Mokhless et al. (16) and Aydogdu et al. (10) analyzed it through urine filtration, a less sensitive method, although with higher specificity. However, we understand that this was not a confounding factor in our analysis. On three analyzes in which this measurement could influence (Figures 2, 4 and 6), the result directly reflected the outcome of the study with more accuracy, Erturhan et al. (15). Then we can infer that, in fact, the improvement generated by alpha blocker is even greater than that was found in the meta-analysis, since two of the studies used as the measuring standard a less consistent method.

To claim that the result of a study with a small sample is statistically significant is required to evaluate the error type 1, when p is less than 5%. This ensures that the result is actually true, as all the analyzes in this meta-analyzes. To state that a study is reproducible is necessary to evaluate the error type 2, when the power is greater than 80%. This ensures that, if the study would be remade elsewhere would have the same result. This is the case for the analysis of calculus and expulsion of calculi smaller than 5mm (Figures 2 and 4). However, studies with power less than 80% requires a larger sample to affirm reproducibility, as in the case of analysis relative to pain episodes and expulsion of calculi larger than 5mm (Figures 3 and 6).

The study followed the ethical and confidentiality principles of information that are recommended, since it is an analysis of results already published in other articles, and the formal approval of a research ethics committee was not necessary.

CONCLUSIONS

The use of an alpha-1 adrenergic blocker is related with a greater incidence of expulsion of ureteral calculi, smaller or greater than 5mm, and fewer episodes of pain when compared to ibuprofen. However, it is necessary larger samples to enhance the power analysis of the expulsion of ureteral calculi larger than 5mm and the episodes of pain.

CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Factors associated with the Journal Impact Factor (JIF) for Urology and Nephrology Journals

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ABSTRACT

Purpose: The Journal Impact Factor (JIF) is an index used to compare a journal's quality among academic journals and it is commonly used as a proxy for journal quality. We sought to examine the JIF in order to elucidate the main predictors of the index while generating awareness among scientific community regarding need to modify the index calculation in the attempt to turn it more accurate.

Materials and Methods: Under the Urology and Nephrology category in the Journal Citations Report Website, the top 17 Journals by JIF in 2011 were chosen for the study. All manuscripts' abstracts published from 2009-2010 were reviewed; each article was categorized based on its research design (Retrospective, Review, etc). T and correlation tests were performed for categorical and continuous variables respectively. The JIF was the dependent variable. All variables were then included in a multivariate model.

Results: 23,012 articles from seventeen journals were evaluated with a median of 1,048 (range=78-6,342) articles per journal. Journals with a society affiliation were associated with a higher JIF (p=0.05). Self-citations (rho=0.57, p=0.02), citations for citable articles (rho=0.73,p=0.001), citations to non-citable articles (rho=0.65,p=0.0046), and retrospective studies (rho=-0.51,p=0.03) showed a strong correlation. Slight modifications to include the non-citable articles in the denominator yield drastic changes in the JIF and the ranking of the journals.

Conclusion: The JIF appears to be closely associated with the number of citable articles published. A change in the formula for calculating JIF to include all types of published articles in the denominator would result in a more accurate representation.

ARTICLE INFO

Key words:
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INTRODUCTION

The Journal Impact Factor (JIF) is an index annually published by The Journal Citation Reports (JCR). It was established by Eugene Garfield to compare investigators' and journals' research influence on its time (1). The JIF for a particular journal is calculated by the ratio of total number of citations received in a determined year to the total number of citable articles published by that journal in the previous 2 years (Figure-1) (2, 3). The non-citable articles criteria include editorials, news, meeting reports, etc. Original research and review articles are the only article types that meet the definition of citable articles according to the Institute of Scientific Information (3, 4). Web of

Figure 1 - JIF Calculation formula.

$$JIF_A \text{ for } YYYY = \frac{\text{All citations in YYYY to articles published in A during } (YYYY-1)+(YYYY-2)}{\text{All citable articles published in A during } (YYYY-1)+(YYYY-2)}$$

Science (Thomson Reuters Inc.) is a citation service accessible through an indexing database and search engine, ISI Web of Knowledge^{SM} (WoK) (5-9).

The numerator of the JIF formula includes the citations of all articles of a given journal whereas its denominator excludes the number of non-citable articles published; thus making the formula potentially manageable. While this flaw has been regarded as insignificant by the index creator, it has also generated significant debate and skepticism among editors and authors who disagree. The creator himself has accepted the issue yet considering it "statistically significant only in rare cases" (2, 10-14). The exclusion of non-citable articles from the denominator can hypothetically improve the JIF by increasing the number of self-citations, non-citable articles or reducing the number of citable articles (15-17). Regardless of this situation, the JIF is held as the gold standard measure to judge journals' quality with a general lack of awareness of its formula, purpose, and meaning. All of which is now coming under greater scrutiny (18-21, 14). This debate is not merely academic as many universities are now using "publishing in high JIF journals" as an evaluation criterion for promotion. Publication in high JIF journals also affects success in competing for extramural grant funding (22).

We sought to examine the JIF among nephrology and urology journals in order to elucidate the main predictors of the index while generating awareness among scientific community about the current formula used to calculate the JIF and its flaws. We also attempt to show that small discrepancies in the formula do influence the overall calculation and the subsequent ranking list.

MATERIALS AND METHODS

Journal Selection

The top 40 journals ranked by JIF in the Journal Citation Reports for 2011 in the urology and nephrology category were selected for the study. We excluded journals that were not published in English and published articles of one specific kind of research design (basic science, retrospective studies, review articles). Twenty-three journals out of the 40 journals initially selected were excluded for containing only review (16) and basic research (7) publications.

Variables Selection

All of the archived abstracts from each journal's website for years 2009-2010 were individually reviewed. This is the period used for JIF calculation that is reported for 2011. We categorized each of the articles according to research design (basic science, non-clinical experimental and translational research, clinical trial, retrospective study, prospective study, case report and case series, cross-sectional study, review article, meta-analysis, systematic review, and guideline). If the study design could not be ascertained from the abstract, the manuscript's methods section was read in order to obtain a more precise classification. Appendix 1 outlines the articles' sorting criteria for research design (Appendix 1). The number of self-citations, citable and non-citable articles, and citations made to citable articles, and citations made to non-citable articles were also identified (15, 16). Other variables examined included society affiliation, US vs. non-US journals, number of articles published by the journal, average pages per issue, and US and non-US articles.

ISI Web of Knowledge^{SM} Citations Data Validation

Data values for JIF, self-citations, citable and non-citable articles published during 2009 and 2010, as well as the total number of citations used to calculate the 2011 JIF were retrieved and examined from the JCR web site (7, 8). The JCR website, however, did not individually disclose the citations made to citable and non-citable articles that were used to calculate the 2011 JIF. As an

alternative, two other databases (Web of Science and MEDLINE) from the WoK website were used (5, 6, 9). The data for US and non-US articles was also acquired through the WoK website (6, 9).

Because the sum of citations to citable and non-citable articles (total number of citations) retrieved from the WoK website did not directly match the total number of citations acquired from the JCR website, we sought to validate the WOK website data by generating an alternative JIF utilizing the data extracted from the WOK website. The goal for this intermediate step was to accurately use the WOK website data a posteriori during our statistical analysis in lieu of the JCR website data (not individually disclosed).

The alternative JIF was calculated using the citations data acquired from the WoK website. This JIF was correlated with the official JIF published by JCR through the Spearman's rank-order correlation test. A correlation with a p-value less than 0.05 and a slope close to 1 were defined as the required criteria for validation.

Statistical Analysis

The number of articles published during 2009 and 2010 issues published per year, average pages per issue, self-citations, issues published per year, average articles published per issue, citations made to citable articles, citations made to non-citable articles, and the categorical variables were analyzed as independent variables. All other continuous variables were analyzed as a ratio relative to the total number of publications in the period of interest

$$\left(\text{i.e.}\frac{\text{number of review articles}}{\text{total number of publications made during 2009 \& 201}}\right)$$

because they depended on the total number of articles published.

The association between the variables and JIF was examined using the JIF as the dependent variable. A two tailed Welch's t-test and Spearman's rank-order correlation test were performed for categorical and continuous variables respectively. All variables were then included in a multivariate linear regression model using a stepwise variable selection method. An alternative multivariate model excluding the counts for citable articles was analyzed in order to account for co-linearity of other variables with the variable "citable articles".

A hypothetical JIF (JIF′) was created with the data obtained from the JCR website, one that includes the non-citable articles in the denominator of the index calculation (Citations made to Citable and Non-citable articles/Citable and Non-citable articles). A Welch's t-test was also used to compare the JIF published by the JCR with the hypothetically created JIF. All computations were performed using SAS 9.3 (SAS Institute, Cary, NC). P-values of <0.05 were considered to be statistically significant. All the assumptions were verified for every test.

RESULTS

A total of 12/17 journals were society affiliated, 8 were US originated, and 9 were non-US originated. Table-1 summarizes all the variables used in the analysis (Table-1). A total of 23,012 article abstracts were examined with a median of 1,048 (range=78–6,342) articles per journal.

ISI Web of Knowledge℠ Citations Data Validation

While utilizing the WOK website data 11 journals increased their JIF, 5 decreased their JIF, and 1 was unaffected. The Journal of Urologic Oncology: Seminars and Original Investigations, and The Journal Neurourology and Urodynamics had an important citations count miss-match between databases, which resulted in a JIF decrement of 0.5 points or more. The JIF calculated using the WoK website data correlated well with the JIF calculated using the JCR website data (rho=0.91, p<0.001, m=0.96) (Figure-2). Once validated, the data gathered from the WoK website was used to calculate a hypothetical JIF excluding citations accounted to non-citable articles (Table-2). This exclusion represented a consistent drop in the JIF value throughout the 17 journals, promoting at the same time ranking position variations.

Statistical Analysis

Journals with a society affiliation were associated with a higher JIF compared to journals without a society affiliation (mean JIF=4.46 vs. 2.80, p=0.05). US journal origin vs. non-US journal origin did not demonstrate an association

with JIF (mean JIF=4.08 vs. 3.88, p=0.86) (Table-1). A significant difference was found while comparing the JIF published by the JCR with the hypothetical JIF including the non-citable articles in the denominator of the calculation (mean JIF=3.97 vs. mean JIF'=2.34, p=0.02) (Table-2).

The bivariate analysis also found some of the examined variables to be significantly associated with JIF: self-citations (rho=0.57, p=0.02), retrospective studies (rho=-0.51, p=0.03), citations to citable articles (rho=0.73, p=0.001), citations to non-citable articles (rho=0.65, p=0.0046), and case reports/case series (rho=-0.47, p=0.05) (Table-1). The remaining variables examined were not found to be good predictors of JIF.

The multivariate linear regression analysis demonstrated that citations made to citable articles (p<0.001) are the most important predictor of a high JIF, whereas the number of citable articles (p=0.015), total number of articles published (p<0.001) and case reports/case series (p=0.002) are the most important predictors of low

Table 1 - Journals' Summary Statistics and Analyses Results.

Dependent Variable	Total Number	Median (Range)	-	-
Journal Impact Factor	17	3.22 (2.10-9.66)	-	-
Categorical Variables	Total Number	JIF Mean (CI-95%)	*t*-value (df)	*p*-value
US Journals	9	4.08 (2.01-6.16)	-0.18 (15)	0.86
Non-US Journals	8	3.88 (2.20-5.55)	-	-
Society Affiliated Journals	12	4.46 (2.86-6.07)	-2.14 (13)	0.05*
Non-society Affiliated Journals	5	2.80 (2.06-3.54)	-	-
Continuous Variables	Total Number	Median (Range)	Rho (R^2)	*p*-value
*Average Pages Per Issue	179.3	154.9 (31.54-424.58)	0.36 (0.13)	0.16
Total Number of Publications	23012	1048 (78-6342)	0.30 (0.09)	0.25
US Articles	7961	357 (20-1712)	0.46 (0.22)	0.06
Non-US Articles	15051	535 (58-4630)	-0.46 (0.22)	0.06
Citable Articles	9598	488 (31-1437)	-0.29 (0.08)	0.26
Citations to Citable Articles	36859	2497 (58-4630)	0.73 (0.53)	0.001*
Non-citable Articles	13595	467 (2-1437)	0.29 (0.08)	0.26
Citations to Non-citable Articles	1790	87 (0-234)	0.65 (0.42)	0.0046*
Journal Self-Citations	5255	240 (0-1339)	0.57 (0.32)	0.02*
Retrospective	2616	54 (14-613)	-0.51 (0.26)	0.03*
Clinical Trials	621	18 (6-108)	-0.01 (0.00)	0.96
Basic Science Research	2776	128 (5-287)	-0.05 (0.00)	0.84
Prospective	1659	64 (8-337)	-0.44 (0.20)	0.07
Case Report/Case Series	683	9 (2-199)	-0.47 (0.22)	0.05*
Cross-Sectional	313	7 (1-127)	-0.05 (0.00)	0.83
Review Articles	824	42 (2-117)	-0.03 (0.00)	0.89
Meta-analysis	53	1 (0-13)	0.03 (0.00)	0.92
Systematic Review	41	1 (0-10)	-0.18 (0.03)	0.50
Guidelines	45	0 (0-25)	0.23 (0.05)	0.37

Figure 2 - The scattered plot exhibits the correlation between Journal Impact Factors calculated using data retrieved from Web of Knowledge, and data retrieved from Journal Citation Reports. *Slope (m).

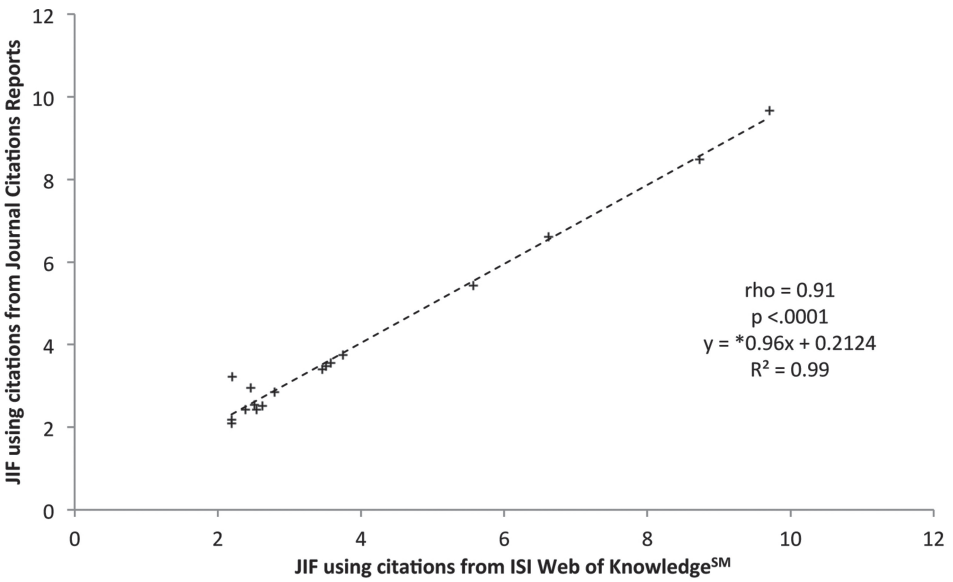

Table 2 - Data Extracted From Journal Citations Reports and Web of KnowledgeSM

	JIF-JCR	JIF-WOK*	JIF'	NCA	CA	Total Cites-WOK$^{\Delta}$	Total Cites-JCR
J Am Soc Nephrol	9,66	9,71	7,49	143	493	4785	4764
Eur Urol	8,49	8,73	3,70	592	456	3982	3876
Kidney Int	6,61	6,62	3,63	475	578	3825	3818
Am J Kidney Dis	5,43	5,57	2,02	824	488	2718	2652
J Urology	3,75	3,75	0,85	4905	1437	5383	5383
J Sex Med	3,55	3,58	1,24	1413	756	2707	2685
Prostate	3,49	3,51	3,45	4	361	1267	1258
Nephrol Dial Transplant	3,40	3,46	2,77	272	1193	4126	4051
Urol Oncol-Semin Ori	3,22	2,20	2,61	46	199	438	640
Neurourol Urodynam	2,96	2,46	0,61	1016	262	645	775
Bju Int	2,84	2,80	1,44	988	1016	2840	2890
Am J Nephrol	2,54	2,51	2,35	23	282	708	716
Pediatr Nephrol	2,52	2,62	0,58	1815	548	1437	1380
Urology	2,43	2,54	1,32	956	1140	2900	2768
Prostate Cancer P D	2,42	2,39	2,21	11	114	272	276
Bmc Nephrol	2,18	2,19	2,18	0	68	149	148
Periton Dialysis Int	2,10	2,19	1,36	112	207	467	434

JIF-JCR mean = 3.97 vs. JIF' mean = 2.34, t-Test (29) = 2.41, p-value= 0.02
JIF: Journal Impact Factor, WOK: Web of KnowledgeSM, CA: Citable Articles, NCA: Non-Citable Articles
*JIF using data extracted from Web of Knowledge.
JIF' calculated including Non-citable articles in the denominator; Formula: JIF=Total Cites-JCR/(NCA+CA)
$^{\Delta}$Calculated by adding up Citations to CA and NCA.

JIF (Table-3). Appendix 2 summarizes the multivariate model excluding the variable citable articles.

DISCUSSION

The findings of this study emphasize the need to modify the JIF formula in order to make it more accurate and acceptable. No prior publications have focused on this topic in Urology, nor has a detailed statistical analysis been performed to weight the different variables that are included in the JIF. Variables such as citations to citable and non-citable articles, self-citations, and low number of citable articles published have been hypothesized as predictors of high JIF (15, 16, 17). These assumptions were held as true during this analysis and confirm them.

Regardless of having readily available counts of total number of citations the JCR website does not routinely disclose the individual counts for citations made to citable and non-citable articles, and such numbers can only be obtained indirectly through the WoK website (7, 8). The data acquired from the WoK and JCR websites were compared in order to assess the feasibility of the analysis. Regardless of being under the same umbrella, differences between the two datasets were found. Due to these discrepancies, we sought to validate the citations data retrieved from the WoK website against the data published in the JCR website. With the proper computing, a strong validation was indeed achieved with excellent similarity between the two datasets (rho=0.91, slope=0.96) (Figure-2).

The validation process enabled us to prove that citations to non-citable articles do significantly improve the JIF (rho=0.65, p=0.0046) (15, 16). Also, a high proportion of retrospective studies and case reports/case series had a negative effect over the JIF. With few exceptions retrospective studies and case reports/case series are regarded as a resource of low evidence level, hence tend to have a low impact in the scientific community and do not receive as many citations as designs regarded as higher level of evidence (i.e. meta-analyses) (23). Therefore the inclusion of non-citable articles and exclusion of case reports/case series and retrospective studies may serve as positive predictors of JIF. Low citable article counts and high self-citation rates also appear to predict the JIF (Table-1). The latter echoes the result of a previous analysis including 6 anesthesiology journals (17).

Citations made to citable articles were the most important variable influencing the JIF. Articles reporting the results of large clinical trials, basic science research, cross-sectional studies, reviews, systematic reviews, and meta-analysis did not demonstrate a significant correlation with the JIF nor were they independent predictors of JIF when controlling for all other factors. This could be due to the fact that research design variables are included under the umbrella of citable articles making them collinear variables. However, a sensitivity analysis was performed in order to control for co-linearity among the research design variables by eliminating the citable articles variable. The results were not modified (Appendix 2).

Table 3 - Results for the Multiple Linear Regression Model.

Multivariate Model	Estimate	Standard Error	Adjusted Estimate (*t*)*	*p*-value
Intercept	5.13	0.87	5.9	-
Total Articles published	-0.0014	0.00022	-6.36	<0.001
Citable Articles	-2.98	1.045	-2.85	0.015
Case Reports/Case Series	-28.03	7.3	-3.84	0.002
Citations to Citable Articles	0.0015	0.00016	9.38	<0.001

*Adjusted estimate by variable scale (normalized estimate: Estimate/Standard Error).
Model R^2 = 0.9

Because citable articles are fundamental for the JIF calculation, such model was performed only to account for co-linearity and does not provide any other useful insight.

The journals with highest JIF in our cohort grossly were those that accepted the least number of retrospective studies and case reports/case series. The concern with this fact is that journal editors could place greater emphasis on the "citability" of an article rather than the quality and nature of the scientific question. Much of the literature in Urology focuses on certain subspecialties such as oncology, which makes these articles potentially more citable and getting higher priority for publication. The citability of an article could also be linked to the topical nature of the article or in other words, its conformation to the existing trend. In example, multiple articles on certain popular topics such as robotic surgery could be published and crowd out excellent work in basic science and less popular areas.

One other interesting finding was that the JIF of journals linked to professional societies had a higher value. This may represent the fact that members of the society are more likely to be aware of and cite articles from the journal of their society. This may be an unrecognized value to the journals and to the societies since publishing in higher JIF journals is becoming increasingly important to academicians and increasing JIF is important to journals.

When a hypothetical JIF including the non-citable articles in the denominator was created, a significant difference was found when compared to the JIF published by the JCR (p=0.02). This analysis contrasts with the belief of finding "significant discrepancies only in rare cases" (2). Moreover, this hypothetical modification of the JIF generated multiple rank changes among the journals. This issue builds a compelling case regarding the need to modify the formula to calculate the JIF since it is regarded as gold standard of journal quality and often looked at as a criterion for promotion or extramural grant funding (Table-2) (18-22).

The limitations of this study include the fact that we examined the correlation of articles to JIF over a two-year snapshot in time. Factors involved and associations could change over time and these data do not have the ability to capture that. We identified and analyzed a set of journals in a non-randomized manner. Hence we are making the assumption that these findings hold true for journals that are not the top 40 in the Urology/Nephrology category. These findings also apply only to English language journals. The biggest limiting factor lies on the lack of access to citations counts for each research design, which is essential to assess the influence of research designs over the JIF prediction. Despite these shortcomings, this is the first study to perform a detailed analysis of JIF among urology journals. These data can provide us with some understanding of what predicts JIF.

CONCLUSIONS

Variations to the formula to account for non-citable articles in the denominator do represent a statistical significant difference when compared to the JIF as it is currently calculated. A change in the formula for calculating JIF to include all types of published articles in the denominator would result in a more accurate and accepted representation. The JIF appears to be predicted by the number of citations made to citable articles and non-citable articles, self-citations, case reports/case series, retrospective studies and citable articles published. Number of citations made to each different research design is necessary in order to assess their influence on the JIF.

CONFLICT OF INTEREST

None declared.

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Appendix 1 - Articles' Sorting Criterion According to Research Design.

Retrospective studies	Retrospective designs, including retrospective center and professional experience; retrospective and prospectively generated databases.
Clinical Trials	Experimental clinical studies such as randomized and non-randomized, blinded and non-blinded clinical trials.
Basic science research	Basic science research, animal experimental and observational research, non-clinical meta-analysis, and translational research.
Prospective studies	Prospective observational studies, including longitudinal studies, and short follow up cohort observational studies. No databases where included in these group as they belong to retrospective studies.
Case Report/Case Series	Case reports, case series, description of novel procedures and merely descriptive articles.
Cross-sectional studies	Cross-sectional studies. By definition, data collection takes place in over a fixed amount of time any follow up. Data retrospectively acquired defaulted the abstract to be classified as a retrospective study.
Review articles	Review articles without any fixed methodology or criteria for revision.
Meta-analysis	Meta-analyses, systematic review and meta-analyses.
Systematic reviews	Systematic reviews without meta-analyses.
Guidelines	Guidelines, professional consensus and recommendations, reference material, expert opinion, and best practices.
Non-citable articles	Editorials, letters, replies, commentaries, erratum, diaries, debates, summaries, news, biographical articles, meeting abstracts, meeting podiums, meeting posters, points and counterpoints and other journal specific categories.

Appendix 2 - Results for the Multiple Linear Regression Model Excluding Citable Articles.

Multivariate Model	Estimate	Standard Error	Adjusted Estimate (Estimate/SE)	*p*-value
Intercept	2.28	0.46	4.96	-
Total Publications	-0.001	0.00022	-4.55	<0.001
Non-Citable Articles	2.75	1.032	2.66	0.02
Case Reports/Case Series	-28.95	7.4	-3.91	0.002
Citations to Citable Articles	0.00147	0.00016	9.19	<0.001

Model R^2 = 0.9

ORIGINAL ARTICLE

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Adjuvant chemotherapy for locally advanced upper tract urothelial carcinoma: updated results of the Seoul National University Hospital experience

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ABSTRACT

Objectives: The objective of this study was to update the long-term outcome in the treatment of locally advanced upper tract urothelial carcinoma (UTUC) after radical nephroureterectomy (RNU) regarding the role of adjuvant chemotherapy.
Materials and methods: Clinical data from 138 patients who underwent RNU for locally advanced UTUC (pT3/4 or pN+) were analyzed.
Results: The adjuvant chemotherapy group comprised 66 patients, and other 72 patients did not receive adjuvant chemotherapy. Cisplatin-based chemotherapy was the most common regimen, depending on the patient's eligibility and renal function. The median follow-up period was 48.7 months (interquartile range: 29.2-96.9 months). The 3-and 5-year disease-specific survival (DSS) rates were 76.0% and 69.9% for the non--adjuvant chemotherapy group versus 74.6% and 54.5% for the adjuvant chemotherapy group (p=0.301, log-rank test). Overall survival (OS) rates for the same time period were 70.1% and 62.9% for the non-adjuvant chemotherapy group versus 73.8% and 53.2% for the adjuvant chemotherapy group (p=0.931, log-rank test). On multivariate analysis, adjuvant chemotherapy could not predict DSS and OS after surgery. When patients who received cisplatin-based adjuvant chemotherapy (n=59) were compared to those who did not receive adjuvant chemotherapy, similar results were found.
Conclusions: There does not appear to be a significant DSS or OS benefit associated with adjuvant chemotherapy. Prospective randomized clinical trials are necessary to verify the effect of adjuvant chemotherapy on locally advanced UTUC.

ARTICLE INFO

Key words:
Urinary Tract; Carcinoma, Transitional Cell; Chemotherapy, Adjuvant; Survival

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INTRODUCTION

Upper tract urothelial carcinoma (UTUC) is a rare disease that accounts for approximately 5% of all urothelial malignancies (1). Although radical nephroureterectomy (RNU) has been considered standard care for treating localized UTUC, 45-60% of patients with locally advanced disease will relapse after extirpative surgery alone (2). In a large multicenter collaborative study of 1.363 patients treated with RNU, Margulis et Al. (3) reported 5-year survival rates of 74.7%, 54%, 35.3%, and 12.2% for pT2, pT3, N+and pT4, respectively. Contemporary analyses indicate that there has been no improvement in survival rates in the past several decades for patients with high-grade disease (4).

Adjuvant chemotherapy with agents for metastatic disease may be reasonable in treating locally advanced UTUC associated with poor survival. However, there is no standardized therapy conferring a survival benefit after RNU, as there have been no controlled trials that explored the efficacy of adjuvant chemotherapy in this setting. Most evidence for the treatment of patients with UTUC may be extrapolated from experience with bladder cancer.

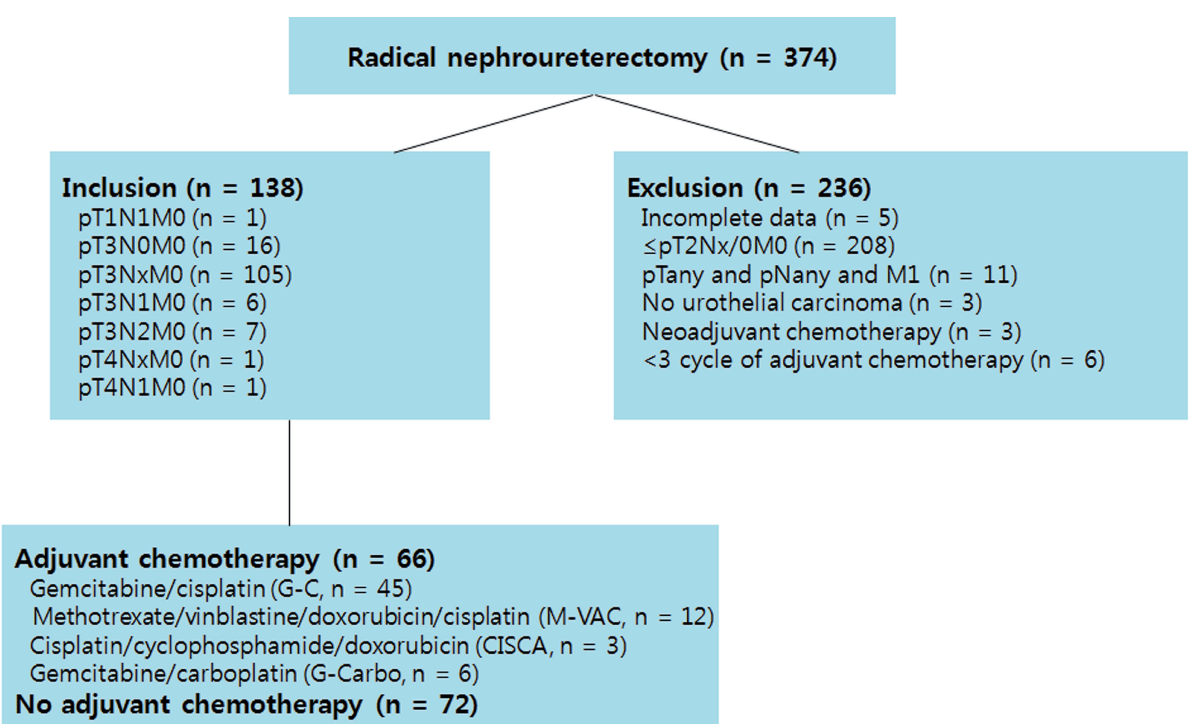
The rarity of UTUC has resulted in a paucity of literature on adjuvant chemotherapy and its role in the treatment of high-risk UTUC (5). Previously, we reported the efficacy of adjuvant chemotherapy in patients with invasive UTUC (6). In this study, we sought to give an update by reporting the long-term outcome and role of adjuvant chemotherapy in the treatment of locally advanced UTUC after RNU.

MATERIALS AND METHODS

This study was approved by the institutional review board. We performed a retrospective review of 374 patients who underwent radical nephroureterectomy (RNU) at Seoul National University Hospital from 1993 to 2010. RNU was performed according to standard procedures, and the regional lymph nodes were generally resected if intraoperatively palpable or preoperatively enlarged during evaluation. Patients with incomplete data, localized disease (≤ pT2Nx/0M0), distant metastasis (pTany and pNany and M1), no urothelial carcinoma, administration of neoadjuvant chemotherapy or administration of less than 3 cycles of adjuvant chemotherapy were excluded. To meet criteria for adjuvant chemotherapy, treatment must have been started within 3 months of undergoing RNU. Cisplatin-based chemotherapy was the most common regimen, depending on patient eligibility and renal function, as described previously (Figure-1) (6).

Pathological specimens were evaluated by a staff pathologist with genitourinary expertise. All specimens were histologically confirmed to be urothelial carcinoma. Staging was done according to the 2010 American Joint Committee on Cancer classification and grading according to the 1998

Figure 1 - Study flow diagram.



WHO system. Lymphovascular invasion (LVI) was defined as the presence of tumor cells within an endothelium-lined space without underlying muscular walls. The presence of concomitant carcinoma in situ (CIS) was assessed in every representative section. Tumor location was defined as renal pelvic, ureteral or both. Tumor multifocality was defined as the synchronous presence of 2 or more pathologically confirmed tumors in any location (renal pelvis, ureter or both). Tumor necrosis was defined as the presence of microscopic coagulative necrosis in more than 10% of the tumor.

Patients were evaluated every 3-4 months for the first two years, every 6 months for the next two years, and then annually thereafter. Follow-up consisted of history taking, physical examination, blood tests, urine cytology, cystoscopy, chest X--ray, abdominopelvic computed tomographic (CT) scan, and bone scan. Survival was evaluated from the date of surgery to last follow-up or death. Patients who were alive with or without disease were censored from the relevant analyses. Cause of death was determined by the responsible physicians and death certificates. Perioperative deaths occurring within 30 days of surgery were censored from disease-specific survival analyses.

Statistical Analyses

For statistical analysis, the characteristics of adjuvant and non-adjuvant groups were compared. A chi-squared or Fisher's exact test was used for categorical variables and a Student's t-test for age and body mass index. Outcomes were measured by disease-specific survival (DSS) and overall survival (OS) based on chemotherapy status between the cohorts. Survival was calculated using the Kaplan-Meier method and compared using a log-rank test. To adjust for the effect of potential confounders, multivariate analyses using Cox proportional hazards model were conducted. Significant variables showing less than 0.05 of two sided p-value in the univariate analyses were entered into a multivariate analysis. The assessed variables were gender, age, body mass index, American Society of Anesthesiologists (ASA) score, previous or concomitant bladder cancer, preoperative ureteroscopy, bladder cuffing, tumor location, multifocality, hydronephrosis, tumor grade, concomitant CIS, LVI, tumor necrosis, margin status and pN stage. Pathologic T stage was not included in the multivariate model, as most pathologic T stage was pT3. However, administration of adjuvant chemotherapy was regarded as a main variable of interest and forced into the multivariate model. The same analysis was also performed for testing in a subgroup that received only cisplatin-based adjuvant chemotherapy. All statistical tests were performed with SPSS software (SPSS Inc., Chicago, IL, USA). All reported P values were two-sided, and significance was set at $P<0.05$.

RESULTS

Table-1 compares the characteristics of patients who did not receive adjuvant chemotherapy (n=72) and those who received adjuvant chemotherapy (n=66) or cisplatin-based adjuvant chemotherapy (n=59). Patients who received adjuvant chemotherapy were younger than those who did not (p=0.001). The adjuvant chemotherapy group was more likely to have a lower ASA score (p=0.006), less previous or concomitant bladder cancer (p=0.001) and more concomitant CIS (p=0.014). Other variables were similar between groups.

Overall median follow-up period was 48.7 months (interquartile range: 29.2-96.9 months). The median follow-up was 46.8 months (interquartile range: 28.9-101.3 months) for the non--adjuvant chemotherapy group and 52.8 months (interquartile range: 33.3-110.1 months) for the adjuvant chemotherapy group (p=0.469). In the entire population, 69 (50.0%) died of any cause and 52 deaths (37.7%) were attributable to UTUC. The 3-and 5-year DSS rates were 76.0% and 69.9% for the non-adjuvant chemotherapy group versus 74.6% and 54.5% for the adjuvant chemotherapy group (p=0.301, log-rank test) (Figure-2A). OS rates for the same time period were 70.1% and 62.9% for the non-adjuvant chemotherapy group versus 73.8% and 53.2% for the adjuvant chemotherapy group (p=0.931, log-rank test) (Figure-2B).

Table-2 shows the multivariate Cox regression model for predicting DSS after surgery in total patients. Bladder cuffing, LVI, and margin status were significantly associated with DSS, while

Table 1. Patient characteristics.

Variables	No ACH	ACH	P value†
Total	72	66	
Gender			**0.276**
Male	58 (80.6%)	48 (72.7%)	
Female	14 (19.4%)	18 (27.3%)	
Age, year	67.3 (57.4-73.0)	60.3 (54.1-65.7)	**0.001**
BMI, cm/kg^2	24.4 (22.4-25.6)	23.9 (21.2-25.7)	**0.552**
ASA score			**0.006**
1	21 (29.2%)	30 (45.5%)	
2	41 (56.9%)	35 (53.0%)	
3	10 (13.9%)	1 (1.5%)	
Bladder cancer*			**0.001**
No	51 (70.8%)	61 (92.4%)	
Yes	21 (29.2%)	5 (7.6%)	
Preoperative ureteroscopy			**0.650**
No	62 (86.1%)	55 (83.3%)	
Yes	10 (13.9%)	11 (16.7%)	
Bladder cuffing			**0.311**
No	17 (23.6%)	11 (16.7%)	
Yes	55 (76.4%)	55 (83.3%)	
Tumor location			**0.219**
Renal pelvis	41 (56.9%)	29 (43.9%)	
Ureter	19 (26.4%)	24 (36.4%)	
Both	12 (16.7%)	13 (19.7%)	
Multifocality			**0.956**
Absent	57 (79.2%)	52 (78.8%)	
Present	15 (20.8%)	14 (21.2%)	
Hydronephrosis			**0.522**
Absent	41 (56.9%)	34 (51.5%)	
Present	31 (43.1%)	32 (48.5%)	
Pathologic T stage			**0.062**
pT1	2 (2.8%)	0 (0.0%)	
pT3	70 (97.2%)	64 (97.0%)	

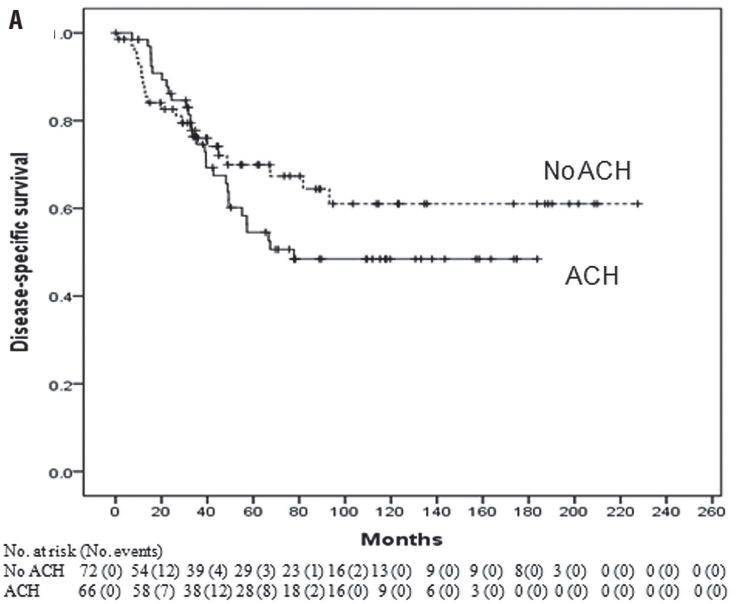
pT4	0 (0.0%)	2 (3.0%)	
Tumor grade			**0.073**
G1	6 (8.3%)	1 (1.5%)	
G2	39 (54.2%)	33 (50.0%)	
G3	27 (37.5%)	32 (48.5%)	
Concomitant CIS			**0.014**
Absent	71 (98.6%)	58 (87.9%)	
Present	1 (1.4%)	8 (12.1%)	
LVI			**0.280**
Absent	56 (77.8%)	46 (69.7%)	
Present	16 (22.2%)	20 (30.3%)	
Necrosis			**0.473**
Absent	65 (90.3%)	57 (86.4%)	
Present	7 (9.7%)	9 (13.6%)	
Margin status			**0.479**
Negative	69 (95.8%)	61 (92.4%)	
Positive	3 (4.2%)	5 (7.6%)	
Pathologic N stage			**0.080**
pN0	9 (12.5%)	8 (12.1%)	
pNx	60 (83.3%)	46 (69.7%)	
pN+	3 (4.2%)	12 (18.2%)	

Data presented are number (%) or median (interquartile range).
†Compared to no adjuvant chemotherapy group.
*****Previous or concomitant.
ACH: adjuvant chemotherapy; B**MI:** body mass index; **ASA:** American Society of Anesthesiologists; **CIS:** carcinoma in situ; **LVI:** lymphovascular invasion.

other variables were not. In multivariate analysis, bladder cuffing was the sole independent prognostic factor for DSS (p=0.002). Univariate analysis revealed that age, ASA score, previous or concomitant bladder cancer, bladder cuffing, LVI and margin status were significant predictors of OS. In the multivariate model, age (p=0.005), previous or concomitant bladder cancer (p=0.031), bladder cuffing (p=0.005) and margin status (p=0.027) remained significant predictors of OS. In contrast, ASA score and LVI were not predictors of OS. Adjuvant chemotherapy could not predict DSS and OS after surgery.

We compared the non-adjuvant chemotherapy group (n=72) to the cisplatin-based adjuvant chemotherapy group (n=59) (Supplementary Table-1). There was no significant difference between the two groups in terms of DSS (p=0.193, log-rank test) (Supplementary Figure-1A) and OS (p=0.719, log-rank test) (Supplementary Figure-1B). Supplementary Table-2 shows the multivariate Cox regression model for predicting DSS and OS after surgery. In univariate analysis, bladder cuffing, LVI, and margin status were significantly associated with DSS. Multivariate analysis revealed that bladder cuffing (p=0.001) and LVI (p=0.045) were

Figure 2 - Kaplan-Meier analysis for (A) disease-specific survival and (B) overall survival after radical nephroureterectomy stratified by the administration of adjuvant chemotherapy in all patients.



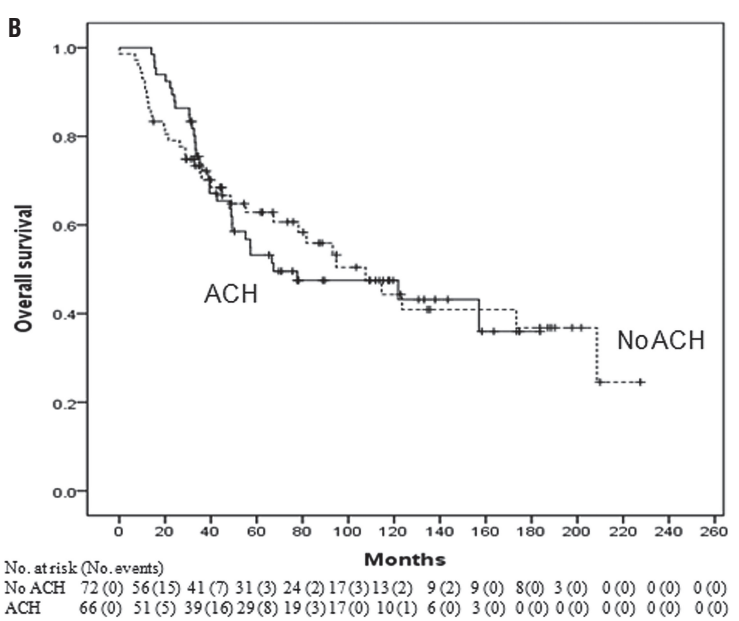


Table 2 - Multivariate Cox proportional hazards regression analysis of disease-specific survival and overall survival.

	DSS		OS	
	HR (95% CI)	P value	HR (95% CI)	P value
Age			1.045 (1.013-1.077)	0.005
ASA score				
1			Reference	
2/3			1.327 (0.723-2.435)	0.362
Bladder cancer*				
No			Reference	
Yes			1.905 (1.061-3.422)	0.031
Bladder cuffing				
Yes	Reference		Reference	
No	2.615 (1.430-4.782)	0.002	2.211 (1.266-3.862)	0.005
LVI				
Absent	Reference		Reference	
Present	1.791 (0.994-3.226)	0.052	1.669 (0.947-2.944)	0.077
Margin status				
Negative	Reference		Reference	
Positive	2.458 (0.938-6.443)	0.067	1.879 (1.074-3.286)	0.027
ACH				
No	Reference		Reference	
Yes	1.255 (0.705-2.237)	0.440	1.430 (0.809-2.526)	0.218

*Previous or concomitant.
DSS, disease-specific survival; OS, overall survival; HR, hazard ratio; CI, confidence interval; ASA, American Society of Anesthesiologists; LVI, lymphovascular invasion; ACH, adjuvant chemotherapy.

independent prognostic factors for DSS. Univariate analysis showed a statistically significant association between OS and age, ASA score, previous or concomitant bladder cancer, bladder cuffing, LVI and margin status. In multivariate analysis, age (p=0.002), previous or concomitant bladder cancer (p=0.034), bladder cuffing (p=0.002) and LVI (p=0.029) were independent prognostic factors for OS. Adjuvant chemotherapy could not predict DSS and OS after surgery.

DISCUSSION

Although UTUC is morphologically similar to bladder cancer, there are occasional phenotypic and genotypic (genetic and epigenetic) differences between UTUC and bladder cancer (4). Furthermore, the natural history of UTUC is different from bladder cancer, with >60% of UTUCs and only 15-25% of bladder cancer presenting with invasion at diagnosis (1, 7). Therefore, data generated from bladder cancer studies cannot always be extrapolated to patients with UTUC (8).

Urothelial carcinoma of the bladder is considered to be relatively chemosensitive. The survival benefit of neoadjuvant chemotherapy before radical cystectomy in patients with invasive bladder cancer has been demonstrated (9). However, the efficacy of adjuvant chemotherapy after radical cystectomy remains controversial (10). Results of adjuvant chemotherapy studies for UTUC are also contradictory. For UTUC, adjuvant chemothe-

rapy provided a therapeutic benefit in some studies (6, 11, 12), while there was no significant difference in outcomes between adjuvant and non-adjuvant chemotherapy groups in others (13-18). This discordance probably accounts for the inconsistent use of chemotherapy for locally advanced UTUC. Still, most patients are not candidates for cisplatin-based chemotherapy after RNU, primarily due to impaired renal function. Lane et al. (19) reported that 61% of all patients and 49% of high-risk patients who could have received chemotherapy preoperatively were unable to receive treatment after RNU.

There is no definite evidence showing that conventional M-VAC regimen significantly prolongs survival as an adjuvant treatment arm for patients with locally advanced UTUC. Lee et al. (18) investigated 27 patients who underwent RNU for pT3N0M0 UTUC with a median follow-up of 47 months. Sixteen patients received chemotherapy (M-VAC regimen with three-four cycles) after RNU, and 11 did not. No evidence of significant differences in recurrence-free survival and DSS could be found between the two groups. In the study by Soga et al. (17), adjuvant M-VAC could prevent bladder tumors following surgery for UTUC but did not show a survival benefit.

Hellenthal et al. (16) published data from an international multicenter study of 542 patients with pT3 or higher UTUC. Of these, 121 patients received adjuvant chemotherapy and chemotherapy that was cisplatin-based in 89% of cases (59% M-VAC and 20% GC). No significant differences in DSS or OS between the two groups were found. The selection of patients with more advanced disease could account for this finding. Another multi-institutional study analyzed 627 patients with T3 or higher UTUC, with 140 patients receiving adjuvant chemotherapy (mostly platinum-based). There was no evidence of extension of DSS or OS for patients treated with adjuvant chemotherapy (14). In other studies, chemotherapy was only administered to patients with higher grade and stage tumors, including metastatic disease. Thus, it is likely that patient selection contributed to inconsistent findings. A multi-center study from 10 Canadian academic centers revealed that adjuvant chemotherapy was not prognostic for improved DSS or OS (15).

However, retrospective studies have identified a benefit from adjuvant chemotherapy. Suzuki et al. (11) investigated the effectiveness of adjuvant chemotherapy in 56 patients with locally advanced bladder cancer or UTUC. Twenty patients underwent adjuvant chemotherapy (M-VAC or MEC) and 36 patients were controls. Adjuvant chemotherapy had a positive survival benefit in patients with node-positive disease, but did not affect survival of all patients. There was no distinction between UTUC and bladder cancer, making it difficult to draw definite conclusions for UTUC. Kawashima et al. (12) evaluated the data of 93 patients with pT3N0M0, and 38 received platinum-based adjuvant chemotherapy. In multivariate analysis, adjuvant chemotherapy was significantly associated with DSS.

Meta-analysis of adjuvant chemotherapy for UTUC demonstrated DSS and OS benefit with cisplatin-based adjuvant chemotherapy (20). However, most studies examining adjuvant chemotherapy were retrospective and may suffer from substantial selection biases. First, patients with the worst prognostic factors were selected to receive adjuvant chemotherapy compared to counterparts undergoing observation; the proportion of patients who had pN+ disease and received adjuvant chemotherapy was higher than those not receiving adjuvant chemotherapy (15, 16). Second, there are few studies with more than 50 patients, due to the low frequency of UTUC. Finally, the utility of adjuvant chemotherapy in UTUC may be limited given the decline in renal function following RNU, and renal excretion and inherent nephrotoxicity of cisplatin. The proportion of patients receiving adjuvant chemotherapy was three to four times smaller than those receiving surgery alone. Patients receiving adjuvant chemotherapy may have better renal function and performance status. Despite possible benefits, there is insufficient evidence to recommend routine use of cisplatin-based adjuvant chemotherapy for UTUC (20).

Previously, we reported on 43 patients with a tumor stage pT2 or higher without metastasis (6). Twenty-two patients received adjuvant chemotherapy. All regimens contained platinum with the M-VAC scheme used most often. The

follow-up period totaled 30.7 months. Results showed higher DSS and OS rates for patients with adjuvant chemotherapy. However, the study included a small number of patients and may have a selection bias if the chemotherapy group had a better performance status or less comorbidity than the non-chemotherapy group.

In this update, which is the largest single--center study to date to the best of our knowledge, we enrolled 138 patients with pT3, pT4, or N+and M0 UTUC. Sixty-six patients underwent adjuvant chemotherapy, and 72 patients were solely controlled. The median follow-up period was 48.7 months. We found that adjuvant chemotherapy did not significantly correlate with DSS or OS in patients with high-risk disease compared to patients receiving no adjuvant treatment. This study included a homogeneous group of patients with stage III or IV UTUC who initially received the same surgical treatment at a single institution.

Despite this advantage, limitations include the small number of patients and its retrospective non-randomized nature. Furthermore, the regimen and number of chemotherapy cycles varied. Still, we didn't consider disease-free survival (DFS) as a potential endpoint other than OS and DSS. If we analyzed the DFS in UTUC patients receiving adjuvant chemotherapy, it might have been that adjuvant chemotherapy prolongs the DFS. A large prospective randomized trial to verify our findings is expected, though it will be difficult to perform due to low incidence of UTUC.

CONCLUSIONS

There does not appear to be a significant DSS or OS benefit associated with adjuvant chemotherapy. A prospective randomized clinical trial is needed to verify the effect of adjuvant chemotherapy on locally advanced UTUC and to determine whether this is due to the inherent biases of retrospective analysis, the limited efficacy of adjuvant chemotherapy, or the use of suboptimal regimen. In addition, efforts should be made to develop new chemotherapeutic agents and establish reliable criteria for patient selection in performing adjuvant chemotherapy.

ACKNOWLEDGEMENTS

This study design and the use of patients' information stored in the hospital database were approved by the Institutional Review Board (IRB) at the Seoul National University Hospital. The approval number is H-1407-024-592. We were given exemption from getting informed consents by the IRB because the present study is a retrospective study and personal identifiers were completely removed and the data were analyzed anonymously. Our study was conducted according to the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

ABBREVIATIONS

ASA = American Society of Anesthesiologists
CIS = carcinoma in situ
DSS = disease-specific survival
GC = gemcitabine and cisplatin
LVI = Lymphovascular invasion
MEC = methotrexate, epirubicin, and cisplatin
M-VAC = methotrexate, vinblastine, adriamycin and cisplatin
OS = overall survival
RNU = radical nephroureterectomy
UTUC = upper tract urothelial carcinoma

CONFLICT OF INTEREST

None declared.

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Supplementary Table-1: Patient characteristics.

Variables	No ACH	ACH (cisplatin-based only)	P value†
Total	72	59	
Gender			**0.298**
Male	58 (80.6%)	43 (72.9%)	
Female	14 (19.4%)	16 (27.1%)	
Age, year	67.3 (57.4-73.0)	60.3 (54.2-65.7)	0.001
BMI, cm/kg^2	24.4 (22.4-25.6)	23.8 (21.2-25.7)	0.476
ASA score			**0.017**
1	21 (29.2%)	25 (42.4%)	
2	41 (56.9%)	33 (55.9%)	
3	10 (13.9%)	1 (1.7%)	
Bladder cancer*			**0.003**
No	51 (70.8%)	54 (91.5%)	
Yes	21 (29.2%)	5 (8.5%)	
Preoperative ureteroscopy			**0.628**
No	62 (86.1%)	49 (83.1%)	
Yes	10 (13.9%)	10 (16.9%)	
Bladder cuffing			**0.348**
No	17 (23.6%)	10 (16.9%)	
Yes	55 (76.4%)	49 (83.1%)	
Tumor location			**0.138**
Renal pelvis	41 (56.9%)	24 (40.7%)	
Ureter	19 (26.4%)	23 (39.0%)	
Both	12 (16.7%)	12 (20.3%)	
Multifocality			**0.868**
Absent	57 (79.2%)	46 (78.0%)	
Present	15 (20.8%)	13 (22.0%)	
Hydronephrosis			**0.486**
Absent	41 (56.9%)	30 (50.8%)	
Present	31 (43.1%)	29 (49.2%)	
Pathologic T stage			**0.066**
pT1	2 (2.8%)	0 (0.0%)	
pT3	70 (97.2%)	57 (96.6%)	
pT4	0 (0.0%)	2 (3.4%)	
Tumor grade			**0.106**
G1	6 (8.3%)	1 (1.7%)	
G2	39 (54.2%)	30 (50.8%)	
G3	27 (37.5%)	28 (47.5%)	
Concomitant CIS			**0.022**
Absent	71 (98.6%)	52 (88.1%)	
Present	1 (1.4%)	7 (11.9%)	
LVI			0.387
Absent	56 (77.8%)	42 (71.2%)	
Present	16 (22.2%)	17 (28.8%)	

Necrosis			**0.336**
Absent	65 (90.3%)	50 (84.7%)	
Present	7 (9.7%)	9 (15.3%)	
Margin status			**0.466**
Negative	69 (95.8%)	54 (91.5%)	
Positive	3 (4.2%)	5 (8.5%)	
Pathologic N stage			**0.028**
pN0	9 (12.5%)	6 (10.2%)	
pNx	60 (83.3%)	41 (69.5%)	
pN+	3 (4.2%)	12 (20.3%)	

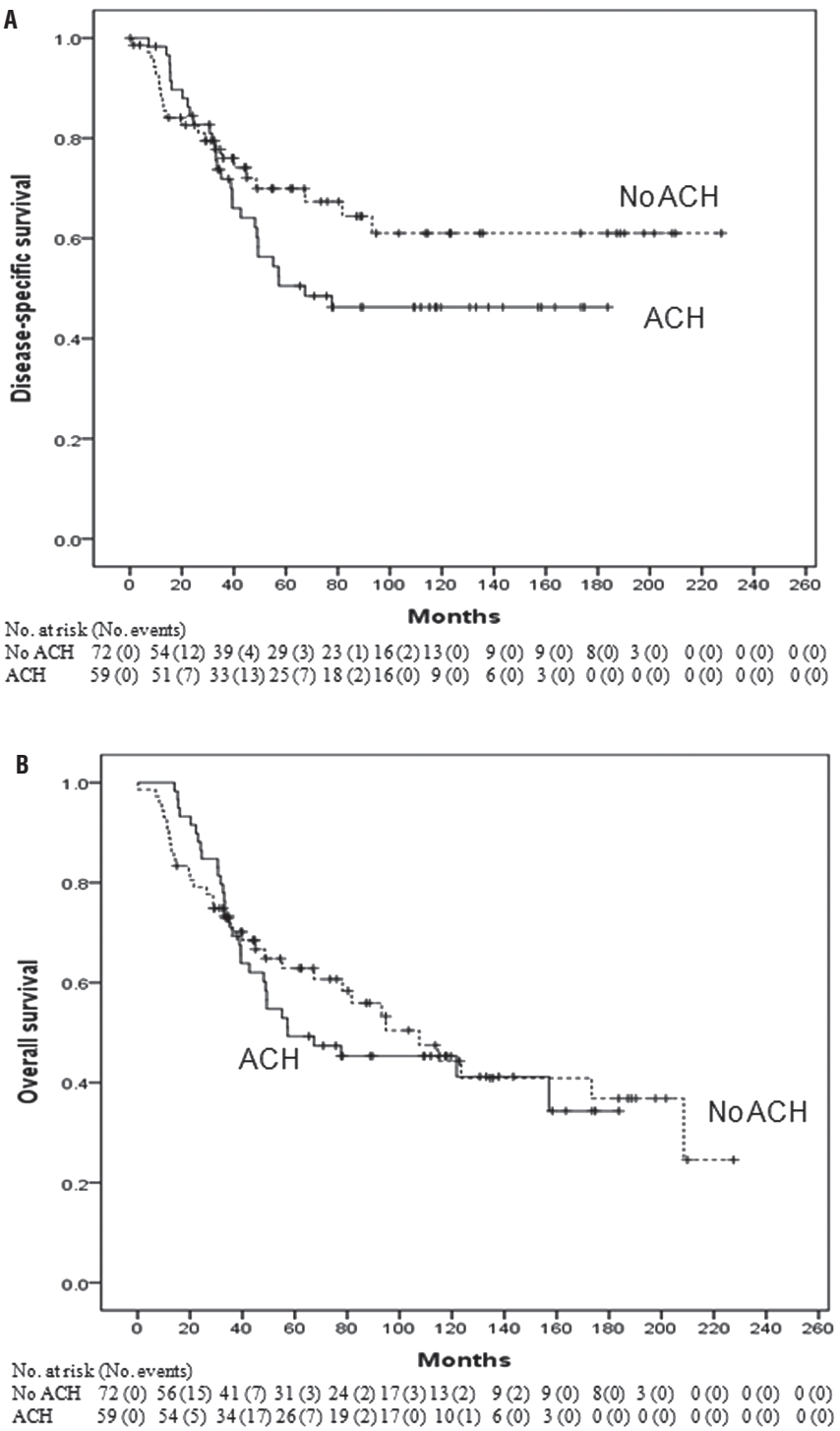
Data presented are number (%) or median (interquartile range).
†Compared to no adjuvant chemotherapy group.
*Previous or concomitant.
ACH, adjuvant chemotherapy; BMI, body mass index; ASA, American Society of Anesthesiologists; CIS, carcinoma in situ; LVI: lymphovascular invasion.

Supplementary Table-2: Multivariate Cox proportional hazards regression analysis of disease-specific survival and overall survival in patients who received cisplatin-based adjuvant chemotherapy or not.

	DSS		OS	
	HR (95% CI)	P value	HR (95% CI)	P value
Age			1.050 (1.018-1.084)	0.002
ASA score				
1			Reference	
2/3			1.310 (0.706-2.431)	0.392
Bladder cancer*				
No			Reference	
Yes			1.880 (1.048-3.372)	0.034
Bladder cuffing				
Yes	Reference		Reference	
No	2.708 (1.473-4.978)	0.001	2.399 (1.374-4.189)	0.002
LVI				
Absent	Reference		Reference	
Present	1.834 (1.014-3.317)	0.045	1.868 (1.066-3.273)	0.029
Margin status				
Negative	Reference		Reference	
Positive	2.261 (0.860-5.948)	0.098	1.852 (0.761-4.512)	0.175
ACH				
No	Reference		Reference	
Yes	1.374 (0.766-2.464)	0.287	1.647 (0.911-2.977)	0.098

*Previous or concomitant.
DSS, disease-specific survival; OS, overall survival; HR, hazard ratio; CI, confidence interval; ASA, American Society of Anesthesiologists; LVI, lymphovascular invasion; ACH, adjuvant chemotherapy.

Supplementary Figure-1: Kaplan–Meier analysis for (A) disease-specific survival and (B) overall survival after radical nephroureterectomy stratified by the administration of adjuvant chemotherapy in patients who received cisplatin-based adjuvant chemotherapy or not.



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Clinical significance of serum and urinary HER2/neu protein levels in primary non-muscle invasive bladder cancer

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ABSTRACT

Objective: We aimed to compare serum and urinary HER2/neu levels between healthy control group and patients with non-muscle invasive bladder cancer. Additionally, we evaluated relationship of HER2/neu levels with tumor stage, grade, recurrence and progression.
Materials and Methods: Fourty-four patients with primary non-muscle invasive bladder tumors (Group 2) and 40 healthy control group (Group 1) were included the study. Blood and urinary samples were collected from all patients and HER2/neu levels were measured by ELISA method. Blood and urinary HER2/neu levels and additionally, ratio of urinary HER2/neu levels to urinary creatinine levels were recorded. Demographic data and tumor characteristics were recorded.
Results: Mean serum HER2/neu levels were similar between two groups and statistically significant difference wasn't observed. Urinary HER2/neu levels were significantly higher in group 2 than group 1. Ratio of urinary HER2/neu to urinary creatinine was significantly higher in group 2 than group 1, (p=0,021). Serum and urinary HER2/neu levels were not associated with tumor stage, grade, recurrence and progression while ratio of urinary HER2/neu to urinary creatinin levels were significantly higher in high-grade tumors. HER2/neu, the sensitivity of the test was found to be 20.5%, and the specificity was 97.5%, also for the urinary HER2/neu/urinary creatinine ratio, the sensitivity and specificity of the test were found to be 31.8% and 87.5%, respectively.
Conclusions: Urinary HER2/neu and ratio of urinary creatinine urine were significantly higher in patients with bladder cancer compared to healthy subjects. Large series and controlled studies are needed for use as a tumor marker.

ARTICLE INFO

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HER2-neu-derived peptide (654-662) [Supplementary Concept]; Serum; Urinary Bladder Neoplasms

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INTRODUCTION

Bladder cancer is the second most frequently diagnosed genitourinary malignancy after prostate cancer. Approximately 70,000 new cases of bladder cancer were detected in the United States of America (USA) in 2007, and bladder cancer is estimated to occur in 500,000 people (1, 2). The most common histological type of bladder cancer is transitional cell carcinoma, which is responsible

for up to 95% of all bladder cancers (3).

Because of the invasive and uncomfortable nature of cystoscopy, new methods are being investigated for diagnosing bladder cancer and identifying the risks of recurrence and progression. Urinary cytology is a non-invasive method that has been used for many years. The sensitivity of urinary cytology is poor (20-60%), however, its specificity, which is dependent on the tumor grade, surpasses 90%, particularly in high-grade tumors (4-6). The low sensitivity and grade-dependent specificity of urinary cytology as well as the significant role of the pathologist's experience in determining accuracy have necessitated research for new tumor markers to diagnose bladder cancer. Because tumor suppressor genes and oncogenes have been reported to be associated with bladder cancer and may have the potential for developments in technology and molecular biology, these new molecules are perceived to contain tumor markers for bladder cancer.

The HER2/neu (C-erbB-2) is a member of the epidermal growth factor receptor (EGFR) family and is responsible for cell growth and proliferation by activating the tyrosine kinase pathway. HER2/neu (C-erbB-2) has been shown to exhibit over-expression in tumor tissues of breast, colon, gastric, lung and bladder cancer (7-11). The expression of HER2/neu can be determined using different methods, such as immunohistochemistry (IHC), enzyme-linked immunosorbent assay (ELISA), fluorescent in situ hybridization (FISH), and polymerase chain reaction (PCR) (12). Many studies have investigated HER2/neu expression in tumor tissues in patients with bladder cancer. However, according to the literature review conducted in the present study, only one study has evaluated the HER2/neu protein level in serum and urinary samples (13).

The present study aimed to compare the HER2/neu protein levels in serum and urine samples of the patients with non-muscle invasive bladder cancer (NMIBC) and the same levels in the healthy control subjects in similar age groups.

Urinary HER2/neu concentrations were normalized to the concentrations of urinary creatinine because of the concentration of the urine itself may affect the interpretation of a urinary biomarker and to reduce the variations due to dilution (14). Additionally, we aimed to analyze the correlation of HER2/neu protein levels with the stage, grade, recurrence and progression of tumors.

MATERIALS AND METHODS

The present study was performed in accordance with the Declaration of Helsinki between September 2012 and October 2013, with the approval (28.08.2012-25/P) of the Local Ethics Committee. A total of 84 patients (n=62 men, 22 women) were included in the study. Group-1 (control group) was composed of 40 healthy volunteers (n=25 men, 15 women) and Group-2 was composed of 44 patients (n=37 men, 7 women), who were diagnosed with NMIBC (Ta, T1-WHO 2009). During the collection of urinary and serum samples, patients with infection, macroscopic hematuria, radiologically evident invasive bladder cancer and known patients with other primary malignancy were excluded from the study. The demographic data, body-mass index (BMI), and history of smoking were recorded for all of the patients. The tumor stage (Ta and T1 using UICC 2002 TNM staging system), grade (low-or high-grade according to the WHO 2004 grading system), presence of carcinoma in situ (CIS), size and number of tumors, and recurrence and progression of tumors were recorded in a follow-up period for the patients with bladder cancer. Before surgery, the patients with bladder cancer were submitted to the NMP22 Bladder Check test (Matritech Inc., Newton, MA, USA) and urinary cytology. NMP22 test results were obtained according to the manual's guidelines, four drops of urine were dropped on the kit, and we observed and recorded the changes after a 30 minute wait. A color band in the test position indicates a positive result. Fresh urine samples were collected from the patients using sterile containers for cytology, the samples were examined by a pathologist who had no prior knowledge of the patient group. In the cytology examination, the presence of atypical and malignant cells was considered as malignant cytology, whereas the absence of these types of cells was considered as benign cytology. After surgery, the patients were administered intravesical treatment

quantitative data were analyzed using the chi-squared test, and when the conditions of the chi-squared test were not fulfilled, the analyses were conducted using Fisher's exact test. The correlation was checked using Spearman's correlation analysis. The receiver operating characteristics (ROC) curve was analyzed to calculate the predictive values, whereas the Kappa statistic was used to determine sensitivity and specificity. A $p<0.05$ was considered to be statistically significant. The analyses were conducted using the SPSS 21.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

The mean ages of the patients were 62±9.6 years (range, 34-80 years) in Group-1 and 63.9±11.1 years (range, 43-84 years) in Group-2 (p=0.488). Group-1 was composed of 15 females (37.5%) and 25 males (62.5%), and Group-2 was composed of 14 females (31.8%) and 30 males (68.2%) (p=0.256). The history of smoking variable was significantly higher in patients with bladder cancer (88.6-62.5%; p=0.005). BMI was similar in both groups (p=0.345); BMI was 26.5±2.6kg/m^2 (range, 21-32kg/m^2) in Group-1 and 27.2±4.3kg/m^2 (range, 21-42kg/m^2) in Group-2 (p=0.345).

The characteristics of the patients who were diagnosed with bladder cancer are summarized in Table-1.

Between both groups, there was no significant difference in their serum HER2/neu levels (p=0.395) (Figure-1A). The urinary HER2/neu levels and the ratio of the urinary HER2/neu to creatinine were significantly higher in patients with bladder cancer (p=0.011, p=0.021, respectively) (Figures 1B and 1C) (Table-2).

The serum HER2/neu level, urinary HER2/neu level, and urine HER2/neu/urinary creatinine ratio according to smoking status were evaluated. Nevertheless, similar levels were observed both for smokers and non-smokers (p=0.306, p=0.732, p=0.535, respectively). The urinary HER2/neu le-

Table 1 - Characteristics of the patients with bladder cancer.

	Median	Range	Mean±SD	
Follow-up duration (month)	11	9-17	11.6±2.5	
Size of tumor (mm)	30	7-80	35.0±17.7	
Number of tumor	2	1-10	2.4±2.0	
			n	%
Tumor stage	Ta		13	29.5
	T1		31	70.5
Grade	Low grade		25	56.8
	High grade		19	43.2
CIS	Present		38	86.4
	Not present		6	13.6
NMP 22	Negative		22	50.0
	Positive		22	50.0
Cytology	Benign		24	54.5
	Malignant		20	44.5
Recurrence	No		28	63.7
	Yes		16	36.3
Progression	No		41	93.2
	Yes		3	6.8

Figure 1 - Comparison of patients and controls: a) Serum HER2/ner; b) Urine HER2/neu levels and c) Urine HER2/neu/Urinary creatinine ratio.

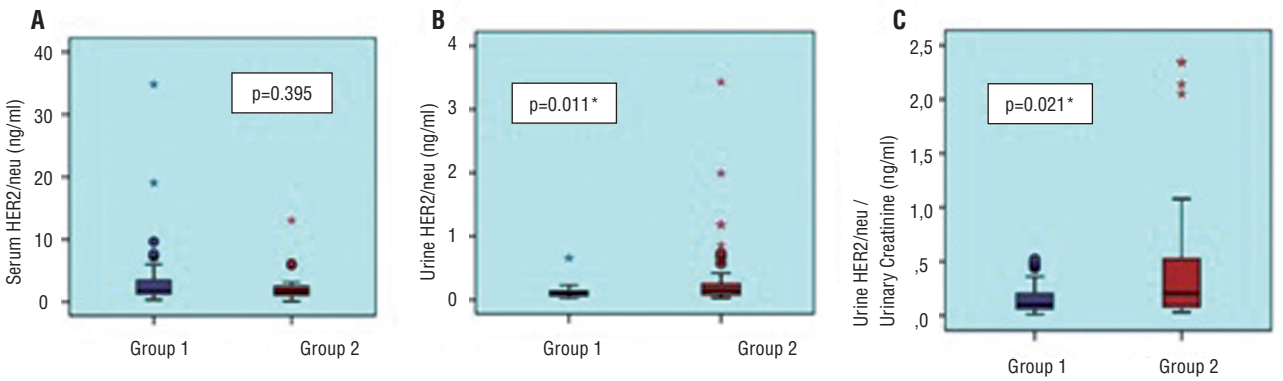


Table 2 - Comparison of HER2/neu protein levels of patients and controls.

	Control group (n=40)		Bladder cancer group (n=44)		p
	Mean±SD	Range	Mean±SD	Range	
Urine Her2/neu (ng/mL)	0.12±0.10	0.0-0.7	0.35±0.61	0.0-3.4	0.011
Urine Her2/neu / Urine creatinine (ng/mg)	0.16±0.15	0.0-0.5	0.45±0.62	0.0-2.4	0.021
Serum Her2/neu (ng/mL)	3.87±6.08	0.3-34.8	5.84±17.97	0.1-106	0.395

Mann-Whitney U test

vels, urinary HER2/neu/creatinine ratio, and serum HER2/neu levels did not show any significant difference between Ta and T1 (p=0.877, p=0.857, p=0.857, respectively). Although no significant difference was observed between high-and low-grade cancer in the urinary and serum HER2/neu levels (p=0.162, p=0.297, respectively), high-grade cancer was found to have a significantly higher urinary HER2/neu/urinary creatinine ratio compared with low-grade cancer (p=0.035). The urinary and serum HER2/neu levels and urinary HER2/neu/urinary creatinine ratio were found to be similar in patients with tumor recurrence and progression and in patients who did not have recurrence and progression. Although there was no relationship between the cytology results and the HER2/neu levels in patients with bladder cancer, those with positive NMP22 were found to have a significantly higher urinary HER2/neu/urinary creatinine ratio (0.55±0.63, 0.35±0.61, p=0.038) and significantly lower serum HER2/neu levels (10.05±24.95ng/mL, 1.63±1.25ng/mL, p=0.015) (Table-3).

The HER2/neu levels were not correlated with age, BMI, and the size and number of tumors (Table-4).

Using the cut-off value of 0.4ng/mL for urinary HER2/neu, the sensitivity of the test was found to be 20.5%, and the specificity was 97.5%. Using the cut-off value of 0.4ng/mL for the urinary HER2/neu/urinary creatinine ratio, the sensitivity and specificity of the test were found to be 31.8% and 87.5%, respectively. The positive predictive values were calculated to be 90% and 73.7%, respectively, for the urinary HER2/neu level and urinary HER2/neu/urinary creatinine ratio (Figure-2).

DISCUSSION

Studies of tumor markers for the diagnosis and follow-up of patients with bladder cancer have been ongoing for years. The ideal tumor marker is expected to be easy to administer and interpret, to be cost-effective, and to have high

Table 3 - Comparison of HER2/neu levels according to the groups with bladder cancer.

		Urine HER2/ neu (ng/mL)		Urine HER2/neu/ urine Creatinine (ng/mg)		Serum HER2/ neu (ng/mL)	
		Mean ± SD	p	Mean ± SD	p	Mean ± SD	p
Smoking	smokers	0.19±0.27	0.732	0.34±0.46	0.535	2.95±2.45	0.306
	Non-smokers	0.26±0.51		0.31±0.49		5.51±15.53	
Tumor stage	Ta	0.33±0.53	0.877	0.44±0.61	0.857	9.52±28.98	0.857
	T1	0.36±0.65		0.46±0.64		4.30±10.88	
Grade	Low	0.27±0.43	0.162	0.31±0.49	**0.035**	8.25±23.63	0.297
	High	0.46±0.78		0.64±0.74		2.67±2.81	
NMP22	Negative	0.26±0.44	0.160	0.35±0.63	**0.038**	10.05±24.95	**0.015**
	Positive	0.45±0.74		0.55±0.61		1.63±1.25	
Cytology	Benign	0.26±0.41	0.579	0.39±0.60	0.333	8.69±0.60	0.651
	Malignant	0.47±0.78		0.53±0.65		2.43±0.65	
Recurrence	No	0.34±0.66	0.634	0.39±0.57	0.420	7.85±22.32	0.575
	Yes	0.38±0.53		0.57±0.71		2.33±2.91	
Progression	No	0.37±0.63	0.596	0.47±0.64	0.895	5.86±18.57	0.969
	Yes	0.17±0.03		0.24±0.16		5.55±6.51	

Kruskal-Wallis/Mann-Whitney U test

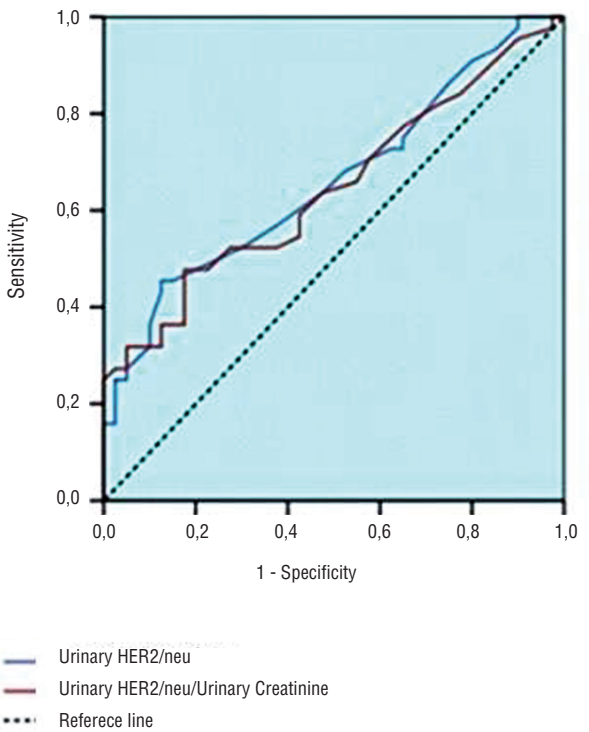
Table 4 - Correlation of HER2/neu levels to age, BMI and the size and number of tumors.

		Age (year)	BMI (kg/m^2)	Size of Tumor (mm)	Number of Tumor
Urine HER2/neu	r	-0.014	-0.022	0.277	-0.057
	p	0.897	0.843	0.068	0.711
Urinary HER2/neu/ Urinary Creatinine	r	0.178	0.122	0.108	-0.070
	p	0.105	0.269	0.483	0.649
Serum HER2/neu	r	-0.073	-0.102	-0.056	0.172
	p	0.512	0.363	0.722	0.277

Spearman's rank correlation

specificity to avoid false positive results and high sensitivity to avoid undetected tumors (16). However, to date, no alternative tumor marker has been discovered for the diagnosis and follow-up of bladder cancer that can replace cystoscopy and cytology. In this study, although the serum HER2/neu levels of the patients with NMIBC were noted to be similar to those of the control group, the urinary HER2/neu levels and urinary HER2/neu level/urinary creatinine ratio were significantly higher in patients with bladder cancer. There may be similar results for the serum HER2/neu levels because only patients with NMIBC were included in the study. There is, however, limited information in the literature considering this issue. A study from Korea analyzed a total of 38 patients with bladder cancer, including 12 patients with muscle invasive bladder cancer, and found similar serum

Figure 2 - ROC curve for urinary HER2/neu level urinary HER2/neu/creatinine ratio.



HER2/neu levels between the cancer patients and the control group. However, they did not perform a subgroup analysis, which involved only patients with muscle invasive bladder cancer (13).

In the present study, the ELISA method was preferred for measuring the HER2/neu levels. In addition to ELISA, the FISH, IHA and PCR methods can be utilized to determine the HER2/neu level. There is no precise information regarding the superiority of any of these methods. Whereas FISH and IHA methods measure the HER2/neu level in tumor tissue, ELISA is capable of measuring the HER2/neu level in body fluids. There are no available data concerning ELISA, FISH and IHA, evaluated on the resected tissues from the same neoplastic cases. Several studies have indicated over-expression of the HER2/neu protein and gene amplification in bladder cancer. Though over-expression of the HER2/neu protein reaches 81%, gene amplification is found in approximately 60% of the patients with bladder cancer (15). Ecke et al. evaluated the urinary HER2/neu level using the ELISA method and obtained significantly high values in patients with bladder cancer (12). When Kim et al. applied the ELISA method to analyze the HER2/neu levels in serum and urine samples, they did not observe a significant difference in the serum HER2/neu levels; however, they reported a significant difference in the urinary HER2/neu levels. Moreover, the urinary HER2/neu/urinary creatinine ratio was analyzed in this study, and a significantly higher ratio was obtained in the patients with bladder cancer (13).

Although there was no correlation between the urinary and serum HER2/neu levels and the stage and grade of tumors, the HER2/neu/urinary creatinine ratio was observed to be significantly higher in patients with high-grade tumor. Although Lönn et al. reported a correlation between the HER2/neu levels and NMIBC, Kim et al. did not find any relationship between HER2/neu levels and tumor grade (13, 17). In the present study, no correlation was observed between HER2/neu levels and the recurrence and progression of tumor. Skagias et al. used the IHC method to analyze the pathologies of 80 patients diagnosed with bladder cancer, and they identified HER2/neu over-expression in 52% of the patients. In the same study, although the HER2/neu expression was found to be correlated with tumor grade, cancer-specific survival, and overall survival, the HER2/neu expression did not show any correlation with recurrence (16). When Rink et al. examined the tumor cells circulating in the serum samples of the patients scheduled for radical cystectomy, they observed high HER2/neu levels in 23% of the patients and emphasized the correlation between the high HER2/neu levels observed in recurrence and survival (18).

After examining the specimens using the FISH method, Fleischmann et al. reported that HER2/neu positivity was more frequently observed in patients with lymph node metastasis (19). Kim et al. identified the correlation between positive cytology and high urinary HER2/neu level. However, the urinary HER2/neu level was correlated with the cytological findings in the current study, and the urinary HER2/neu/urinary creatinine ratio was found to be significantly higher in NMP22 positive patients. Similarly, this study did

not find any correlation between the HER2/neu levels and the size and number of tumors, and the age and BMI of the patients (13).

The number of smokers was higher in the group of patients with bladder cancer compared with the control group. Although smoking is known to increase the risk of bladder cancer by 3-fold, no relationship was detected between smoking and the HER2/neu levels in this study. Additionally, no HER2/neu mutation was observed in the study of Li et al., who genetically examined a patient group comprising 230 smokers (20).

In two different studies analyzing the urinary HER2/neu levels as potential tumor markers, the sensitivity of the test varied between 71.1% and 88.9%, whereas the specificity varied between 62.5% and 84% (12, 13). Compared with other relevant studies, the sensitivity of the urinary HER2/neu level was lower and the specificity was higher in the present study. In addition to the serum and urinary HER2/neu levels, the urinary HER2/neu urinary creatinine ratio was analyzed in this study, the findings were considered to be similar to the results relative to the urinary HER2/neu level, which may be attributable to the use of different kits for the tests. There is neither a specified and commonly accepted predictive value for urinary HER2/neu nor an accepted technique for measuring the HER2/neu level. Cytology, a tumor marker with high specificity, has been used for years. Although its specificity exceeds 90%, particularly in patients with high-grade bladder tumor, its sensitivity reaches 60% (16). Because of the high specificity, guidelines recommend that cytology be used along with cystoscopy, especially in the follow-up of high-risk patients. Although the specificity of urinary HER2/neu was observed to be 97.5% in both high and low-grade cancer patients in the present study, considerably low sensitivity was obtained in the HER2/neu tests. Because the specificity of urinary HER2/neu level is high and not dependent on the tumor grade, urinary HER2/neu may be a new alternative to cytology.

Excluding the studies conducted in search of tumor markers, there are several studies that evaluate the potential therapeutic use of the monoclonal antibody trastuzumab and EGFR-HER2 inhibitor lapatinip in bladder cancer (15, 21). However, there is currently insufficient information in the literature regarding the benefit of these agents for the treatment of bladder cancer. Nevertheless, measuring the HER2/neu levels before and after treatment may be important, as in breast cancer, when these agents are administered to treat bladder cancer. Larger, randomized, controlled trials may shed light on this issue in the near future.

Several limitations of our study should be noted. These limitations include the following: the small sample size, the inclusion of only non-muscle invasive bladder cancer, a single center study, no standard measurement technique of HER2/neu protein and, finally, the short follow-up. The ELISA method and commercial kits were employed to determine the HER2/neu levels. There is no standardization on this subject, and the method used to ensure more accurate results should be identified. Enriching the study with immunohistochemical tissue examinations and genetic methods, in addition to the ELISA method, might have clarified the uncertainty.

CONCLUSIONS

Although the urinary HER2/neu level and the ratio of urinary HER2/neu level to urinary creatinine level using the ELISA method were significantly higher in patients with NMIBC compared with the healthy patient group, the serum HER2/neu levels were similar in both groups. Although no correlation was observed between the urinary HER2/neu level and tumor grade, tumor stage, cytology, and NMP22 results, the urinary HER2/neu/urinary creatinine ratio was found to be correlated with the tumor grade and NMP22 positivity. The urinary HER2/neu test was found to have high specificity but low sensitivity for patients with non-muscle invasive bladder cancer. Additional studies are necessary to better understand the clinical importance of HER2/neu protein in patients with bladder cancer.

CONFLICT OF INTEREST

None declared.

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Loss of TIMP-1 immune expression and tumor recurrence in localized prostate cancer

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ABSTRACT

Introduction and objective: Overexpression of MMPs has been related to biochemical recurrence after radical prostatectomy. TIMP1 and TIMP2 are controllers of MMPs and the aim of this study is to evaluate the expression levels of MMPs and their regulators using immunohistochemistry in tissue microarray of localized prostate cancer (PC).
Materials and Methods: Immune-expression of MMP-9, MMP-2, TIMP1, TIMP-2, MMP-14 and IL8, were analyzed by immunohistochemistry in radical prostatectomy specimens of 40 patients with localized PC who underwent surgery between September 1997 and February 2000. Protein expression was considered as categorical variables, negative or positive. The results of the immune-expression were correlated to Gleason score (GS), pathological stage (TNM), pre-operatory PSA serum levels and biochemical recurrence in a mean follow up period of 92.5 months.
Results: The loss of TIMP1 immune-expression was related to biochemical recurrence. When TIMP1 was negative, 56.3% patients recurred versus 22.2% of those whose TIMP1 was positive (p=0.042). MMP-9, MMP-2, IL8 and MMP-14 were positive in the majority of PC. TIMP-2 was negative in all cases.
Conclusion: Negative immune-expression of TIMP1 is correlated with biochemical recurrence in patients with PC possibly by failing to control MMP-9, an important MMP related to cancer progression.

ARTICLE INFO

Key words:
Prostatic Neoplasms; Matrix Metalloproteinases; Prognosis; Diagnosis; Gene Expression

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INTRODUCTION

Degradation of basal membranes and extracellular matrix (ECM) is essential for tumor invasion and development of metastases, and matrix metalloproteinases (MMPs) are potent proteolytic enzymes that are known to play a key role in these processes. Within the MMP family, Matrix Metallproteinase 2 (MMP-2) (gelatinase A, 72 kDa) and Matrix Metalloproteinase 9 (MMP-9) (gelatinase B, 92 kDa) cleave type IV collagen and gelatin, which are the main structural components of the basal membrane (1). MMP-9 and MMP-2 expression has been implicated in the development and progression of many tumors, such as bladder (2), colorectal (3), lung cancer (4) and prostate cancer (5).

MMPs are transcriptionally regulated. MMP-2 is mainly regulated by its zymogen inhibitor, tissue inhibitor of metalloproteinase 2 (TIMP-

2), and by its major activator, membrane type-1 MMP (MT1-MMP), also known as MMP-14. MT1--MMP specifically activates the pro-gelatinase, MMP-2, on the tumor cell surface in vitro through the formation of a complex with TIMP-2 (6). IL8 upregulates MMP-2 in tumor cells, which is thought to be responsible for its angiogenic activity (7). MMP-9 is mainly regulated by TIMP-1 and has been reported that reversion-inducing cysteine-rich protein with Kazal motifs (RECK) inhibits both MMP-2 and MMP-9 (8).

The balance between secreted MMPs and their specific regulators plays an important role in the maintenance of connective tissue homeostasis in normal and pathological tissues (9). In neoplastic diseases, including prostate, an imbalance between MMPs and their inhibitors, leading to an excess of degradative activity, is assumed to be related to the invasiveness capacity of tumor cells (10-12).

In a previous study, we have analyzed the gene expression of MMPs and their regulators in PC by qRT-PCR, and found that MMP-9 was upregulated probably as a consequence of the under--expression of its negative regulators. Moreover, the levels of MMP-9 were higher in patients with preoperative PSA>10ng/mL, and most importantly in those who have presented biochemical recurrence (8). We also noted that TIMP-2, MT1-MMP and IL8 were overexpressed and would be possibly responsible for the decrease in MMP-2 expression in PC tissue (5).

To validate our previous findings, we decided to search for protein expression of MMPs and its regulators by immunohistochemistry in a tissue microarray representative of radical prostatectomy specimens of men followed by a mean period of 92.5 months, trying to find new prognostic markers for the disease.

PATIENTS AND METHODS

Patients

The study was conducted using surgical specimens from 40 patients with clinically localized PC (pT2/3N0M0) who underwent radical prostatectomy in our institution between 1993 and 2007. These cases were randomly selected from our database (Table-1). All patients underwent surgery by the same surgeon, and they were followed by PSA measurement in the first 5 years each 6 months and then annually with a mean follow--up of 92.5 months. We included patients with PC and subjects that provided informed consent to participate in the study and that allowed their biological samples to be genetically tested. We excluded from the study patients who undergone adjuvant or neoadjuvant treatment. Approval for the study was given by the Institutional Board of Ethics (no. 0453/08).

All surgical specimens were formalin--fixed and totally paraffin-embedded. The slides most representative of tumor from each patient were selected by considering the area that best represented the whole tumor. Two areas from each tumor were marked with permanent ink and were included in the TMA.

The immunohistochemistry heat antigen retrieval process using citrate buffer (1mM, pH 6.0) was performed. The slides were incubated overnight at 4ºC with the monoclonal antibodies specified in Table-2. The LSAB system was used for immunostaining (Dako Cytomation, CA). Co-

Table 1 - Demographic characteristics of 40 men submitted to radical prostatectomy to treat prostate cancer.

Age (years)	
Mean	63
Min - Max	41 - 79
PSA (ng/ml)	
Mean	12
Min - Max	2.0 - 37.0
< 10 n (%)	18 (45.0)
≥ 10 n (%)	22 (55.0)
Stage	
pT2 n (%)	22 (55.0)
pT3 n (%)	18 (45.0)
Gleason Score	
< 7 n (%)	14 (35.0)
≥ 7 n (%)	26 (65.0)

Table 2 - Antibodies utilized.

Antibody	Manufacturer	Diluition
MMP-9	ABnova	1:10
MMP-2	Abcam	1:100
TIMP-1	Abcam	1:100
TIMP-2	Abcam	1:100
MMP-14	Abcam	1:100
IL-8	Abcam	1:100

lor was developed by reaction with a 3, 3'diaminobenzidine substrate-chromogen solution followed by counterstaining with Harris hematoxylin. Slides were dehydrated, cover slipped and observed under a light microscope. The expression of each marker was evaluated by a single pathologist (KRML) who has considered the cases as categorically negative or positive. The results were then correlated with Gleason score that was classified as low grade (Gleason score≤6) or high grade (Gleason score≥7), pathological stage (TNM 2010) considered as organ-confined (pT2) or non-organ-confined (pT3) and pre-operative serum PSA levels <or≥10ng/mL. In addition, we analyzed the immunohistochemistry results with disease behavior considering biochemical recurrence when PSA was >0.4ng/mL.

Statistical Analysis

To compare the clinical characteristics of patients with PC, we used the Mann-Whitney, chi-squared and Fisher exact tests. For descriptive analysis of MMP-9, MMP-2 and its regulators expression according to pathological stage, Gleason score and PSA, we constructed a box plot, and for comparison between categories, we used the Mann-Whitney test. Statistical analysis was performed using SPSS 15.0 for Windows, and significance was set at $p \leq 0.05$.

RESULTS

The analysis of MMPs and TIMPs by immunohistochemistry was performed in a tissue microarray conferring a standardization of the technique and microscopic analysis. Expression was categorized as positive when stain was strong or moderate and negative when there was a weak or no staining. The staining was always diffuse with no focal reaction. The expression of MMPs and their regulators were located in the cytoplasm except MMP-14 that showed interstitial positivity in some cases. MMP-9 and MMP-2 were positive in 91.4% and 77.7% of the cases respectively. TIMP-1, TIMP-2, MMP-14, IL8 were expressed by 47.2%, 0.0%, 65.7%, 63.9% of the cases respectively (Figures 1 and 2).

Figure 1 - Photomicrograph illustrating immunohistochemical reactions. A: positive reaction of MMP-9 in cancer tissue; B: negative reaction of MMP-9 in normal prostate tissue; C: positive reaction of MMP-2 in prostate cancer; D: negative reaction of MMP-2 in normal prostate tissue; E: negative reaction of TIMP-1 in prostate cancer; F: positive reaction in control group.

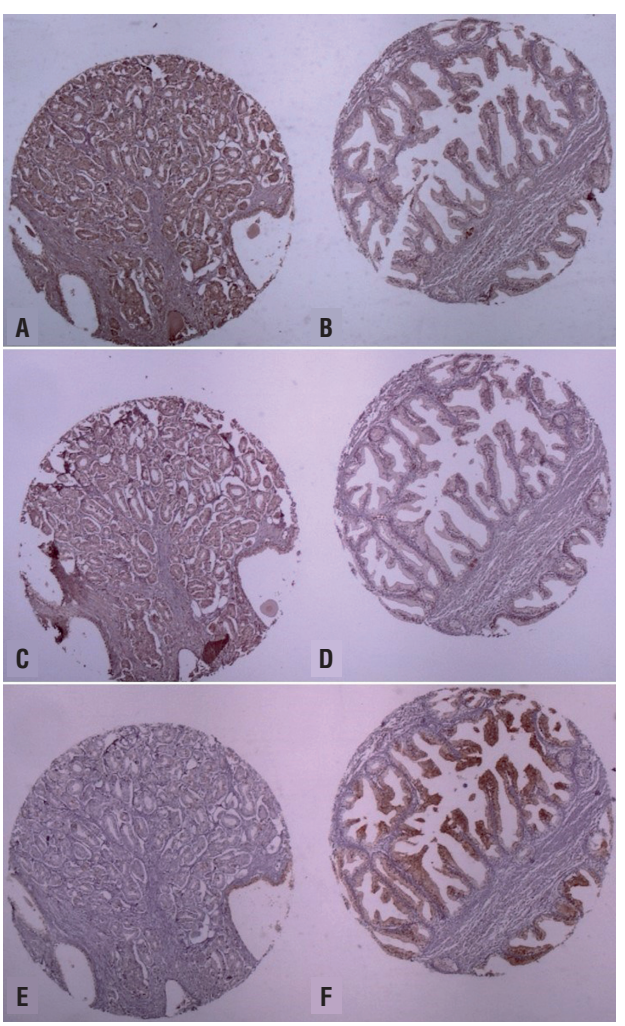
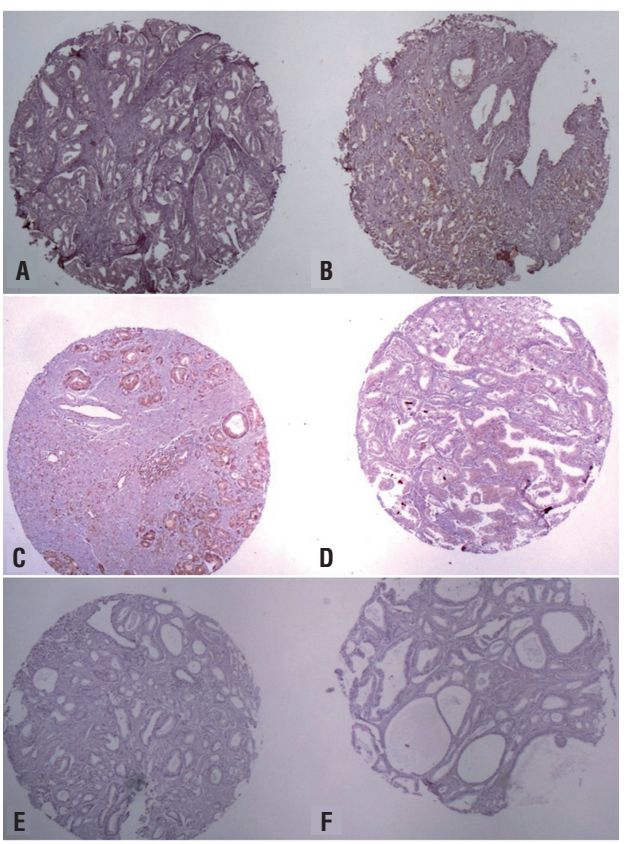


Figure2-Photomicrographillustratingimmunohistochemical reactions. A: positive reaction of MMP-14 in cancer tissue; B: negative reaction of MMP-14 in normal prostate tissue; C: positive reaction of IL-8 in prostate cancer; D: negative reaction of IL-8 in normal prostate tissue; E: negative reaction of TIMP-2 in prostate cancer; F: positive reaction in control group.



Analysis of the protein expression according to prognostic factors of PC is shown in Table-3. We found no statistical differences regarding the expression of any protein studied according to these prognostic variables. Statistical analysis of TIMP-2 protein was not possible, because this protein was negative in 100.0% of cases.

The loss of TIMP-1 immune-expression was related to biochemical recurrence. When this protein was positive only 22.2% of cases had biochemical recurrence whereas tumor recurrence occurred in 56.3% when TIMP-1 was negative (p=0.048) (Table-4). Kaplan-Meier curve showed a median biochemical recurrence free survival of 105 months for patients with TIMP-1 positive against 62.8 months for patients with TIMP-1 negative (Figure-3).

DISCUSSION

In the present study, we demonstrated that MMP-2, MMP-9 and MMP-14 are positive in prostate cancer and its regulators are negative in the majority of cases. Prostate cancer is the most common cancer in men, and the advanced metastatic disease is currently incurable. It is the most common male malignancy and the second leading cause of death among men in many countries, including Brazil. In the United States, 238.590 new cases and 29.720 deaths related to PC were estimated for the year 2013 (13).

Due to the lack of efficient parameters to identify potentially aggressive tumors in many cases, clinicians are frequently unable to identify patients at greater risk of disease progression. Therefore, novel molecular makers that can more precisely indicate the biological behavior and prognosis of PC are urgently needed. Extensive studies have revealed that tumor invasion, metastasis, and angiogenesis require ECM degradation, mainly by MMPs (14).

The MMP is abundantly expressed in malignant tumors, regardless of their origin and a significant correlation between the increased expression of MMP and a worse prognosis in terms of survival could be demonstrated in several cancers (15, 16). As a result, the possibility of using their expression levels as prognostic markers have been suggested.

We have previously demonstrated that MMP-9 gene is overexpressed in 82.3% of PC cases (8), and in this study we aimed to validate the gene expression results with the protein expression using immunohistochemistry. The results were confirmed since MMP-9 protein was expressed by 91.4% of cases. This phenomenon has been considered a frequent event in the process of prostate carcinogenesis, but few studies evaluated their regulators and their importance in disease progression (14).

In our cases, MMP-2 and MMP-14 were immune-expressed by the majority of PC cases (77.7%); on the contrary, the MMP-2 and MMP-

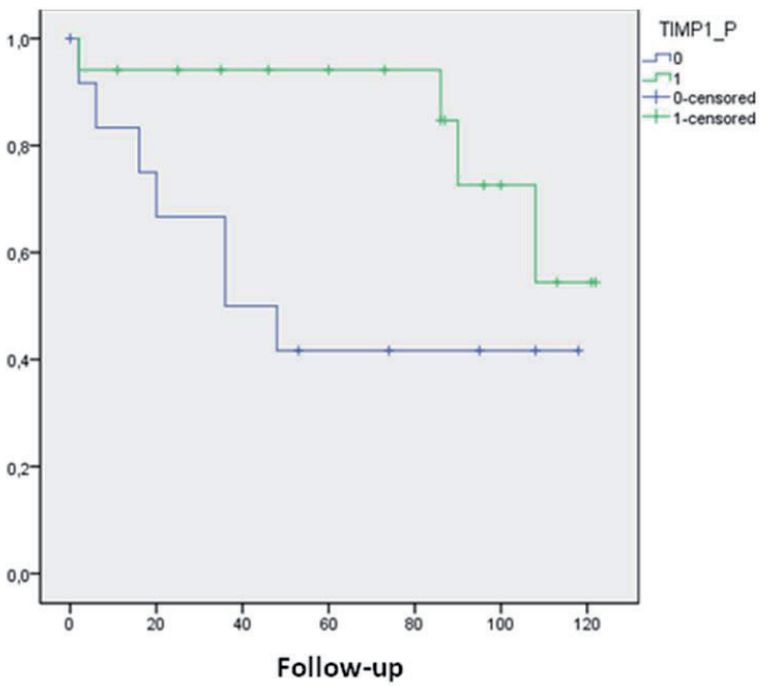
Table 3 - Protein expression and Gleason score, pathological stage and PSA-value.

	Gleason Score Median (Q1-Q3)			Pathological Stage Median (Q1-Q3)			PSA-value Median (Q1-Q3)		
	< 7	≥ 7	p	pT2	pT3	p	< 10	≥ 10	p
MMP-2			0.777			0.288			1.000
Negative	37.5%	62.5%		75.0%	25.0%		50.0%	50.0%	
Positive	32.1%	67.9%		53.8%	46.2%		50.0%	50.0%	
MMP-9			0.266			0.373			0.581
Negative	66.7%	33.3%		33.3%	66.7%		33.3%	66.7%	
Positive	31.3%	68.8%		60.0%	40.0%		50.0%	50.0%	
TIMP-1			0.637			0.409			0.738
Negative	29.4%	70.6%		66.7%	33.3%		47.1%	52.9%	
Positive	36.8%	63.2%		52.6%	47.4%		52.6%	47.4%	
MMP14			0.160			0.298			0.428
Negative	15.4%	84.6%		45.5%	54.5%		38.5%	61.5%	
Positive	44.0%	56.0%		64.0%	36.0%		52.0%	48.0%	
IL8			0.221			0.297			0.137
Negative	23.1%	76.9%		50.0%	50.0%		30.8%	69.2%	
Positive	43.5%	56.5%		68.2%	31.8%		56.5%	43.5%	

Table 4 - Protein expression accordding to biochemical recurrence.

	Biochemical recurrence		
	No	Yes	p-value
MMP-2			0.248
Negative	42.9%	57.1%	
Positive	66.7%	33.3%	
MMP-9			0.311
Negative	33.3%	66.7%	
Positive	63.3%	36.7%	
TIMP-1			**0.042**
Negative	43.8%	56.3%	
Positive	77.8%	22.2%	
MMP-14			0.259
Negative	54.5%	45.5%	
Positive	73.9%	26.1%	
IL-8			0.340
Negative	54.5%	45.5%	
Positive	71.4%	28.6%	

Figure 3 - Kaplan-Meir curve of biochemical recurrence-free survival according to TIMP-1 immune-expression. Kaplan-Meier curve shows a median biochemical recurrence free survival of 105 months for patients with TIMP-1 positive (1-green line) against 62.8 months for patients with TIMP-1 negative (0-blue line).



14 genes were found to be under-expressed in most cases of the PC cases (5). Our results are similar to those published by Lichtinghagen et al. (2002) (17), who showed MMP-2 under-expression in prostate cancer tissue using RT-PCR. Conversely, they observed higher expression of MMP-2 at the protein level using immunohistochemistry, a result later confirmed by Brehmer et al. (18), indicating that there is a discrepancy between the levels of MMP-2 mRNA and protein expression in prostate cancer.

At the post-translational level, all MMPs are under control of specific tissue inhibitors (TIMPs) that bind proximally to the catalytic domain of MMPs, preventing substrate attachment. TIMPs are not simply regulators of MMP activity, they also have multifunctional roles that include promotion of the cell growth (9) and inhibition of angiogenesis (19). Four TIMPs have been identified. They inhibit all MMPs, forming non-covalent complexes with the active forms. Among them, TIMP-1 and TIMP-2 selectively binds pro-MMP-9 and pro-MMP-2 respectively (9, 10). Singh et al. (20) found that combined evaluation of MMP-9, TIMP-1 and TIMP-2 in plasma may facilitate clinical decision making for improved management of oral cancer. We showed that TIMP-1 and TIMP-2 are under-expressed in PC compared to BPH, and we confirmed our results, because TIMP-2 was negative in 100% of the cases and TIMP-1 was negative in 52.1%. We believe that the TIMPs control over MMPs is responsible for this discrepancy that literature has published.

Interestingly, we were able to find a relationship between the expression of TIMP1 protein and biochemical recurrence. When TIMP-1 was negative biochemical recurrence occurred in only 22.2% of the cases. Furthermore, we found a median of biochemical-free survival time of 105 months for patients with TIMP-1 positive versus 62.8 months in those where TIMP-1 was negative. At the post-translational level, all MMPs are under control of specific TIMPs that bind proximally to the

catalytic domain of MMPs, preventing substrate attachment. TIMPs are not simply regulators of MMP activity, they also have multifunctional roles that include cell growth promotion (8) and inhibition of angiogenesis (19). Four TIMPs have been identified. Among them, TIMP-1 selectively binds pro-MMP-9 and is considered the main inhibitor of MMP9.

Considering all this dynamic involving MMPs and their regulators, it seems that immunohistochemistry should be more useful to study their roles in PC behavior than the mRNA profile. Also, being an easier, cheaper and widespread available method, it could be included in clinical practice as a useful prognostic parameter orienting the choice of primary or adjuvant treatment. Larger studies are necessary to confirm our statement.

ABBREVIATIONS

BPH = Benign prostatic hyperplasia
cDNA = Complementary deoxyribonucleic acid
ECM = Extracellular matrix
MMP = Matrix metalloproteinase
BC = Prostate cancer
qRT-PCR = Quantitative real-time polymerase chain reaction
RECK = Reversion-inducing cysteine-rich protein with Kazal motif
RNA = Ribonucleic acid
TIMP-1 = Tissue inhibitor of metalloproteinases 1
TIMP-2 = Tissue inhibitor of metalloproteinases 2
IL-8 = Interleukin 8

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CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Safety of 12 core transrectal ultrasound guided prostate biopsy in patients on aspirin

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ABSTRACT

Objective: To prospectively assess safety outcome of TRUS guided prostate biopsy in patients taking low dose aspirin.
Materials and methods: Consecutive patients, who were planned for 12 core TRUS guided prostate biopsy and satisfied eligibility criteria, were included in the study and divided into two Groups: Group A: patients on aspirin during biopsy, Group B: patients not on aspirin during biopsy, including patients in whom aspirin was stopped prior to the biopsy. Parameters included for statistical analysis were: age, serum prostate specific antigen (PSA), prostate volume, hemoglobin (Hb %), number of hematuria episodes, number of patient reporting hematuria, hematuria requiring intervention, number of patient reporting hematospermia and number of patient reporting rectal bleeding.
Results: Of 681 eligible patients, Group A and B had 191 and 490 patients respectively. The mean age, prostate volume, serum PSA and pre-biopsy hemoglobin were similar in both Groups with no significant differences noted between them. None of the post--biopsy complications, including number of hematuria episodes (p=0.83), number of patients reporting hematuria (p=0.55), number of patients reporting hematospermia (p=0.36) and number of patients reporting rectal bleeding (p=0.65), were significantly different between Groups A and B respectively. None of the hemorrhagic complication in either group required intervention and were self limiting.
Conclusion: Continuing low dose aspirin during TRUS guided prostate biopsy neither alters the minor bleeding episodes nor causes major bleeding complication. So, discontinuation of low dose aspirin prior to TRUS guided prostate biopsy is not required.

ARTICLE INFO

Key words:
Prostate; Biopsy; Aspirin; Hemorrhage

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INTRODUCTION

Growing life expectancy and resultant ageing population, along with increasing awareness and use of serum prostate specific antigen (PSA) for prostate cancer screening led to increase in transrectal ultrasound (TRUS) guided prostate biopsy, a gold standard procedure for histopathological diagnosis of prostate cancer, in urological practice.(1-3) 10-12 systematic cores for initial diagnosis have been suggested by European Association of Urology (EAU) 2014 guidelines (level of evidence 2a, grade of recommendation B).(4) A high proportion of patients requiring TRUS guided prostate biopsy for diagnosis of prostate cancer are on medications like aspirin, warfarin, etc. for associated co-morbidities (3). With 12 core TRUS guided prostate biopsy, although minor and self limiting, hemorrhagic complications like hematuria, hematospermia and rectal bleeding were reported in 33-39%, 12-36% and 14-27%, respectively (3, 5, 6).

Literature regarding TRUS guided prostate biopsy in aspirin users report variable results, some observed no difference in bleeding complications, while others reported higher rate of minor bleeding complications (1, 6-8). In this study, we intended to prospectively assess safety outcome of TRUS guided prostate biopsy in low dose aspirin users.

MATERIALS AND METHODS

This prospective study was performed in our hospital in the period between April 2011 and November 2014. Indications for biopsy were serum PSA>4ng/mL and/or abnormal digital rectal examination. Consecutive patients, planned for 12 core TRUS guided prostate biopsy, were included in the study, whereas patients with: a) History of bleeding disorder; b) Patient on anticoagulant other than aspirin; c) <or>12 biopsy cores and; d) patients who did not sign informed consent, were excluded. Patients were non-randomly divided into two Groups: Group A) patients on low dose (75mg per day) aspirin during biopsy; Group B) patients not on aspirin during biopsy, including patients in whom aspirin was stopped prior to the biopsy.

Biopsy procedure: All patients got proctoclysis enema in the morning of biopsy. Ciprofloxacin 500mg orally was given 1 hour prior to biopsy and continued twice daily for 5 days. For analgesia, either 2% lignocaine jelly, eutectic mixture of lignocaine and prilocaine (EMLA) cream or periprostatic nerve block was used depending on patient choice. TRUS was performed in left lateral decubitus position using 7.5Hz bi-planar probe to assess prostate volume and then 12-core TRUS guided prostate biopsy was done using 18G disposable biopsy gun. Each biopsy core was collected in separate jar and sent for histopathological examination. Patients were observed for 2 hours post procedure and then sent home with advice to report about complications, if any. Patients were followed up at three weeks with biopsy report and query was made regarding complications.

Data included for analysis included

Before biopsy: Age, serum prostate specific antigen (PSA), prostate volume, hemoglobin (Hb %), platelet count, serum creatinine.

After biopsy: Number of hematuria episodes, number of patient reporting hematuria, hematuria requiring intervention, number of patient reporting hematospermia and number of patient reporting rectal bleeding.

Statistical Analysis

Recorded study parameters were arranged on a Microsoft excel spreadsheet (Microsoft, Seattle, WA USA) and SPSS (IBM SPSS Statistics 21.0; IBM SPSS, 2012) software package was used for analysis. The continuous data were expressed as mean±standard deviation and analyzed by Student t-test whereas categorical data were expressed as number/percentages and analyzed by Fisher exact tests. P values<0.05 were considered statistically significant.

RESULTS

Of 783 TRUS guided prostate biopsy during the study period, 681 satisfied eligibility criteria and data of these patients were analyzed for the study. Of these, 191 patients were receiving aspirin during the biopsy, while in rest of 490 patients either the aspirin was stopped prior to biopsy or were not receiving aspirin. Table-1 summarized the baseline characteristics of patients in both Groups. The mean age (67.25 vs. 66.97, p=0.66), prostate volume (61.14 vs. 62.51, p=0.41), serum PSA (31.25 vs. 29.70, p=0.66), pre-biopsy hemoglobin (12.92 vs. 12.78, p=0.15), platelet count (137.94 vs. 142.67, p=0.27) and serum creatinine (1.21 vs. 1.17, p=0.26) were similar in the two Groups (A vs. B) with no significant differences noted between them. Although Group A had significantly higher number of patients with cardiovascular disease (182 vs. 68, P<0.0001) compared to Group B, other co-morbidities including cerebrovascular disease (23 vs. 40, p=0.18), diabetes (70 vs. 160, p=0.50) and chronic obstructive pulmonary diseases (30 vs 56, p=0.20) had similar distribution among the patients of both Groups (A vs. B).

Table-2 summarizes the post-biopsy complications. None of the complications, including the mean number of hematuria episodes (1.87 vs. 1.83, p=0.83), number of patient reporting hematuria (86 vs 247, p=0.55), number of patient

Table 1 - Baseline characteristics.

	Group A (n=191)	Group B (n=490)	P value*
Age (years)	67.25±7.44	66.97±7.72	0.66
Prostate Volume (mL)	61.14±18.96	62.51±20.09	0.41
Serum PSA (ng/mL)	31.25±43.82	29.70±40.87	0.66
Hb (gm %)	12.92±1.04	12.78±1.13	0.15
Platelet Count (x10^3 per microlitre)	137.94±46.34	142.67±52.20	0.27
Serum Creatinine (mg/dL)	1.21±0.45	1.17±0.38	0.26

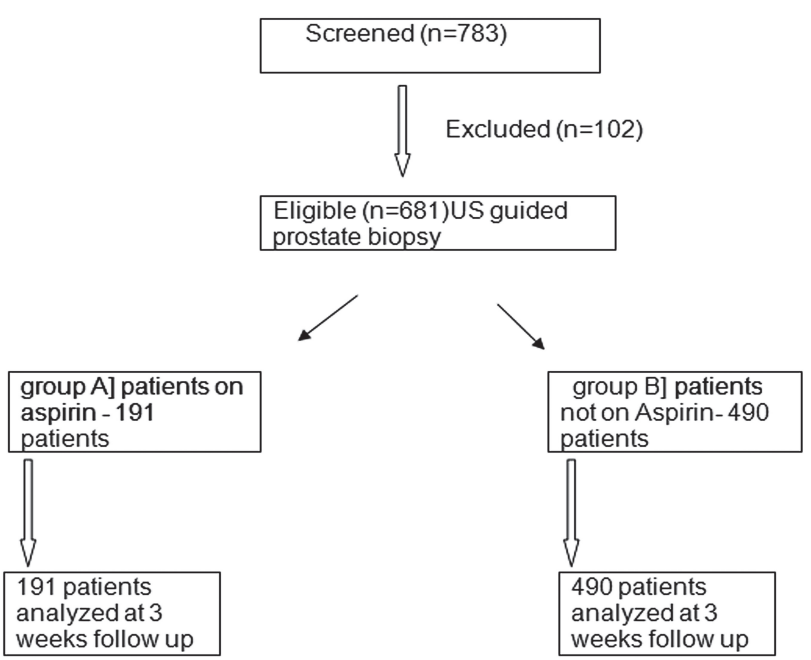
*Student t-test.

Table 2 - Post-biopsy complications.

	Group A (n=191)	Group B (n=49 0)	P value*
Number of hematuria episodes	1.87±1.22	1.83±1.48	0.83#
Number of patient reporting hematuria	86 (45.02%)	247 (50.40%)	0.55
Hematuria requiring intervention	0	0	
Number of patient reporting hematospermia	21 (10.99%)	40 (8.16%)	0.36
Number of patient reporting rectal bleeding	29 (15.18%)	65 (13.26%)	0.65

#Student t-test, *Fisher exact tests

Figure 1 - Allocation and dispersion of patients.



reporting hematospermia (21 vs. 40, p=0.36) and number of patient reporting rectal bleeding (29 vs. 65, p=0.65), were significantly different between Groups A and B respectively. None of the hemorrhagic complication in either Group required intervention and were self-limited.

DISCUSSION

Adoption of serum PSA screening for prostate cancer resulted in high number of TRUS guided prostate biopsies (6). 12 core prostate biopsy, with addition of laterally directed cores, improved cancer detection rate with complications not significantly different from sextant biopsies (9). There are no clear guidelines regarding TRUS guided prostate biopsy in patients on aspirin. At our center, we did not stop aspirin routinely prior to biopsy unless advised specifically by cardiologist because rebound thromboembolic complication following aspirin discontinuation is a known risk. It is suggested that low-dose aspirin should be discontinued only if bleeding risk outweigh the cardiovascular risk of aspirin discontinuation (10-12). However, Connor SEJ and Wingate JP in a survey among practicing radiologists and urologists observed that 52% of radiologists and 27% of urologists stopped aspirin prior prostatic biopsy (13).

Chowdhury R et al., in a prospective study involving 216 patients on low dose aspirin, observed that between aspirin users and non-users, hematuria, (33.8% vs. 37%), rectal bleeding (14.4% vs. 11.5%) and hematospermia (12% vs. 13.8%) rates did not vary significantly. They concluded that cessation of low dose aspirin prior to biopsy is not necessary (3). Halliwell O et al., in a prospective assessment of 1022 aspirin users and non-users patients, observed higher hematuria (72 vs. 61%, p<0.001), duration of hematuria (4.05 vs. 2.85 days, p<0.01), rectal bleeding (21 vs. 13%) among aspirin users. Hematospermia was not significantly different between both Groups. They also observed that although bleeding rates were higher with aspirin use, no patient required intervention for bleeding complications (1). Giannarini G et al. in a prospective trial including 200 patients observed that low dose aspirin did not increase bleeding rates but it prolonged the duration of hematuria and rectal bleeding (8). Carmignani L et al., in meta--analysis of TRUS guided prostate biopsy in patients taking aspirin, included 3218 patients and observed that compared to control, aspirin users have significantly higher rate of mild hematuria (p=0.001), whereas rate of rectal bleeding (p=0.33) and hematospermia (p=0.24) were not significantly altered. They came to conclusion that stopping aspirin prior to TRUS guided prostate biopsy is not necessary since it did not increase risk of moderate to severe hematuria (14). Culkin DJ et al. in their review of anticoagulant therapy in urological practice, which included 79 articles, recommended that prostate biopsy is safe in patients on low dose aspirin with a risk of minor bleeding approximately three times higher than in controls (15).

In our study, we did not observe significant difference in number of hematuria episodes, number of patient reporting hematuria, rectal bleeding and hematospermia among the two groups. None of the patients required intervention for their hemorrhagic complication. Our result conform to some studies reported in literature (3, 8).

Limitations of the study include: a) non randomized nature and; b) follow-up limited to 3 weeks. The prospective randomized study among TRUS guided prostate biopsy patients taking low dose aspirin may be required for definitive conclusion.

CONCLUSIONS

Continuing low dose aspirin during TRUS guided prostate biopsy neither alters the minor bleeding episodes nor causes major bleeding complications. So, discontinuation of low dose aspirin prior to TRUS guided prostate biopsy is not required.

CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Serum Levels of Trace Elements in Patients with Testicular Cancers

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ABSTRACT

Introduction: Trace elements are primary components of biological structures; however, they can be toxic when their concentrations are higher than those needed for biological functions.
Materials and Methods: In the present study serum levels of trace elements were measured in 30 patients (mean age was 26.9±11.2 years) newly diagnosed with germ cell testicular cancer and 32 healthy volunteers (mean age: 27.4±10.8) by using furnace atomic absorption spectrophotometer. Serum samples were stored at-20°C until assays.
Results: In patients with germ cell testicular cancer, the diagnosis was seminoma in 15, mix germ cell tumor in 7, embryonal carcinoma in 4, yolk sac tumor in 2 and teratoma in 2 patients. There was stage I testicular tumor in 19 patients (63.3%) while stage II in 6 patients (20.0%), stage IIIA in 4 patients (13.3%) and stage IIIC in one patient (3.4%). It was found that serum Co, Cu, Mg and Pb levels were increased ($p<0.05$), whereas Fe, Mn, and Zn levels were decreased in patients with testicular cancer ($p<0.05$).
Conclusions: These alterations may be important in the pathogenesis of testicular cancers; however, further prospective studies are needed to identify the relationship between testicular cancer and trace elements.

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Key words:
Testicular Neoplasms; Trace Elements; Tumor Markers, Biological

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INTRODUCTION

Testicular cancers are the most commonly seen cancers in men aged 15-40 years (1). They comprise 1% of all cancers in men. Germ cell tumors comprise 90-95% of all primary testicular tumors. In the past, overall survival rate was approximately 10%, while it increased up to 90% by advances in tumor markers and imaging techniques, and cisplatin-based chemotherapy regimens. The incidence of testicular cancer varies according to race and socioeconomic status. The likelihood of incident testicular cancer is higher in Scandinavian countries, while it is lower in Asian countries (2).

The trace elements are primary components of biological structures; however, they can exert toxic effect when their concentrations are higher than those needed for biological functions. Moreover, the toxicity can also be true for other non-essential elements which have very similar atomic properties and ability to mimic the reactivity of trace elements. Thus, this above-mentioned

toxicity/duality leads biological system to develop ability of recognizing and delivering the metal to its target without enabling it to involve in toxic reactions (3, 4). Proteins are the primary compounds involved in this recognition and transport. The majority of the associations between a trace element and other biological molecules result in undesirable chemical modifications in these molecules.

In living organisms, biological mechanisms have been developed in order to use vital trace elements including zinc and copper and to minimize toxic effects of heavy metals such as cadmium, mercury and lead (5). Oxidative processes are most intensive in a background where an imbalance is present in trace elements involved in the structure of enzymes accounted for antioxidant defense (6). Alterations in the ion content of trace elements including iron, copper and zinc can affect activity of antioxidants (7). In several cancers, it has been reported that significant differences occur in the normal distribution of iron, copper and zinc (8). In some trials, serum Cu/Zn ratio was employed as chemoprophylaxis (9). Moreover, it has been also used to evaluate and assess the prognosis in patients with cancer (10, 11).

In the present study, serum levels of trace elements were measured and it was found that Mn, Co, Cu, Mg, Fe and Zn concentrations were altered in sera of the patients with testicular cancer in comparison to healthy subjects.

MATERIALS AND METHODS

The study included 30 men with germ cell testicular cancer. Mean age was 26.9±11.2 years. All patients were lifetime non-smokers and had no history of alcohol addiction, drug abuse, antioxidant use and metabolic diseases. No comorbid disease was present in any of the patients. All patients had newly diagnosed testicular cancer and preoperative blood samples were taken. Thirty two healthy male subjects (mean age: 27.4±10.8) were randomly selected as controls among volunteers who had no history of smoking, alcohol consumption, drug or antioxidant use, and known comorbid disease. The patient and control groups had similar socioeconomic status.

According to 2009 TNM tumor staging system, there was stage I testicular tumor in 19 patients (63.3%) while stage II in 6 patients (20.0%), stage IIIA in 4 patients (13.3%) and stage IIIC in one patient (3.4%). The study was conducted in accordance to Helsinki Declaration, 1989 Revision. All participants gave written informed consent before participation in the study.

Blood Samples

Blood samples were drawn at morning after 12 hours fasting and stored on ice at 4°C. Then, sera were separated by centrifugation at 3000 rpm for 10 minutes. Serum samples were stored at -20°C until assays.

Measurements of Mineral-Heavy Metal Levels

Two milliliters of HNO3/H2O2 mixture (2:1) were added to 0.7 g of the serum samples. The mixture was placed into the water bath at 70°C for 30 min and stirred occasionally. Then, one mL of the same acid mixture was added, and the mixture was transferred into a Teflon vessel bomb for the microwave oven. The bomb was closed, and the solution was placed inside the microwave oven. Radiation was applied for 3 min at 450 W. After addition of 0.5mL of the same acid mixture, radiation was repeated for 3 min. After cooling for 5 min, 2.0mL of 0.1mol/L HNO^3 was added, and the solution was transferred to a Pyrex tube. After centrifugation, the clear solution was used to determine Mn, Cd, Cu, Pb, Fe, Mg, Co and Zn levels. They were measured by using atomic absorption spectrophotometer technique with a UNICAM-929 spectrophotometer (Unicam Ltd, York Street, Cambridge, UK).

Statistical Analysis

All data were analyzed by using SPSS for Windows version 13.0. Descriptive statistics of the traits evaluated were expressed as mean, standard deviation, minimum and maximum values. Mann--Whitney U test was used for intergroup comparisons. $P<0.05$ was considered as significant.

RESULTS

There was seminoma in 15, mix germ cell tumor in 7, embryonal carcinoma in 4, yolk sac

tumor in 2 and teratoma in 2 patients. The demographic characteristics are presented Table-1. Trace elements levels are presented in Table-2. No statistical difference was detected between patients with testicular cancer and controls regarding age and body mass index (BMI).

Trace element levels were compared between groups. It was found that Co, Cu, Mg and Pb levels were significantly higher in the patient group compared to the controls. It was also found that Zn, Fe and Mn levels were significantly lower in the patient group compared to controls. Cd levels were found to be higher in patients with testicular tumors compared to controls, but this didn't reach statistical significance.

DISCUSSION

Traces elements are accepted as essential components of biological structures, which play complex roles in the cancer development or inhibition. There are many questions regarding their essential and toxic effects on human health above the concentrations needed for their biological functions. In this context, there are conflicting results in the literature (12, 13).

Table 1 - Demographic characteristics.

Demographic characteristics	Patients (n = 30)	Control (n = 32)	p Value
Age (years) (mean±SD)	26.9±11.2	27.4±10.8	>0.05
BMI (kg/m^2)	27.0±2.7	28.0±2.1	>0.05

Table 2 - Descriptive Statistics and Comparison Results According to the Groups for Specifications.

		N	Mean	Std. Dev.	Min.	Max.	p
Cd	Patient	30	0.008636	0.020831	0.00194	0.07781	0.083
	control	32	0.001109	0.000114	0.00101	0.00135	
Co	Patient	30	0.002506	0.001108	0.00107	0.00432	0.001
	control	32	0.001102	0.000986	0.00101	0.00143	
Cu	Patient	30	1.0944	0.3992	0.01	1.84	0.001
	control	32	0.84	0.13474	0.52	0.98	
Fe	Patient	30	0.917971	0.404285	0.0433	1.588	0.001
	control	32	2.430144	0.189439	2.0121	2.7998	
Mg	Patient	30	24.4	2.88236	15.83	28.47	0.001
	control	32	0.7527	0.18918	0.11	0.99	
Mn	Patient	30	0.010167	0.008136	0.00145	0.03986	0.001
	control	32	0.7527	0.189182	0.11114	0.98991	
Pb	Patient	30	0.035	0.019655	0.00276	0.07757	0.001
	control	32	0.001204	0.000131	0.001	0.0015	
Zn	Patient	30	0.90998	0.330196	0.036	1.72	0.001
	control	32	2.96368	0.443655	2.125	4.021	

Mn is an essential element which is required for several enzyme activities. It has a major role in the antioxidant defense system and comprises part of SOD enzyme (14). Therefore, it can be suggested that decreased serum Mn concentrations with disturbance of antioxidant mechanism can make target organs sensitive to carcinogens. Decreased serum Mn concentrations were reported in patients with bladder and renal cancers (15, 16). Our study showed that serum Mn concentrations were lower in patients with testicular cancer compared to controls. According to our hypothesis, Mn can result in oxidant/antioxidant imbalance in patients with testicular cancer, as being a trace element affecting oxidation status.

Zinc plays an anti-carcinogenic role through structural stabilization of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and ribosome. Zinc is also important in the functions of several transcription factors and proteins that are involved in the recognition of specific DNA sequences and regulation of gene transcription. Zinc has a protective effect against free-radical injury (17). In previous studies, it has been reported that serum Zn concentrations were decreased in patients with ovarian, cervical, bladder and renal cancer (15-19). It seems that it is necessary to determine serum zinc levels in patients with ovarian cancer in comparison with healthy controls in order to fully elucidate the relationship between serum Zn levels and ovarian cancer. In addition, in a previous study, there were normal or elevated zinc concentrations only in the sera of the cases with primary liver cancer in contrast to decreased serum zinc levels in remaining cases, suggesting a differential sign favoring transformation from hepatocirrhosis to cancer (20). Our study demonstrated decreased Zn concentrations in patients with testicular cancer compared to controls.

Fe is a trace element which is physiologically essential but dangerous in biochemical aspect. Either excess or deficiency of Fe can lead to oxidative DNA damage, although it is an important nutritional element (21). Moreover, it was proposed that low iron levels may have a role in the prevention of infection and cancer (22).

There is substantial evidence supporting the hypothesis of a relationship between testicular cancer and disruption of iron metabolism and that testicular cancer may be a clinical manifestation of iron-induced testicular damage, presumably through an underlying free-radical mechanism (23). In our study, results indicated that there was a significant difference in serum Fe levels between patient and control groups.

Physiological Cd doses lead to an increased endothelial permeability through inhibition of endothelial proliferation and induction of cell death mediated by DNA damage, which is also inhibited by zinc (24). It has been reported that doubling of soil cadmium is associated with an increased risk for lung cancer by 57% (25). It was also shown that serum Cd levels were increased in patients with bladder cancer (3). In cadmium carcinogenicity, proto-oncogene activation, tumor suppressor gene inactivation, disrupted cell adhesion and inhibition of DNA repair are the implied cellular and molecular mechanisms (26, 27). In addition, it has been reported that elevated Cd concentration may result in prostate, kidney and lung cancers (28). In the past decade, it has been reported that the injection of cadmium metal powder and various cadmium compounds induce sarcoma in local and interstitial cell testicular tumor in a systemic manner (29). In our study, Cd levels were found to be higher in patients with testicular cancer compared to controls. However, the difference didn't reach statistical significance.

In our study, it was also demonstrated that serum levels of Pb, Co, Cu and Mg were increased when compared to controls. It is well-known that Pb and Cd are toxic and carcinogenic metals (25). It has been suggested that Pb has a predisposing role in the carcinogenesis with inhibition of DNA synthesis and repair, oxidative injury and interaction with DNA-binding proteins and tumor suppressor proteins (30, 31). Exposure to inorganic lead at early life induces teratoma and pre-neoplasm of renal and urinary bladder (32). Recent research indicated negative health consequences of lower level of lead exposure, including impaired functions of renal tubular cells, inhibition of sperm formation, fetal damage, decelerated velocity of motor nerves, and central nervous system dysfunction as well as hypertension and other cardiovascular diseases (33, 34). It was also sho-

wn that Pb levels were significantly increased in patients with malignant glioma compared to controls (35). In our study, Pb levels were found to be significantly higher in the patient group compared to controls.

Co is a constituent of vitamin B12 in humans. In human studies, evidence is inconclusive regarding relationship between inhalational exposure to cobalt and cancer. In the only available study on oral exposure to cobalt, no correlation was reported between cobalt and cancer deaths. In a study on workers refining and processing cobalt and sodium, it was shown that there was an increase in deaths from lung cancer in workers exposed to cobalt. Effects of exposure to cobalt salts were investigated in only one study. Preliminary results indicated an increased risk for lung cancer in those working at cobalt production, but follow-up didn't show any increase in the risk for cancer (36). However, that study was limited by the small number of workers who developed cancer. In our study, Co levels were found to be significantly higher in the patient group compared to controls.

Cu plays a role in the production of hemoglobin, myelin, collagen and melanin as an essential nutrient (4). In recent studies, it was shown that normal immune function requires adequate Cu intake (3, 4). It is well-known that serum Cu levels increase in several malignancies such as osteosarcoma, gastrointestinal tumors and lung cancer (37). In some studies, it was shown that there was an increase in Cu levels within tumor cells or lung epithelial lining fluid of patients with lung cancer (38, 39). In our study, serum Cu levels were found to be significantly higher in the patient group compared to controls.

The observed effects of magnesium on either tumor transplant-or chemical-induced cancers depended on the duration of Mg supplementation or deficiency (40). Optimal Mg supplementation may have prophylactic effects against some neoplasms, but Mg alone isn't recommended for therapeutic purposes as cancer cells have also high metabolic requirements (40). Recently, in rats, it was shown that Mg supplementation inhibited increased DNA synthesis at colon epithelium. In that study, it was suggested that the finding might be related to suppression of oncogene-induced bowel carcinogenesis by Mg (41). In our study, serum Mg levels were found to be significantly higher in patients with testicular cancer compared to controls.

In our study, serum levels of trace elements were assessed in germ cell testicular cancer. Serum levels of trace elements can differ in different germ cell testicular cancer as in serum levels of tumor markers. Further studies are needed to evaluate this issue. Germ cell testicular cancers (other than choriocarcinoma) initially spread to retroperitoneal lymph nodes. Retrospective studies with larger series which compare early stage, non-metastatic and metastatic tumors are necessary in order to investigate effects of metastasis on alterations in serum trace element levels.

In conclusion, an association was observed between testicular cancer and trace elements in the present study. We also think that increases in Co, Cu, Mg and Pb levels and decreases in Zn, Mn and Fe levels can play important roles in the induction of testicular cancers. However, future prospective studies on the reasons of alteration in the serum concentrations of trace elements in patients with testicular cancer seem to be well grounded. Further prospective studies are needed to clarify the relationship between various stages of testicular cancer and serum levels of trace elements.

CONFLICT OF INTEREST

None declared.

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Beyond biology: the impact of marital status on survival of patients with adrenocortical carcinoma

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ABSTRACT

Purpose: To analyze the association of marital status and survival of patients with ACC using a population-based database.
Material and Methods: Patients with ACC were abstracted from the Surveillance Epidemiology and End Results (SEER) database from 1988-2010 (n=1271). Variables included marital status (married vs single/divorced/widowed (SDW)), gender, age, race, tumor (T) and node (N) classification, receipt of surgery, and SEER stage. Statistical analysis was performed using Cox proportional hazard models to generate hazard ratios and 95% confidence intervals.
Results: There were 728 (57.3%) females and median age was 56 years (IQR 44-66). Patients who were alive were more frequently married (65.6% vs 61.6%, p=0.008), female (61.1% vs 58.0%, p=0.001), younger (median 51 vs 57 years, p=0.0001), submitted to adrenalectomy (88.6% vs 63.8%, p<0.0001), and more favorable SEER stage (localized-64.9% vs 29.9%; regional–25.1% vs 30.1%; distant 4.8% vs 31.5%, p<0.0001) compared to patients dead of disease (DOD). On multivariable analysis, factors significantly associated with all-cause mortality were SDW status (HR 1.28, 95% CI 1.09-1.51), age, non-operative management, and N+ disease. Risk factors for disease-specific mortality included SDW status (HR 1.30, 95% CI 1.07-1.56), age, non-operative management, T-classification, and N+ disease.
Conclusions: Marital status is significantly associated with survival in patients with ACC. Our results suggest that the decreased survival seen among SDW individuals highlights an area for further research and needed intervention to reduce disparity.

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INTRODUCTION

Adrenocortical carcinoma (ACC) is a rare malignancy with a reported incidence of 0.5-2 per million, a recurrence rate of 60-80%, and 5-year overall survival of 20-47% (1, 2). Despite advances in imaging and treatment regimens over the past 20 years, survival outcomes in patients with ACC continue to remain poor. Therefore, clinicians must seek additional factors to optimize outcomes in this select group of patients.

The effect of marital status on disease specific survival (DSS) in patients with cancer has been reported across several malignancies, although the reason for a survival benefit provided by marriage has not been completely elucidated (3-9). In a recent study analyzing the impact of marital status on the 10 leading causes of cancer--related death in the US, Aizer et al. found that single-divorced-widowed (SDW) patients were at greater risk of presentation with metastatic disease, under treatment and cancer specific mortali-

ty (3). These results suggest that SDW patients with malignancy represent an at-risk population that may benefit from structured support and intervention.

Apart from the known risk factors that impact survival such as TNM classification, we sought to identify other significant factors specific to survival outcomes. Given the poor survival associated with ACC and paucity of literature reporting the effect of socioeconomic variables on survival in these patients, the objective of this study was to assess the impact of marital status on overall survival (OS) and DSS in patients with ACC. Furthermore, we sought to identify other non-clinical or pathologic factors that may be associated with greater risk of mortality. Our hypothesis was that SDW patients and patients with poorer socioeconomic status (SES) would have worse OS and DSS compared to married patients and those with more favorable SES.

MATERIALS AND METHODS

Study Population: The study cohort consisted of patients from all 18 registries comprising the Surveillance, Epidemiology and End Results (SEER) database from 1988-2010. The SEER database reports cancer specific outcomes from specific geographic areas representing 28% of the US population (10). Patients ≥18 years of age with ACC were identified in the SEER database utilizing the primary site codes C74.0 and C74.9, and International Classification of Diseases for Oncology, 9th edition (ICD-9) code 1940 for a study cohort of 1271 patients. Patients were divided into three groups (alive, dead of disease (DOD) and dead of other causes (DOC)).

Description of Covariates: Demographic variables of interest included marital status (married vs single/divorced/widowed (SDW)), gender, age at diagnosis, race (African American vs Caucasian vs Hispanic vs other), SEER registry, and median census county data for educational attainment (<9th grade vs <high school vs >Bachelor degree), poverty level, % foreign born, % unemployed, and household income. Clinical and pathologic variables included receipt of surgery (adrenalectomy vs none vs other), laterality (left vs right vs bilateral), American Joint Committee on Cancer (AJCC) 7th edition tumor (T) and node (N) classification, metastasis (yes/no), SEER stage (localized vs regional vs distant vs unstaged), and median OS (SEER survival data–censoring date September 10, 2013).

Statistical analysis

Descriptive statistics for demographic and clinicopathological variable comparisons was performed using t-test and Chi square test. Survival estimates were calculated using the Kaplan-Meier method for OS and DSS by marital status, gender, age at diagnosis, race, T-classification, and N-classification. Cox proportional hazard analysis was performed to generated hazard ratios for risk factors of mortality. The model was constructed and analyses were performed using backward selection, removing all insignificant variables until the best-fit model was achieved. In this model, T--classification and N-classification were adjusted for, while SEER stage was not adjusted for in order to refrain from including confounding variables. Statistical analyses were performed using SAS 9.3 (SAS Institute, Cary, NC). All tests were two-sided and with a statistical significance set at $p<0.05$.

RESULTS

Population Demographics

There were 728 females (57.3%) and 543 males (42.7%) with a median age of 56 years (IQR 44-66). There were 422 patients who were alive (33.2%), 685 patients (53.9%) DOD and 164 patients (12.9%) DOC (Table-1). A significantly higher percentage of alive patients were married (65.6% vs 61.6% vs 51.8%; p=0.008), female (61.1% vs 58.0% vs 44.5%; p=0.001) and younger at diagnosis (median 51 years vs 57 years vs 65 years; p<0.001) compared to those DOD and DOC, respectively. There was no significant difference for educational status, poverty/income, foreign born status, and unemployment between the groups.

Clinicopathologic Analysis

Patients who were alive had a higher frequency of adrenalectomy (88.6% vs 63.8% vs

Table 1 - Demographics of 1271 patients with adrenocortical carcinoma.

Variable	Alive	Dead of Disease	Dead other Causes	p-value
Patients, n (%)	422 (33.2)	685 (53.9)	164 (12.9)	
Marital Status, n (%)				0.008
Married	277 (65.6)	422 (61.6)	85 (51.8)	
SDW	134 (31.8)	241 (35.2)	74 (45.1)	
Unknown	11 (2.6)	22 (3.2)	5 (3.1)	
Gender, n (%)				0.001
Male	164 (38.9)	288 (42.0)	91 (55.5)	
Female	258 (61.1)	397 (58.0)	73 (44.5)	
Median age, years (IQR)	51 (42, 61)	57 (44, 66)	65 (56, 74)	<0.001
Race, n= (%)				0.44
Caucasian	320 (75.8)	533 (77.8)	121 (73.8)	
Hispanic	40 (9.5)	64 (9.4)	19 (11.5)	
AAM	27 (6.4)	42 (6.1)	16 (9.8)	
Other	35 (8.3)	46 (6.7)	8 (4.9)	
Median <9th Grade Education, % (IQR)	5.7 (3.9, 8.9)	5.9 (3.7, 9.9)	5.9 (3.8, 8.9)	0.88
Median <High School Education, % (IQR)	13.9 (10.0, 20.1)	14 (9.9, 20.3)	13.5 (11.3, 18.9)	0.78
Median >Bachelor Degree, % (IQR)	29.2 (21.6, 35.6)	29.2 (23.2, 39.6)	30.1 (23.3, 38.4)	0.05
Median <Poverty, % (IQR)	13.4 (10.5, 16.3)	12.3 (10.2, 16.3)	12.2 (9.9, 16.3)	0.56
Median Foreign Born, % (IQR)	15.4 (6.9, 28.8)	17.8 (7.7, 30.5)	16.3 (9.4, 29.4)	0.38
Median Unemployed, % (IQR)	9.1 (7.9, 9.8)	9.2 (7.5, 9.8)	9.2 (7.7, 9.8)	0.85
Median Household Income, % (IQR)	56,550 (48,340, 67,010)	57,580 (51,770, 70,570)	58,820 (54,090, 70,570)	0.08

SDW = single/divorced/widowed; **IQR =** interquartile range; **AAM =** African American; **SEER =** Surveillance Epidemiology and End Results

67.7%; p<0.0001) compared to patients DOD or DOC, respectively (Table-2). Furthermore, patients who were alive had more favorable T classification (p<0.0001), more favorable N classification (p<0.0001), less frequency of metastatic disease (1.7% vs 25.4% vs 17.1%; p<0.0001), and have more favorable SEER stage (p<0.0001) compared to patients DOD or DOC. Median survival was 9 months (IQR 3-24) for patients DOD and 10 months (IQR 1-51) for patients DOC (Figure-1A). DSS was statistically significantly associated with marital status (Figure-1B, p=0.009), age at diagnosis (decade) (Figure-1D, p<0.0001), T-classification (Figure-1E, p<0.0001), and N-classification (Figure-1F, p<0.0001), but was not associated with gender (Figure-1C, p=0.18).

Risk Factors for Mortality

On multivariable analysis, significant factors associated with increased risk of all-cause mortality were SDW status (HR 1.28, 95% CI 1.09-1.51), older age (HR 1.43, 95% CI 1.31-1.55), % <9th grade education (HR 1.06, 95% CI 1.00-1.13), non-operative management (HR 3.18, 95%

Table 2 - Clinical and pathologic variables of 1271 patients with adrenocortical carcinoma.

Variable	Alive	Dead of Disease	Dead other Causes	p-value
Surgical Approach, n (%)				<0.0001
Adrenalectomy	374 (88.6)	437 (63.8)	111 (67.7)	
None	36 (8.5)	212 (30.9)	41 (25.0)	
Other	11 (2.6)	30 (4.4)	9 (5.5)	
Laterality, n (%)				0.08
Left	222 (52.6)	347 (50.7)	96 (58.5)	
Right	190 (45.0)	300 (43.8)	61 (37.2)	
Bilateral	1 (0.3)	8 (1.1)	1 (0.6)	
T Classification, n (%)				<0.0001
TX	39 (9.3)	82 (12.0)	30 (18.3)	
T0	1 (0.2)	0	0	
T1	32 (7.6)	10 (1.5)	14 (8.5)	
T2	228 (54.0)	192 (28.0)	57 (34.8)	
T3	68 (16.1)	91 (13.3)	18 (11.0)	
T4	47 (11.1)	136 (19.8)	31 (18.9)	
N Classification, n (%)				<0.0001
NX	79 (18.7)	244 (35.6)	58 (35.4)	
N0	332 (78.7)	356 (52.0)	89 (54.2)	
N1	11 (2.6)	85 (12.4)	17 (10.4)	
Metastasis, n (%)	7 (1.7)	174 (25.4)	14 (8.5)	<0.0001
SEER Stage, n (%)				<0.0001
Localized	274 (64.9)	205 (29.9)	77 (46.9)	
Regional	106 (25.1)	206 (30.1)	40 (24.4)	
Distant	20 (4.8)	216 (31.5)	28 (17.1)	
Unstaged	22 (5.2)	58 (8.5)	19 (11.6)	
Median Survival, months (IQR)	53 (17, 112)	9 (3, 24)	10 (1, 51)	<0.0001

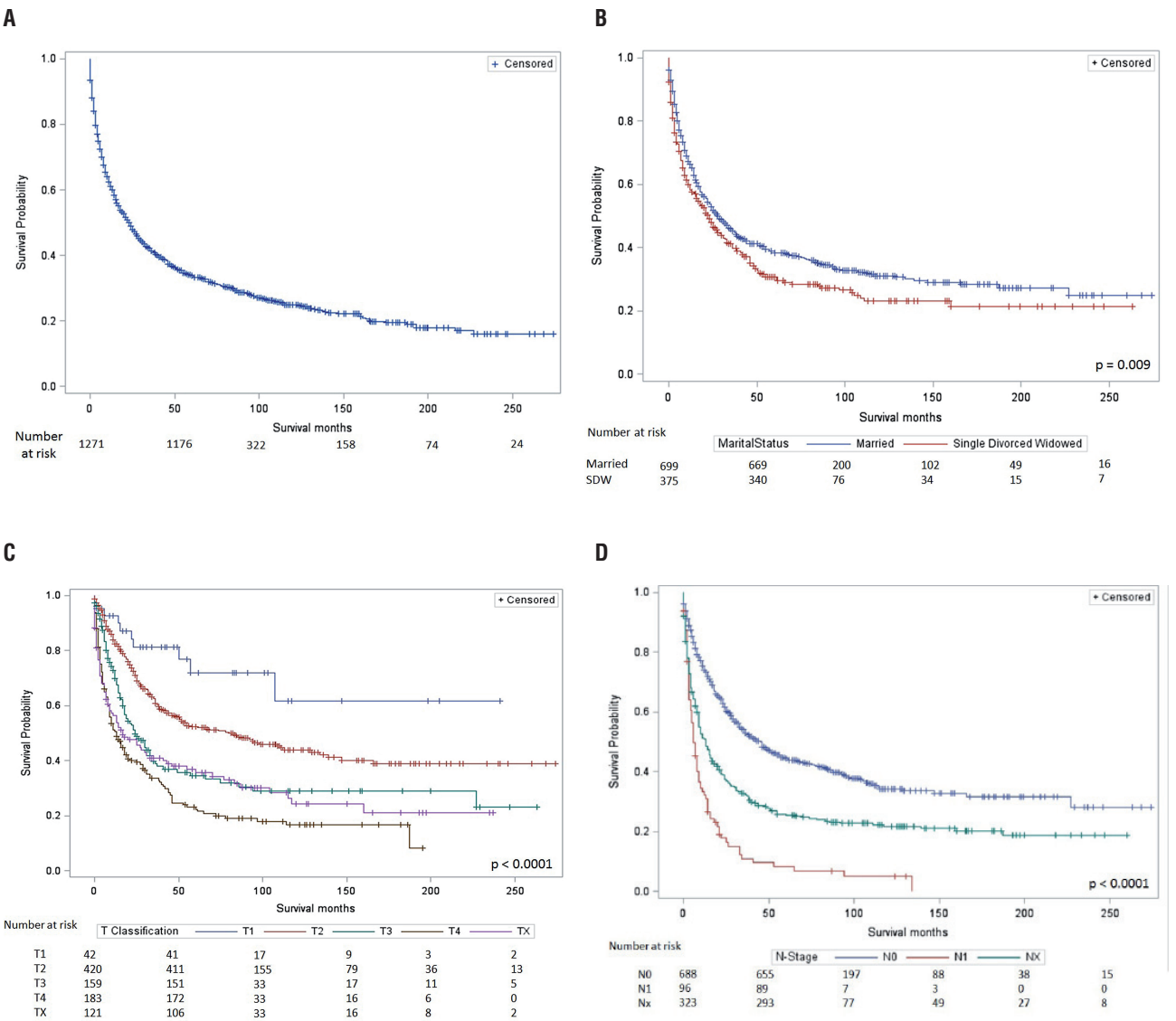
SEER = Surveillance Epidemiology and End Results; **IQR =** interquartile range

CI 2.57-3.95), T-classification (TX vs T1–HR 1.78, 95% CI 1.12-2.82; T3 vs T1–HR 2.28, 95% CI 1.45-3.60; T4 vs T1–HR 2.58, 95% CI 1.66-4.03), and N+ disease (HR 2.27, 95% CI 1.74-2.97). SDW status was also a significant risk factor for disease--specific mortality (HR 1.30, 95% CI 1.07-1.56), as did older age (HR 1.46, 95% CI 1.32-1.61), non--operative management (HR 3.56, 95% CI 2.80-4.52), T-classification (TX vs T1–HR 2.58, 95% CI 1.30-5.13; T2 vs T1–HR 2.19, 95% CI 1.14-4.22; T3 vs T1–HR 3.66, 95% CI 1.87-7.14; T4 vs T1–HR 3.97, 95% CI 2.05-7.69), and N+ disease (HR 2.37, 95% CI 1.76-3.19). Gender was included in the model and additionally in the Kaplan-Meier analysis and there was no significant difference in outcomes on multivariate analysis.

DISCUSSION

This population-based study analyzing factors associated with mortality in patients with ACC demonstrates that SDW patients have significantly worse all cause and cancer specific mortality compared to married patients. Furthermore,

Figure 1 - Kaplan-Meier survival estimates for (A) overall survival; (B) disease-specific survival by marital status (p=0.009); (C) disease-specific survival by T-classification (p<0.0001), and (D) disease-specific survival by N-classification (p<0.0001).



age was also associated with mortality in addition to poor staging characteristics. Although male patients had worse outcomes on univariate analysis, gender did not retain significance as a risk factor on multivariable analysis. In contrast to many studies in other malignancies, there was no significance found for ethnicity or socioeconomic status on outcomes. The importance of this study is that it is the first to show the effect of marital status on mortality outcomes in patients with ACC.

The survival benefit associated with marital status has been described in other urologic malignancies, including bladder, prostate, penile and testis cancer (4-9). Gore and colleagues demonstrated a clear survival benefit among married patients undergoing radical cystectomy identified within the SEER database from 1973-2000 compared to SDW patients (6). This positive effect was an independently significant factor even when controlling for pathological factors, gender

Table 3 -Cox proportional analysis of risk factors for mortality in 1271 patients with adrenocortical carcinoma.

		Overall Mortality HR (95% CI)	p-value	Cancer Specific Mortality HR (95% CI)	p-value
Marital Status	Married	Ref		Ref	
	SDW	1.28 (1.09-1.51)	0.003	1.30 (1.07-1.56)	0.007
Gender	Female	Ref		Ref	
	Male	1.17 (1.00-1.38)	0.049	1.02 (0.85-1.22)	0.84
Age#	Per decade	1.43 (1.31-1.55)	<0.0001	1.46 (1.32-1.61)	<0.0001
Race	White	Ref		Ref	
	African-American	1.29 (0.95-1.77)	0.11	1.17 (0.80-1.72)	0.42
	Hispanic	1.22 (0.92-1.61)	0.18	1.12 (0.80-1.56)	0.50
	Other	1.07 (0.76-1.50)	0.71	0.97 (0.66-1.42)	0.85
%<9th Grade Education	Per 1% change	1.06 (1.00-1.13)	0.039	1.07 (0.99-1.14)	0.055
%< High School Education	Per 1% change	0.96 (0.93-0.99)	0.042	0.96 (0.92-0.99)	0.038
Laterality	Left	Ref		Ref	
	Right	0.89 (0.76-1.04)	0.15	0.87 (0.73-1.05)	0.15
	Bilateral	0.96 (0.42-2.19)	0.92	0.99 (0.43-2.31)	0.99
Surgery	Adrenalectomy	Ref		Ref	
	None	3.18 (2.57-3.95)	<0.0001	3.56 (2.80-4.52)	<0.0001
	Other	1.11 (0.73-1.70)	0.62	1.17 (0.70-1.98)	0.55
T Classification	T1	Ref		Ref	
	TX	1.78 (1.12-2.82)	0.014	2.58 (1.30-5.13)	0.007
	T2	1.44 (0.93-2.22)	0.10	2.19 (1.14-4.22)	0.018
	T3	2.28 (1.45-3.60)	0.0004	3.66 (1.87-7.14)	0.0001
	T4	2.58 (1.66-4.03)	<0.0001	3.97 (2.05-7.69)	<0.0001
N Classification	N0	Ref		Ref	
	NX	0.92 (0.76-1.11)	0.37	0.96 (0.77-1.20)	0.73
	N1	2.27 (1.74-2.97)	<0.0001	2.37 (1.76-3.19)	<0.0001

HR = hazard ratio; **SEER =** Surveillance, Epidemiology and End Results
#Decades: 18-59, 60-69, 70-79, 80+

and race. Sammon et al. analyzed 14,859 patients undergoing radical cystectomy (RC) between 1988 and 2006 and found that never-married males had a higher rate of non-organ confined disease at RC, a trend not observed in never-married females (4). SDW men and women also displayed a higher rate of all-cause mortality and disease specific mortality. In an analysis of the SEER database from 1988-2006, Abdollah et al. identified 163,697 men undergoing radical prostatectomy (RP) with organ confined prostate cancer (7). They found that men who were SDW had more advanced stage at RP and higher cause-specific and all--cause mortality compared to married men. The same group assessed the effect of marital status on OS and cancer-specific mortality for patients with squamous cell carcinoma of the penis (8). Between 1988 and 2006, they identified 1,844 patients with squamous cell carcinoma of the penis and found that unmarried men had a 1.5-fold higher risk for locally advanced disease at surgery and 1.3-fold higher risk of overall mortality. Interestingly, un-

married men did not have an increased of cause--specific mortality in this cohort.

The benefit of marriage in patients with cancer likely represents a stable social construct, although the reasoning is subjective and the effect is likely multifactorial. For example, being married may reflect better access to healthcare compared to unmarried patients. However, better access to healthcare cannot be the total explanation because poor socioeconomic status still adversely affects outcomes in countries that have universal healthcare (3, 11). Psychosocial factors associated with being married may be influential as well. Married patients may be encouraged by their spouses to seek medical attention for worrisome symptoms, seek definitive treatment for conditions, and adhere to prescribed treatment regimens with the encouragement of a supportive spouse (3, 12, 13). The effect of this bond is speculative, however it may be inferred that this relationship may improve adherence to treatment regimens and rigorous follow-up required of cancer patients. The diagnosis of cancer may illicit distress and subsequently depression. By having a supportive partner, married patients have the ability to share the emotional burden of a cancer diagnosis with their spouse (14). Ultimately, patients without a support system associated with marriage may be at risk for poor outcomes and may require additional effort in order to maximize excellent clinical outcomes.

Although the overall and cancer specific mortality rates for patients with ACC is dismal even in married patients, the role of clinicians and multidisciplinary teams is to recognize these disparities in outcomes for the SDW patient and provide avenues to improve survival for these patients to the level of the married patient. Furthermore, interventions to improve outcomes for the SDW patient may prove to be cost-effective in the overall healthcare structure and deliverability of care. Previous studies outside of the urologic community have assessed the impact of promoting support mechanisms in oncology patients (15, 16). In a study from the Massachusetts General Hospital, investigators randomly assigned 107 patients with metastatic non-small-cell lung cancer to receive either early palliative care in conjunction with standard oncologic care or standard oncologic care alone (15). The authors found that patients receiving early palliative care had improved quality of life and mood, required less aggressive end-of-life care and ultimately had longer median survival (11.6 months vs. 8.9 months, p=0.02) compared to the control group. Aoun et al. randomized 26 palliative care patients living alone to having additional care-aid hours in their home and found that these patients had improved quality of life, preservation of self-dignity, ease of burden of everyday living, and reduced loneliness and isolation (16). Although similar studies have not been performed in married vs. SDW patients, particularly for the dismal prognosis associated with ACC, implementing comparable measures to the SDW population should be an area of further research endeavors.

There are some limitations that must be considered when interpreting the data. First, as with any retrospective analysis of a large administrative database, SEER does not provide sufficient granularity to predict causal factors specifically related to marital status and survival such as length of marriage, marital satisfaction, unmarried co-habitational relationships, or long-term homosexual relationships. Secondly, the SEER database does not include details that may impact marital status such as the quality of the relationship, length of marriage, or health of the spouse. Furthermore, patients who are SDW may have a support system equivalent to that often associated with marriage (eg. extended family, coworkers, and friends). Finally, the SEER database does not contain information related to patient comorbidities, which may be an unaccounted confounding factor in the causal association between SDW patients and inferior survival outcomes, nor does it contain information regarding receipt of chemotherapy, which would be important to know for patients with high stage disease or recurrence. The major strength of this study is that it is the first to identify the impact of marital status on survival in ACC. These results suggest that a properly designed and implemented intervention for SDW patients may have a modest impact on this and other malignancies.

CONCLUSIONS

ACC is a disease with an overall poor prognosis due to aggressive biological behavior. SDW status is associated with poorer survival in patients with ACC, suggesting that the decreased survival seen among SDW individuals in other urologic malignancies may also be relevant for patients with ACC. Health care providers caring for unmarried patients with ACC should be aware of the poorer outcomes in these patients, highlighting an area for further research and implementation of improved support systems to reduce this disparity and improve their survival to that of married patients.

ABBREVIATIONS

ACC = adrenocortical carcinoma
SDW = single/divorced/widowed
DSS = disease specific survival
SES = socioeconomic status
SEER = Surveillance, Epidemiology and End Result
DOD = dead of disease
DOC = dead of other causes

CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Effect of mitochondrial potassium channel on the renal protection mediated by sodium thiosulfate against ethylene glycol induced nephrolithiasis in rat model

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ABSTRACT

Purpose: Sodium thiosulfate (STS) is clinically reported to be a promising drug in preventing nephrolithiasis. However, its mechanism of action remains unclear. In the present study, we investigated the role of mitochondrial KATP channel in the renal protection mediated by STS.
Materials and Methods: Nephrolithiasis was induced in Wistar rats by administrating 0.4% ethylene glycol (EG) along with 1% ammonium chloride for one week in drinking water followed by only 0.75% EG for two weeks. Treatment groups received STS, mitochondrial KATP channel opener and closer exclusively or in combination with STS for two weeks.
Results: Animals treated with STS showed normal renal tissue architecture, supported by near normal serum creatinine, urea and ALP activity. Diazoxide (mitochondria KATP channel opening) treatment to the animal also showed normal renal tissue histology and improved serum chemistry. However, an opposite result was shown by glibenclamide (mitochondria KATP channel closer) treated rats. STS administered along with diazoxide negated the renal protection rendered by diazoxide alone, while it imparted protection to the glibenclamide treated rats, formulating a mitochondria modulated STS action.
Conclusion: The present study confirmed that STS render renal protection not only through chelation and antioxidant effect but also by modulating the mitochondrial KATP channel for preventing urolithiasis.

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INTRODUCTION

Nephrolithiasis is a complex disease of kidney where crystals or foreign bodies can act as nidi, upon which ions from the supersaturated urine form microscopic crystalline structures (1). The formation of these crystals in the tubular fluid, followed by crystal retention and accumulation in the kidney are the pre-requisite for the development of renal stone. Free particle model, fixed particle model and Randall's plaque hypothesis are some well-established hypothesis for stone formation and growth (2). However, molecular understanding of all these theories underlines the importance of oxidative stress in the renal stone formation.

Thiosulfate, an endogenous molecule derived from the metabolism of H_2S, is reported to

have antioxidant and chelation property (3). Sodium thiosulfate, FDA approved drug is used to lessen the side effects of cisplatin (4) and widely used in the emergency treatment of cyanide poisoning (5). Due to the availability of the sulfur group for the donations and free radical scavenging potential, STS can essentially act as an anti--urolithiatic agent (6). Few studies by Asplin and his co-workers and Yatzidis showed that thiosulfate can prevent calcium phosphate nephrolithiasis. However, LaGrange et al., 2009 reported negative results for thiosulfate in the prevention of calcium stone disease (7). In fact, in both studies, the mechanism by which STS affects calcium deposition was not clearly mentioned.

Potassium channels in nephrons have varied functions ranging from maintaining ionic equilibrium to regulating the volume during hypotonic stress environments. Their activation depends on location across the nephrons, as they can be activated either by altering pH, calcium, sodium, chloride or ATP levels. Thus, effects of K^+ channels are very complex to study (8). The present study was designed to understand the specific role of ATP sensitive potassium channel using diazoxide (channel opener) and glibenclamide (channel closer) in sodium thiosulfate mediated renal protection from ethylene glycol induced nephrolithiasis in a rat model. The calcium chelating potential of STS was evaluated in vitro using gel diffusion method.

MATERIALS AND METHODS

Chemicals

Diazoxide was purchased from Sigma-Aldrich. All other commercial reagents used were of analytical grade.

Animals

All animal experiments were conducted in accordance with the CPCSEA (Committee for the purpose of conduct and supervision of experiments on animals) guidelines, approved by the institutional animal ethical committee (IAEC No. 214/SASTRA/IAEC), Central Animal Facility, SASTRA University. To demonstrate the anti-urolithiasis property and mechanism of action of sodium thiosulfate we used male albino Wistar rats aged 7 to 8 weeks (180-200g). Animals were kept in polycarbonate cages at a controlled temperature of 25±3°C and 60±10% relative humidity with a 12 h each of dark and light cycle. Rats were acclimatized for one week with standard laboratory diet and tap drinking water before the start of experiment.

Study design

Forty two male Wistar rats were assigned randomly into seven equal groups. All the doses were selected based on previous studies (6, 9).

Group-1 (Normal control): received water ad libitum for 21 days.

Group-2 (Induction control): received 0.4% ethylene glycol (EG) along with 1% ammonium chloride for one week followed by only 0.75% EG in drinking water for two subsequent weeks.

Groups-3 to 7 received the same treatment as group 2 along with the following drug treatments:

Group-3 (STS): received sodium thiosulfate (400mg/Kg b.wt.) intraperitoneally for 21 days.

Group-4 (Diazoxide): received diazoxide (mito K_{ATP} channel opener; 5mg/Kg b.wt.) intraperitoneally for 21 days.

Group-5 (Glibenclamide): received glibenclamide (mito K_{ATP} channel closer; 10mg/Kg b.wt.) intraperitoneally for 21 days

Group-6 (STS+Diazoxide): received diazoxide 30min. before administration of STS for 21 days.

Group-7 (STS+Glibenclamide): received glibenclamide 30min. before administration of STS for 21 days.

Biochemical Parameters

Urine samples from all groups were collected using metabolic cages for 24h and analyzed in triplicates for the levels of urea, creatinine and calcium using respective diagnostic kits from Agappe diagnostics Ltd (India). Whole blood was collected from the retro-orbital sinus on the day of necropsy, centrifuged at 10.000×g for 10 min. and serum chemistry analysis was performed in tripli-

cates for calcium, creatinine, urea, ALP using the respective diagnostic kits purchased from Agappe diagnostics Ltd. & Span diagnostics Ltd. (India).

Antioxidant assays

After necropsy left kidney was cut into four equal sections. Each section was weighed separately, crushed and homogenized in 3mL ice cold Tris buffer (pH=7.4) for performing various assays. Total protein content was measured by Lowry et al., (1956) (10) and used for further calculation. The remaining sample was used for the estimation of various antioxidant levels in kidney homogenate such as TBARS, SOD, GPX and catalase by previously described standard methods (11) while the level of ALP was measured using commercial kit.

Histopathology

After 21 days of treatment, rats were euthanized by carbon dioxide inhalation followed by cervical dislocation. Immediate laparotomy was performed to collect both the kidneys. Isolated kidneys were cleaned off the extraneous tissue, weighed and rinsed with ice-cold normal saline. A section from both kidneys was fixed with 10% v/v neutral formalin and processed through graded alcohol series and xylene, embedded in paraffin, sectioned at 5µm, and stained with hematoxylin and eosin for histopathological examination under a light microscope. Three kidney tissues per group were analyzed for nephropathy, obstruction and stone deposition.

In vitro gel diffusion model

To find the inhibitory effect of STS on calcium oxalate stone formation the gel diffusion assay was performed according to Li et al. with minor modifications (12). A microscope slide was uniformly coated with 3mL of 1% agar. After the agar solidified, two pairs of equidistant wells were made perpendicularly. Sodium oxalate and calcium chloride each 20µl was placed in vertical wells. The horizontal wells were filled either with 20µl distilled water as standard or 20µl of STS at varying concentrations. Then the slide was left in a moist chamber for 24h at room temperature. The calcium and oxalate ions diffuse through the gel and form crystals of calcium oxalate, visible as a cloudy streak in the center. The intensity of crystal formation and size of the crystals was dependent on the molarity of the crystal forming solutions employed. Depending on concentration, the inhibitory substances would modify the density and width of the crystal streak. This was carried out in triplicates with different concentrations (200mM, 100mM, 50mM, and 25mM) of STS. The slides were photographed using Gel Documentation System 'BioRad Chemidoc XRS'. The images were analyzed using Image J software and densitometry plots were obtained. Relative density of the sample with respect to the control was obtained, and the percentage inhibition was calculated by the following formula: inhibition=1-(relative density of the sample/relative density of the control)*100.

Statistical Analysis

Data was expressed as mean±SD. The comparison between groups, at various time points during the experiment was conducted using ANOVA followed by multiple comparison tests, particularly Dunnett's test using GraphPad Prism software version 5.0.

RESULTS

Preliminary observations of the rats indicate that ethylene glycol consumption reduced the body weight while the urine output was elevated at the end of 21 days.

Urine and Serum chemistry

Table-1 shows the levels of urea, creatinine and calcium in the urine, and their corresponding serum concentration is depicted in Table-2. Induction group rats showed a significant decrease in the concentration of urea, creatinine in urine, while its serum concentration was significantly higher as compared to normal control rats. Administration of rats with STS, diazoxide, glibenclamide+STS exhibits near normal levels of urea, creatinine and calcium in both urine and serum as compared to normal control rats.

According to Leibovitch, (13) elevated serum ALP is an indicator of kidney dysfunction, and

Table 1 - Urine Chemistry.

Parameters	Normal control	Induction control	Treatment groups				
			STS alone	Diazoxide alone	Glibenclamide alone	Diazoxide+STS	Glibenclamide+STS
Urea (mg/mL)	11.12±0.9	6.06 ±1.2*	9.07±1.3	10.77±1.5	7.33±0.8*	8.92±1.3	10.29±1.5
Creatinine (µm/L)	12.13±1.4	6.26±1.5*	9.42±0.4	8.99±2.9	3.94±0.2*	6.93±1.8*	7.24±1.5*
Calcium (mg/24hr)	0.95±0.1	2.44±0.2*	1.10±0.3	0.69±0.1*	1.23±0.3	1.28±0.2	0.60±0.1*

Group-1 = served as normal control; **Group-2 =** as a stone induction control; **Group-3 =** was given STS; **Group-4 and Group-5 =** were administered diazoxide and glibenclamide respectively and **Groups 6 and 7 =** were pretreated with diazoxide and glibenclamide respectively half an hour before administration of STS. Data of all results are presented as mean±SD (*) p<0.05, statistically different from normal controls.

Table 2 - Serum Chemistry.

Parameters	Normal control	Induction control	Treatment groups				
			STS alone	Diazoxide alone	Glibenclamide alone	Diazoxide +STS	Glibenclamide +STS
Urea (mg/dL)	16.50±0.9	41.94±1.1*	18.76±1.2	19.55±1.3	29.93±1.2*	16.42±1.6	13.74±1.3
Creatinine (mg/dL)	0.35±0.02	1.03±0.07*	0.38±0.02	0.35±0.06	0.58±0.02*	0.38±0.04	0.35±0.06
ALP (U/L)	57.03±2.3	115.96±2.9*	49.25±2.4	50.73±3.2	76.18±2.4*	70.14±4.2*	41.39±2.1
Calcium (mg/dL)	6.21±0.5	5.14±0.9	4.46±0.7*	3.34±0.7*	1.57±0.2*	4.31±0.7*	4.10±0.9*

Group-1 = served as normal control; **Group-2 =** as a stone induction control; **Group-3 =** was given STS; **Group-4 and Group-5 =** were administered diazoxide and glibenclamide respectively and **Groups 6 and 7** were pretreated with diazoxide and glibenclamide respectively half an hour before administration of STS. Data of all results are presented as mean±SD (*) p<0.05, statistically different from normal controls.

its levels in the blood can be used as an index to assess the effectiveness of the treatment. Administration of STS, diazoxide and glibenclamide+STS to rats reduced the significant elevation of serum ALP activity (Table-2) shown in induction control groups as compared to normal control rats.

Antioxidant status

Ethylene glycol administration to the rat was reported to alter the oxidant and antioxidant balance in the kidney and thereby induces nephrolithiasis in two phases. Initially, it causes the production of free radicals and in the later stage, it initiates infiltration of leukocytes (14). Ethylene glycol treatment significantly (P<0.001) increased the TBARS levels, decreased superoxide dismutase and glutathione peroxidase in the induction control group compared to normal rats. The treatment with STS (400mg/kg) to the rats significantly (P<0.05) reduced the TBARS levels and improved the antioxidant enzymes activities compared to group 2 (Table-3).

Histopathology

Histopathological analysis of renal tissues in the control group showed no calcium oxalate deposits or other abnormalities in different segments of the nephrons (Figure-1A). But in the urolithiasis induction group, a substantial amount of calcium oxalate deposition was observed, and this was present in whole parts of three major areas of the kidney (Figure-1B). Renal tubular dilations with tubular basophilic and epithelial damage were also observed on pathological examination. In sodium thiosulfate treated group the number of calcium oxalate deposits was significantly lower than that in the disease control group with only mild nephropathy in one of the animals in that

Table 3 - Lipid peroxidation and antioxidant levels.

Parameters	Normal control	Induction control	Treatment groups				
			STS alone	Diazoxide alone	Glibenclamide alone	Diazoxide +STS	Glibenclamide +STS
TBARS (mM/100g tissue)	1.78±0.1	4.21±0.5*	2.10±0.4	0.84±0.09*	0.74±0.05*	0.71±0.03*	0.72±0.08*
Superoxide Dismutase (Units/mg protein)	34.7±4.5	17.64±1.2*	31.24±2.4	26.47±1.2*	25.63±1.3*	29.91±2.3	32.30±2.6
Glutathione peroxidase (µg of GSH utilized/min/ mg protein)	22±1.2	12.13±1.1*	19.14±1.6	15.54±1.3*	16.66±1.5*	19.82±1.1	19.57±1.4

Group-1 = served as normal control; **Group-2 =** as a stone induction control; **Group-3 =** was given STS; **Group-4 and Group-5 =** were administered diazoxide and glibenclamide respectively and **Groups 6 and 7 =** were pretreated with diazoxide and glibenclamide respectively half an hour before administration of STS. Data of all results are presented as mean±SD (*) $p<0.05$, statistically different from normal controls.

group (Figure-1C). Rats treated with diazoxide alone showed less obstructive damage (Figure-1D) while glibenclamide treated rats showed severe damage and obstruction (Figure-1E). Apparently more renal damage, inflammation and hemolysis were observed in rats co-administered with STS and diazoxide (Figure-1F) while STS administration along with glibenclamide showed preserved renal tissue with mild obstruction (Figure-1G).

In vitro gel analysis to study chelation effect

In order to re-confirm the inhibitory effect of STS on calcium oxalate crystal, we performed an in-vitro analysis. The calcium oxalate crystals that have been produced in this study were similar to the crystals in the urine of patients with calcium oxalate crystals. The crystals were predominantly of monohydrate type, confirmed by FTIR. According to Figure-2, STS showed a dose-dependent inhibition of calcium oxalate stone formation. Apparently, only 18% direct inhibition was shown with maximum STS concentration of 200mM, indicating an additional tissue based mechanism for its renal protective action.

DISCUSSION

Urinary lithiasis is a multifactorial urological disorder that generally occurs as a result of an imbalance between inhibitors and promoters for renal stone formation (15). The human kidney stones predominantly comprised of calcium oxalate, and few studies have examined the effect of the sodium thiosulfate on calcium oxalate crystallization (16) as well. However, the conclusions from those studies were not consistent as few studies claim the beneficial effect of STS and while others, its negative result (6, 7). In the present study, we investigated the effect of sodium thiosulfate on renal stone formation in both in vivo and in vitro models and evaluated its mechanism of action. Our study results were in agreement with previous reports that suggested anti-urolithiatic property of STS but provides a new direction for its mode of action where STS may modulate mitochondrial K_{ATP} channel in rendering renal protection.

Evidence in the literature showed that sodium thiosulfate reduces calcium phosphate stone formation in the genetic hypercalciuric rat (6). However, very little data on the use of sodium thiosulfate for calcium oxalate nephrolithiasis has been published. Adherence of calcium oxalate to renal tubules is associated with free radical mediated injury and the resultant oxidative stress due to hyperoxaluria, which favors crystal adherence (17). Administration of STS to rats for 21 days not only reduced the stress mediated by ethylene glycol, but also prevented the renal dysfunction measured by biochemical parameters like urea and creatinine in urine and serum. Elevated serum alkaline phosphatase activity is considered to be an indicator of renal damage (18). The increased

Figure 1 - Light microscopic architecture of kidney showing (A) Renal tissue of control (group 1) rats showing no sign of crystal deposition. (B) Renal tissue of urolithiatic rats (group 2) showing crystals deposition and severe obstructive nephropathy (C) Renal tissue of (group 3) STS treated rats showing mild crystal deposition with mild nephropathy. (D) Renal tissue of (group 4) diazoxide treated rats showing mild crystal deposition with moderate obstructive nephropathy (E) Renal tissue of (group 5) glibenclamide treated rats showing prominent crystal deposition with severe obstructive nephropathy (F) Renal tissue of (group 6) diazoxide +STS treated rats showing crystal deposition with severe obstructive nephropathy (G) Renal tissue of (group 7) glibenclamide + STS pretreated rats low crystal deposition with mild nephropathy (H) Crystal's deposition as observed under 40X zoom.

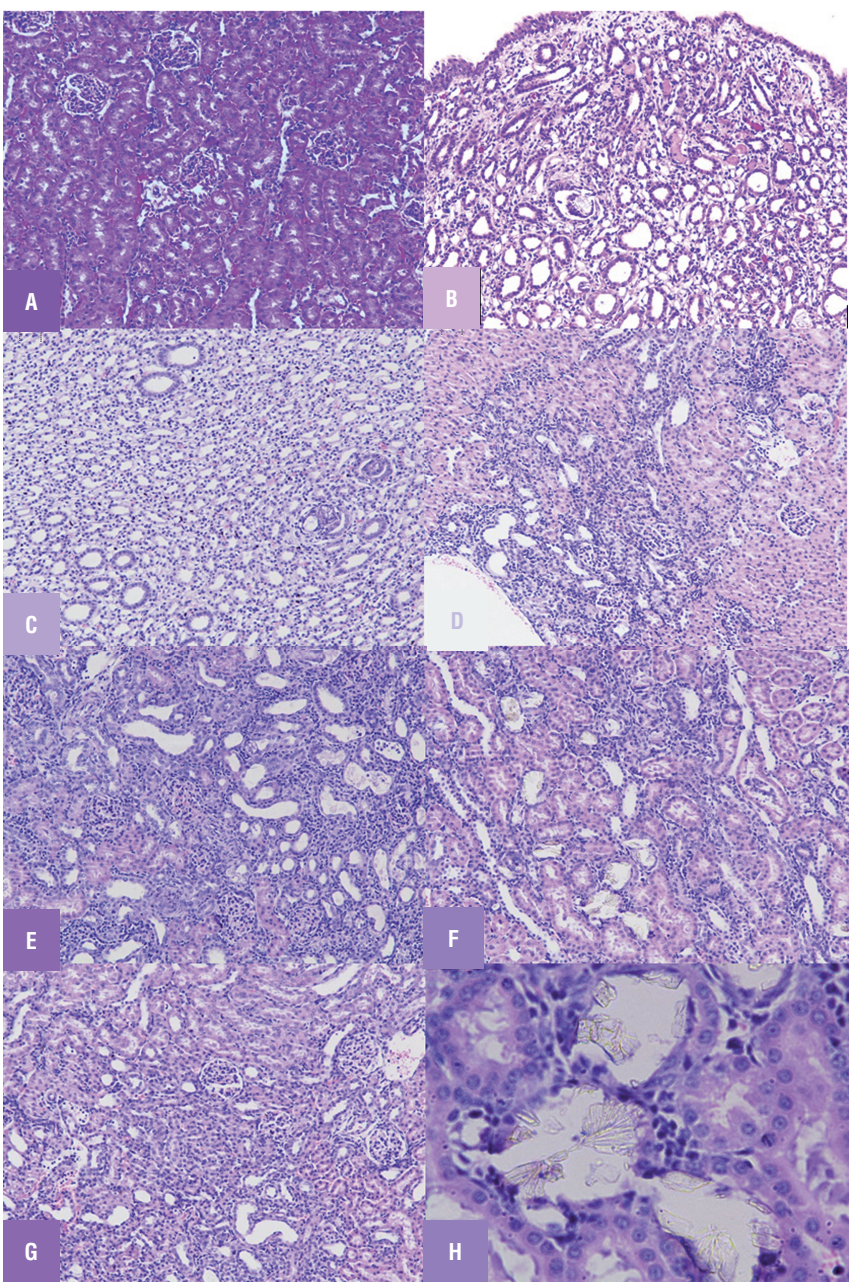
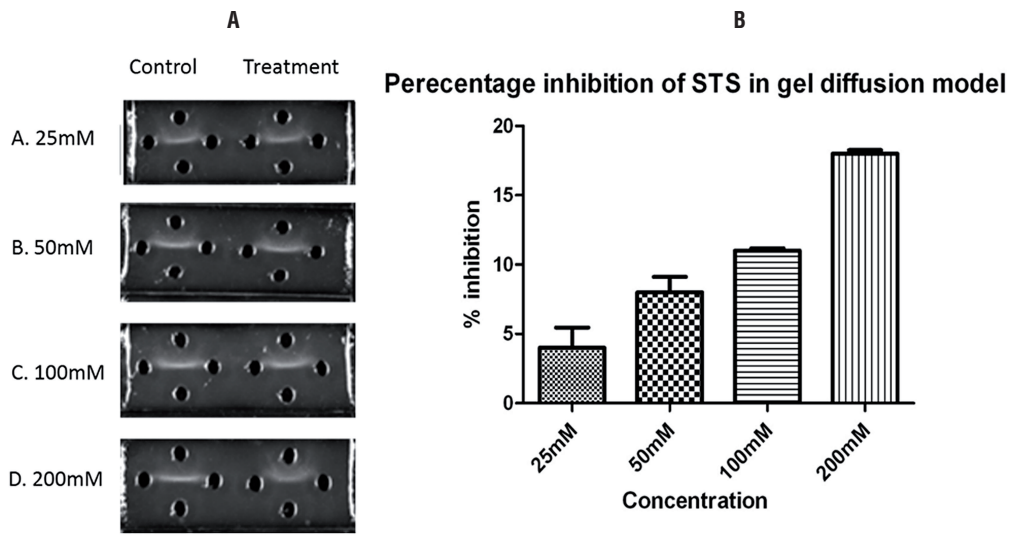


Figure 2 - Images of agar gel slides and graph representing the percentage inhibition produced by STS in calcium oxalate crystal formation represented as a streak.



serum ALP activity may be derived from the injury to the brush border membrane of the renal tubular cells (19). The near-normal activity of ALP in the present study was in agreement with the other group that showed a significant decline of ALP activity after STS administration in uremic rats (20). Further evidence for STS protection was confirmed through histological results where papillary crystalline deposits and calcium parenchyma deposits were absent. The direct interaction of STS on calcium oxalate formation was confirmed by using in vitro gel technique and found around 18% inhibition in calcium oxalate formation with 200mM STS, suggesting indirect action of STS in preventing renal stone formation.

A direct co-relation between renal mitochondrial dysfunction and ethylene glycol induced calcium oxalate formation was reported, where the exposure of the proximal tubule with calcium oxalate crystals resulted in rapid and progressive osmotic swelling and dissipation of transmembrane potential of mitochondria resulted in its damage (21). The peroxidation of protein had greater influence on the nucleation and aggregation property of calcium oxalate crystal growth and that predominantly occur in mitochondria (22). Mitochondrial permeability transition pore (mPTP) opening is a terminal event leading to mitochondrial dysfunction and cell death under conditions of oxidative stress. In fact, the vulnerability of the renal tissue towards oxidative stress depends on the functional cross talks between mPTP and mitochondrial K_{ATP} channel (23). In the present study, we evaluated the specific role of mitochondrial potassium ATP channel in the sodium thiosulfate mediated renal protection.

Acccording to previous study results, it is believed that renal protection mediated by sodium thiosulfate is mainly attributed to its chelation and antioxidant potential (6, 24). However, thiosulfates are metabolized in mitochondria, and thus we anticipated a mitochondria based mechanism for its renal protection. In this connection, we used a mitochondrial potassium channel blocker glibenclamide (binds to sulphonylurea receptor subtypes of K_{ATP} channel) and channel opener diazoxide (binds to ATP binding sites of sulphonylurea receptor subtypes of K_{ATP} channel) to evaluate the renal status as supported by biochemical parameters and histopathology (Figure-1, Tables 1-3).

Glibenclamide showed a prominent renal injury as evidenced from altered serum and urine chemistry that was clearly demarcated in histopathology (Figure-1E, Tables 1 and 2). Several lines of evidence showed that glibenclamide can depolarize mitochondrial membrane leading to calcium overload, one of the major factors responsible for free radical release and injury as evident from the lipid peroxidation and antioxidant marker enzyme levels (Table-3). On the other hand, ATP sensitive potassium channel opener, diazoxide treatment showed well-preserved architecture of the kidney (Figure-1D). It prevented mitochondrial swelling and depolarization that may result in permeability pore transition and leads to tissue injury (25, 26). Diazoxide can also modulate the renin angiotensin system, that may play a significant role in developing renal tubule interstitial fibrosis (27) and resulting stone formation as reported with ethylene glycol induced renal injury. Although the mechanism by which the K_{ATP} opener exert their renal protection have not been clarified yet, it is believed that the opening of K_{ATP} channel preserves mitochondrial functional activities through mild uncoupling and depolarization (28). Thus diazoxide mediated protection is an impact on the mitochondria.

In order to confirm the STS mediated mitochondrial K_{ATP} channel modulatory effect, we administered STS along with diazoxide (mitochondrial K_{ATP} channel opener) and glibenclamide (mitochondrial K_{ATP} channel blocker). We found interesting results, where the protective effect shown by diazoxide treatment alone was negated by STS supplementation. On the other hand, STS supplementation to glibenclamide group showed preserved renal tissue architecture. This inverse relationship of STS is an evidence for its interaction with mito K_{ATP} Diazoxide binds to an ATP-sensitive K^+ transport pathway in kidney mitochondria that affects volume, respiration, and membrane potential and may have a role in the prevention of mitochondrial ATP hydrolysis. Opening of this channel leads to mild uncoupling, blocks calcium entry into mitochondria and leading to renal protection (28, 29). As both diazoxide and STS (mediated through H2S formation) binds to KATP channel in different sites, when diazoxide and STS are given concomitantly, long term or excessive uncoupling may be expected causing ATP hydrolysis and mPTP opening without impairing electron transport, leading to apoptosis. On the other hand, glibenclamide binds to different sulfonylurea subunit blocking potassium entry, thereby exaggerating the ROS production and destabilizing the membrane potential leading to apoptosis (28). When STS is given with glibenclamide, we predict that, H_2S released from STS may bind to Kir6. 1 subunit of mito K_{ATP} channel, thereby reducing the binding efficiency of glibenclamide resulting its limited action of K_{ATP} channel, allowing STS to mediated its renal protection.

The protective mechanism induced by the opening of mito K_{ATP} is well-studied in cardiovascular diseases. Analogous to the heart system, renal protection by diazoxide may well be claimed due to i) Changes in the mitochondrial Ca^{2+} levels ii) Mitochondrial matrix swelling and changes in ATP synthesis iii) Changes in the ROS levels. Sodium thiosulfate is a known calcium chelating agent with antioxidant properties (30) and can render electrons to complex IV upon its metabolism. Furthermore, several lines of the reports suggest that mitochondrial K_{ATP} channel opening may inhibit mitochondrial permeability transition through inhibiting calcium overload and thereby preserve mitochondrial functions. A proven relationship between mitochondrial membrane potential, mitochondrial dependent apoptosis and calcium overload predicts the possibility of thiosulfate mediated calcium signaling mechanism through calcium/calmodulin-dependent protein kinase for its action, proposed for the future study.

The present study enhances the existing knowledge of STS mediated anti urolithiatic mechanism that emphasizes the calcium chelation and antioxidant property of STS alone. Based on our findings, we suggest that thiosulfate modulate the mitochondrial K_{ATP} channel to render renal protection against stone formation.

CONCLUSIONS

Based on the results, we found that the administration of sodium thiosulfate effectively prevented the development of urolithiasis in rats, in

agreement with the findings of Asplin & Onyeka groups. Even though few mechanisms were proposed earlier for the anti-urolithiasis effect of sodium thiosulfate, no conclusive understanding was reached, and the present study confirm the specific role of ATP sensitive mitochondrial K_{ATP} channel in STS mediated renal protective mechanism.

Abbreviations

STS = Sodium thiosulfate
EG = ethylene Glycol
K_{ATP} = Potassium ATP channel
mPTP = mitochondrial permeability transition pore
ROS = reactive oxygen species

CONFLICT OF INTEREST

None declared

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Hemorrhagic Cystitis Requiring Bladder Irrigation is Associated with Poor Mortality in Hospitalized Stem Cell Transplant Patients

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ABSTRACT

Purpose: To evaluate the overall prognosis of post-stem cell transplant inpatients who required continuous bladder irrigation (CBI) for hematuria.
Materials and Methods: We performed a retrospective analysis of adult stem cell transplant recipients who received CBI for de novo hemorrhagic cystitis as inpatients on the bone marrow transplant service at Washington University from 2011-2013. Patients who had a history of genitourinary malignancy and/or recent surgical urologic intervention were excluded. Multiple variables were examined for association with death.
Results: Thirty-three patients met our inclusion criteria, with a mean age of 48 years (23-65). Common malignancies included acute myelogenous leukemia (17/33, 57%), acute lymphocytic leukemia (3/33, 10%), and peripheral T cell lymphoma (3/33, 10%). Median time from stem cell transplant to need for CBI was 2.5 months (0 days-6.6 years). All patients had previously undergone chemotherapy (33/33, 100%) and 14 had undergone prior radiation therapy (14/33, 42%). Twenty-eight patients had an infectious disease (28/33, 85%), most commonly BK viremia (19/33, 58%), cytomegalovirus viremia (17/33, 51%), and bacterial urinary tract infection (8/33, 24%). Twenty-two patients expired during the same admission as CBI treatment (22/33 or 67% of total patients, 22/28 or 79% of deaths), with a 30-day mortality of 52% and a 90-day mortality of 73% from the start of CBI.
Conclusions: Hemorrhagic cystitis requiring CBI is a symptom of severe systemic disease in stem cell transplant patients. The need for CBI administration may be a marker for mortality risk from a variety of systemic insults, rather than directly attributable to the hematuria.

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Bone Marrow Transplantation; Stem Cell Transplantation; Cystitis; Hematuria

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INTRODUCTION

Hemorrhagic cystitis is a significant cause of morbidity in immunocompromised patients on the Bone Marrow Transplant (BMT) service, occurring in up to 30% of hematopoietic stem cell transplant recipients (1-3). The most prevalent causes of hemorrhage include toxic effects of chemotherapy, infection, and radiation cystitis (2). Cyclophosphamide and busulfan are the most common chemotherapeutic agents causing hemorrhagic cystitis, while polyoma BK virus, adenovirus, and cytomegalovirus are the most common infectious agents (4, 5). Polyoma BK viremia, specifically, has been shown to be correlated with increased severity of hemorrhagic cystitis but not with increased mortality (6). Other reported predisposing factors to the development of hemorrhagic cystitis include thrombocytopenia, coagulopathy, and possibly graft-versus-host disease (GVHD) (7).

Often urologic consultation is obtained on these patients when there is a need for continuous bladder irrigation (CBI). The primary goals of this study were to characterize the patients requiring CBI for hemorrhagic cystitis after hematopoietic stem cell transplant and assess all-cause mortality of this cohort.

MATERIALS AND METHODS

A retrospective review was performed of 33 adult patients who received CBI for hemorrhagic cystitis as inpatients on the BMT service at Washington University between 2011-2013. Data collection began after receiving Institutional Review Board approval. Only patients who had undergone prior hematopoietic stem cell transplant were included in the study, and patients who had a history of genitourinary malignancy and/or had undergone a recent surgical urologic intervention were excluded.

Pre-CBI complete blood count values were collected < 24 hours prior to the start of CBI treatment. Infectious diseases were identified with blood culture, urine culture, or quantitative viral polymerase chain reaction analysis, as appropriate. CBI treatment was provided through a triple lumen urinary catheter. All patients initially received intravesical irrigations of saline solution. Clinical variables were retrospectively reviewed, and outcomes were assessed to see if hemorrhagic cystitis was associated with prognosis or systemic disease.

Student's t-test was used to compare continuous, normally distributed variables, and Welch's t-test was used to compare continuous, non-normally distributed variables. All statistical analyses were two-sided using a significance of $p \leq 0.05$.

RESULTS

Patient Characteristics

Mean age of the study population was 48 years (range 23-65), with a median Charleston Comorbidity Index (CCI) of 2 (0-6). Thirty patients had a hematologic malignancy (30/33, 91%), 2/33 patients had myelodysplastic syndrome (MDS) (6%), and 1/33 patient had aplastic anemia (3%). Malignancies included acute myelogenous leukemia (AML) (17/33, 57%), acute lymphoblastic leukemia (ALL) (3/33, 10%), peripheral T cell lymphoma (3/33, 10%), diffuse large B cell lymphoma (2/33, 7%), Hodgkin's lymphoma (2/33, 7%), multiple myeloma (1/33, 3%), Mantle cell lymphoma (1/33, 3%), and Sézary syndrome (1/33, 3%). Details of the patients' characteristics and relation to mortality can be found in Table-1. As expected, malignancy demonstrated a significant association with mortality (p=0.015).

All patients had received a prior hematopoietic stem cell transplant, with a median time from transplant to need for CBI of 2.5 months (range: 0 days-6.6 years). All 33 patients also had previously undergone chemotherapy, with the most prevalent agents including cyclophosphamide (25), cytarabine (21), busulfan (13), vincristine (11), idarubicin (10), and etoposide (10). Fourteen patients had undergone prior radiation therapy (14/33, 42%), none of which had treatment directed to the pelvis. Details of treatment characteristics and relation to mortality can be found in Table-2. Radiation or chemotherapy alone were not independent risk factors for mortality, but obviously may be correlated with stem cell transplant, therefore they cannot be exclusively separated. No specific prior oncologic treatment was uniquely significant over other treatments.

Twenty-eight patients were found to have an infectious disease (28/33, 85%), most commonly BK viremia (19/33, 58%), cytomegalovirus viremia (17/33, 51%), and bacterial urinary tract infection (UTI) (8/33, 24%). Twenty-three patients had pre-existing GVHD (23/33, 70%), frequently of the gastrointestinal (GI) tract (16/33, 48%), skin (6/33, 18%), or liver (5/33, 15%). Median pre-CBI complete blood count values showed patients to be leukopenic at 1.9 x 10^3/µL, anemic at 9.4g/dL and thrombocytopenic at 18 x 10^3/µL. Details of infectious diseases and GVHD and their association with mortality can be found in Table-3.

Two patients eventually required instillation of 1% alum solution to control hemorrhage (2/33, 6%), and 6 patients required cystoscopy with clot evacuation after initiation of CBI (6/33, 18%) for removal of recurrent clots. Neither need

Table 1 - Patient characteristics and malignancy subtype as risk factors for 90-day mortality

Clinical Characteristics	Total (n=33)	Deceased at 90 days (n=24)	P-value
Average Age (in years)	48	49	
Median CCI	2	2	1.000
Malignancy	30	24	0.015
AML	17	14	0.259
MM	1	1	1.000
ALL	3	3	0.545
PTCL	3	2	1.000
DLBCL	2	1	0.477
HL	2	2	1.000
ML	1	1	1.000
SS	1	0	0.273
MDS	2	0	0.068
Aplastic Anemia	1	0	0.273

CCI = Charleston Comorbidity Index; **AML =** acute myelogenous leukemia; **MM =** multiple myeloma; **ALL =** acute lymphoblastic leukemia; **PTCL =** peripheral T cell lymphoma; **DLBCL =** diffuse large B cell lymphoma; **HL =** Hodgkin's lymphoma; **ML =** Mantle cell lymphoma; **SS =** Sezary syndrome.

Table 2 - Prior oncological treatments as risk factors for 90-day mortality.

Treatment	Total	Deceased (at 90 days)	P-value
Radiation	14	9	0.442
Chemotherapy			
Cyclophosphamide	25	17	0.394
Cytarabine	21	17	0.230
Idarubicin	10	8	0.686
Etoposide	10	7	1.000
Busulfan	13	10	1.000
Vincristine	11	9	0.681

for instillation of alum solution nor need for cystoscopy with clot evacuation was shown to be associated with changes in 30- or 90-day mortality (30-day p=0.21, 0.40, 90-day p=0.47, 1.00).

Descriptors of Mortality Analysis

Twenty-eight patients were deceased at the time of last follow-up (28/33, 85%). Average age at death was 49 years, with median time to death from initiation of CBI of 22 days (0-804 days). Of these patients, twenty-two patients died during the same admission of CBI treatment (22/28 or 79% of deaths, 22/33 or 67% of total patients). Thirty-day mortality was found to be 52% (17/33), and 90-day mortality was 73% (24/33).

We assessed factors possibly related to mortality in our cohort. Nearly all of the patients who were deceased at the time of last follow-up

Table 3 - Additional possible risk factors of mortality.

Risk Factor	Total	Deceased (at 90 days)	P-value
Infectious Disease	28	22	0.111
BK Virus	19	14	1.000
CMV	17	12	1.000
VRE UTI	6	4	1.000
Klebsiella UTI	2	1	0.477
Enterobacter UTI	1	1	1.000
GVHD	23	16	0.547
Gastrointestinal	16	13	0.111
Skin	6	3	0.653
Liver	5	5	0.143

CMV = cytomegalovirus; **VRE =** vancomycin-resistant enterococci; **UTI =** urinary tract infection; **GVHD =** graft-versus-host disease

died from cancer related causes and subsequent multi-organ failure (27/28, 96%). Neither mean age nor median CCI at time of admission were shown to be associated with increased 30- or 90-day mortality. Treatment with chemotherapy in general or any specific agent was not shown to be associated with mortality. Similarly, radiation was not associated with increased mortality. Infectious diseases, pre-existing GVHD, neutropenia, anemia, and thrombocytopenia were also not shown to be specifically associated with increased mortality.

DISCUSSION

Our findings demonstrate that hemorrhagic cystitis requiring CBI in patients having undergone stem cell transplant is associated with a high 30-day and 90-day mortality rate. A previous study from our institution showed a 90-day mortality rate of 12% after stem cell transplant (8). The present study, however, demonstrates a markedly higher mortality rate in this cohort with hemorrhagic cystitis requiring CBI, the 90-day mortality rate was 73%. It is important to note that this is an association, but not an assertion of cause. In our best assessment, this cohort's development of hemorrhagic cystitis seems to be part of an overall picture of severe medical illness, rather than a specific cause of mortality. Hence, hemorrhagic cystitis requiring CBI is likely a urological marker of severe systemic insults within this population. Hematuria was not the cause of death in these patients, but rather was just another marker for severe systemic disease.

Hemorrhagic cystitis is a significant cause of morbidity in stem cell transplant patients, with most common etiologies including toxic effects of chemotherapy, infection, and radiation cystitis. However, these patients regularly have many predisposing factors, thereby causality is difficult to assign. The statistical challenge of demonstrating significant relationships is compounded by the relatively small cohort size and overall high mortality risk across the entire cohort, thereby making it unlikely that any patient factor would be more significant than others (Tables 1-3). For example, cyclophosphamide has been shown to be the most common chemotherapeutic agent causing hemorrhagic cystitis in the literature (4, 9, 10). Most of the patients (88%) who received chemotherapy also had other risk factors for development of hematuria such as viruria, bacteria, or radiation, thus cause cannot be clearly identified. This further supports the idea that hemorrhagic cystitis is a symptom of severe systemic disease rather than a causative mortality factor.

In our study, more than half (67%) of the patients died during the same admission in which occurred CBI. The overall 30-day mortality was

52%, and 90-day mortality was 73%. Padilla-Fernandez et al. found a slightly lower overall mortality rate of 51.14% in a population of 52 post--hematopoietic stem cell transplant patients with hemorrhagic cystitis, although the authors did not specifically examine patients with severe enough hemorrhagic cystitis to merit CBI (11). In their study, hemorrhagic cystitis was also not found to be the cause of death in any patient. Nevertheless, this consistently high mortality risk should concern urologists in planning overall treatment and care of these patients. Given that no patients died directly from hemorrhage, and additional interventions were not found to impact mortality, perhaps treatment plans should be made with paramount consideration of patient comfort. Unless a patient is actively exsanguinating due to the severity of their hematuria, care may be limited to CBI, and clinicians should use this high mortality rate to more accurately advise families on prognosis.

Under more typical circumstances, treatments of hemorrhagic cystitis require the patient to be free of bladder clots, which can be achieved with aggressive manual irrigation or cystoscopy with clot evacuation and fulguration. Once the bladder is clear, continuous bladder irrigation is initiated to prevent further clot development and retention. Mild hemorrhages can be treated with sodium chloride instillation, while more severe cases require instillation of aminocaproic acid, alum, silver nitrate, phenol, or formaldehyde (12). In refractory cases, hyperbaric oxygen therapy or embolization of the internal iliac arteries can be employed (13, 14). Surgical options such as urinary diversion or cystectomy are an option if all other therapies are unsuccessful (15). Fibrin glue therapy is a novel technique that has been recently described by Tirindelli et al., who showed that this treatment increased the 6-month probability of survival when used in post-allogenic hematopoietic stem cell transplant patients (16).

Our study has several limitations. This project is both retrospective and hypothesis generating, thus larger-scale prospective studies are necessary to investigate possible associations between hemorrhagic cystitis, predisposing factors, and increased mortality. Nevertheless, a randomized or prospective study may not be practical or possible in this population. In addition, a matched cohort study comparing the mortality rates between similar patients with and without hemorrhagic cystitis would be necessary to truly elucidate a statistically significant risk of mortality in post-stem cell transplant patients with hemorrhagic cystitis.

CONCLUSIONS

Hemorrhagic cystitis requiring CBI is an indicator of severe systemic disease in patients who underwent previous stem cell transplantation via either peripheral blood or BMT. The need for CBI administration may portend an increased risk of mortality for hospitalized stem cell transplant patients. The urologist has a unique perspective when receiving a consultation for CBI in a BMT patient. Counseling of the patient, family, and other providers is paramount. Further studies and improved strategies for management of these patients are needed.

ABBREVIATIONS

CBI = continuous bladder irrigation
BMT = bone marrow transplant
AML = acute myelogenous leukemia
ALL = acute lymphoblastic leukemia
UTI = urinary tract infection
GVHD = graft-versus-host disease
MDS = myelodysplastic syndrome
CCI = Charleston Comorbidity Index
MM = multiple myeloma
PTCL = peripheral T cell lymphoma
DLBCL = diffuse large B cell lymphoma
HL = Hodgkin's lymphoma
ML = Mantle cell lymphoma
SS = Sézary syndrome
CMV = cytomegalovirus
GI = gastrointestinal
VRE = vancomycin-resistant enterococcus

CONFLICT OF INTEREST

None declared.

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Efficacy of botulinum toxin type A 100 Units versus 200 units for treatment of refractory idiopathic overactive bladder

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ABSTRACT

Objective: To evaluate the efficacy and safety of a single intra detrusor injection of BoNTA comparing two different doses (100 U or 200 U) in patients with idiopathic overactive bladder.

Materials and Methods: A randomized prospective study evaluated the efficacy of BoNTA in management of refractory idiopathic overactive bladder and included 80 patients. All patients were assessed initially by taking a history, a physical examination, overactive bladder symptom score, urine analysis, routine laboratory investigations, KUB and pelviabdominal. OABSS was adjusted on all patients postoperative at 1,3,6,9 months also Urodynamic was done for all patients preoperative and postoperative at 3, 6, 9 months.

Results: The mean age was 30.22±8.37 and 31.35±7.61 in group I and II respectively. There was no statistically difference between both groups in all parameters all over the study except at 9 months after treatment. Hematuria was observed 6 and 9 patients in group I and II respectively. Dysuria was observed in 6 and 15 patients in group I and II respectively. UTI was detected in 3 and 7 patients in group I and II respectively.

Conclusion: A single-injection procedure of 100 U or 200 U BoNTA is an effective and safe treatment for patients with IOAB who failed anticholinergic regimens. OABSS and QoL were improved for 6 months; 100 U injections seemed to have comparable results with 200 U. There was a significant difference at month 9 towards 200 U with more incidences of adverse events.

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INTRODUCTION

Detrusor overactivity is defined by the presence of lower urinary tract symptoms of urgency with or without urge urinary incontinence (UUI), usually with frequency and nocturia (1). In the majority of affected patients, the cause of the detrusor overactivity is idiopathic while neurogenic detrusor overactivity occurs mainly in patients with spinal cord diseases (2). Conservative treatments (lifestyle modifications, pelvic floor exercises, bladder training, and anticholinergic regimens) may result in insufficient improvements and in low compliance because of bothersome adverse events (3). Intradetrusor injection of botulinum neurotoxin type A (BoNTA) is emerging as the second-line treatment for refractory OAB symptoms (4).

Botulinum toxin is a purified neurotoxin derived from clostridium botulinum and its

main effect is to inhibit signal transmission at the neuromuscular junction by inhibiting the release of acetylcholine. In addition, botulinum toxin is now thought to have effects on the release of other sensory neurotransmitters such as substance P and ATP, as well as reducing the axonal expression of capsaicin and purinergic receptors (5). Many studies demonstrated significant improvements in OAB symptoms and QoL with BoNTA treatment but they also showed increased post void residual urine, acute urinary retention and urinary tract infections (6). There is no consensus on the dose of BoNTA or BoNTB, injection sites, and the duration between repeat injections (7). This current study aimed to evaluate the efficacy and safety of a single intra detrusor injection of BoNTA alone comparing two different doses (100 U or 200 U) in patients with IOAB.

PATIENTS AND METHODS

This study was a randomized prospective one evaluating the efficacy of BoNTA in management of refractory idiopathic overactive bladder (IOAB) and included 80 patients who presented to the Urology Department of Benha University Hospital from May 2011 to February 2014. An informed written consent was obtained from all patients after the study protocol was approved by the Research Ethics Committee, Faculty of Medicine, Benha University. The inclusion criteria were IOAB refractory to previous anticholinergics with different types of anticholinergic agents, either as a single drug or a combination for >3 months. Exclusion criteria were pregnant women, uncorrectable coagulopathies, active UTI, bladder outlet obstruction, neurogenic bladder, or having a PVR>150 mL at the time of enrollment, and previous radiotherapy or antineoplastic treatment. Additional use of anticholinergics was not allowed during the study period. Patients were randomly classified into two groups I and II. They underwent intradetrusor injection of BoNTA 100 and 200 Unit respectively. All patients were assessed initially by taking a history, a physical examination, overactive bladder symptom score (OABSS) (8) (Table 1), EuroQoL (EQ-5D) visual analogue scale (VAS) (9), measuring the patient's current health-related QoL state; both scales range from 0 to 100 (worst to best), urine analysis, routine laboratory investigations, KUB and pelviabdominal spiral CT and IVP if indicated. Urodynamic evaluation was done in the form of flowmetry and cystometry.

OABSS was developed by Homma et al. (8) which is a single symptom score that employs a self-report questionnaire. There were 4-symptoms evaluated: daytime frequency, nighttime frequency, urgency and urge incontinence for the questionnaire.

The score is the simple sum of the 4-symptom scores.

Injection technique

After dilution with 10cc saline, either 100 or 200 Units BoNTA (Allergan®, Irvine, CA, USA) were used for cystoscopic intradetrusor injection under spinal anesthesia. The injection was performed in 20 sites, using 30-degree lens and a rigid scope with a 6 Fr. injection needle without side holes (Amecath Company®, Egypt).

The injection sites were determined after mapping of the bladder at the anterior, left lateral, right lateral, posterior walls and the trigone (0.5cc at each site). The injection was followed by insertion of a 16 Fr. Foley's catheter, to be removed the next morning after surgery. All patients received peri-operative I.V. antibiotics. For postoperative follow-up, all patients were assessed at 1, 3, 6, 9 months using OABSS, HRQoL as well as urodynamic study at 3, 6, 9 months.

Statistical analysis

The collected data were tabulated and analyzed using SPSS version 16 software (Spss Inc.®, Chicago, ILL Company). Categorical data were presented as number and percentages while quantitative data were expressed as mean and standard deviation. Chi square test (X2) and Student "t" test were used as tests of significance.

The accepted level of significance in this work was stated at 0.05 ($P<0.05$ was considered significant).

Table 1 - Overactive bladder symptom score.

Question	Frequency	Score
How many times do you typically urinate from waking in the morning until sleeping at night?	≤ 7	0
	8-14	1
	≥ 5	2
How many times do you typically wake up to urinate from sleeping at night until waking in the morning?	0	0
	1	1
	2	2
	3	3
How often do you have a sudden desire to urinate, which is difficult to defer?	Not at all	0
	Less than once a week	1
	Once a week or more	2
	About once a day	3
	2-4 times a day	4
	5 times a day or more	5
How often do you leak urine because you cannot defer the sudden desire to urinate?	Not at all	0
	Less than once a week	1
	Once a week or more	2
	About once a day	3
	2-4 times a day	4
	5 times a day or more	5

Patients were instructed to circle the score that best applied to their urinary condition during the past week; the overall score was the sum of the four scores.

RESULTS

Demographic baseline values

Eighty patients (63 women and 17 men) were enrolled in the study. The participants were randomly assigned to one of the two treatment groups, receiving a BoNTA dose of either 100 U (n=40) or 200 U (n=40). The mean (standard deviation) ages were 30.22 (8.37) years for group I and 31.35 (7.61) years for group II. One patient who received 100 U of BoNTA dropped out of the study at month 6 evaluation and another one at month 9. Two patients who received 200 U of BoNTA dropped out of the study at month 9 evaluation. There were no statistically significant differences in baseline characteristics between two groups. There was no statistically difference between both groups in all parameters all over the study except at 9 months after treatment.

EFFICACY (Table 2)

Clinical symptoms

When comparing the mean of OABSS and HRQOL data obtained at months 1, 3, 6 and 9 after treatment to baseline data it was observed significant improvement ($p<0.001$) in both groups. The mean values of OABSS and HRQOL data at months 3, 6 and 9 were significantly ameliorated ($p <0.001$) compared to data at month 1 in both groups. Within-group I analyses at month 9 demonstrated a statistically significant amelioration ($p<0.001$) compared to data at months 3 and 6 (Table 3).

Urodynamic values

Comparison of urodynamic data obtained at months 3, 6 and 9 after treatment to baseline data revealed that the mean of volume at first desire, volume at strong desire, maximal cystome-

Table 2 - Clinical symptoms and PVR Urine Changes.

Variables	BoNTA 100		BoNTA 200	
	N	Mean(SD)	N	Mean(SD)
Frequency				
Baseline	40	1.6 (0.496)	40	1.67 (0.525)
At 1m	40	0.45 (0.503)*	40	0.42 (0.5)*
At 3m	40	0.42 (0.5)*	40	0.33 (0.474)*
At 6m	39	0.51 (0.506)*	40	0.3 (0.464)*
At 9m	38	1.1 (0.508)*†‡Δ	38	0.32 (0.471)*#
Nocturia				
Baseline	40	0.87 (0.965)	40	1.2 (1.202)
At 1m	40	o.23 (0.422)*	40	0.15 (0.361)*
At 3m	40	0.13 (0.334)*	40	0.13 (0.334)*
At 6m	39	0.13 (0.338)*	40	0.12 (0.334)*
At 9m	38	0.36 (0.488)*	38	0.13 (0.342)*#
Urgency				
Baseline	40	4.7 (0.464)	40	4.67 (0.474)
At 1m	40	1.4 (1.37)*	40	1.9 (1.12)*
At 3m	40	1.07 (1.163)*	40	1.45 (1.131)*
At 6m	39	0.97 (1.135)*†	40	1.25 (1.031)*†
At 9m	38	2.57 (0.948)*†‡Δ	38	1.47 (1.202)*#
UUI				
Baseline	40	1.67 (1.899)	40	1.8 (2.002)
At 1m	40	0.77 (1.073)*	40	0.85 (1.098)*
At 3m	40	0.65 (0.975)*	40	0.65 (0.948)*
At 6m	39	0.67 (0.982)*	40	0.72 (1.085)*
At 9m	38	1.26 (1.171)*†‡Δ	38	0.68 (0.162)*#
PVR				
Baseline	40	25.75 (12.83)	40	27.4 (15.05)
At 1m	40	40.0 (21.42)*	40	47.37 (11.87)*
At 3m	40	39.23 (12.48)*	40	42.00 (10.05)*
At 6m	39	38.88 (12.22)*	40	41.79 (10.77)*
At 9m	38	24.21 (8.58)‡Δ	38	29.21 (11.30)‡Δ#

*significant in intragrou *significant in intragroup comparison to "before intervention"
†significant in comparison to "1 month later
‡significant in intragroup comparison to "3 months later"
Δsignificant in intragroup comparison to "6 months later"
#significant in intergroup comparison.
(Paired "t" test was the test of significance)
(N=Number of patients, UUI=Urge Urinary Incontinence, SD=Standard Deviation, PVR=Post Void Residual, M=Month)

Table 3 - Urodynamic Changes.

Variables	BoNTA 100		BoNTA 200	
	N	Mean(SD)	N	Mean(SD)
Volume at first desire (mL)				
Baseline	40	200 (35.73)	40	199 (35.71)
At 3m	40	318 (59.62)*	40	300.2 (44.28)*
At 6m	39	309.2 (58.14)*	40	295.5 (40.86)*
At 9m	38	246.8 (53.78)*‡Δ	38	291.8 (42.82)*‡#
Volume at strong desire (mL)				
Baseline	40	259 (64.40)	40	260.5 (63.08)
At 3m	40	427.5 (58.78)*	40	407.2 (41.44)*
At 6m	39	417.9 (51.2)*	40	401.2 (38.35)*
At 9m	38	313.1 (67.38)*‡Δ	38	392.1 (37.28)*‡#
Detrusor pressure (cm H2O)				
Baseline	40	27.8 (10.12)	40	30.6 (11.09)
At 3m	40	11.1 (6.317)*	40	9.25 (3.01)*
At 6m	39	10.6 (5.36)*	40	9.07 (3.22)*
At 9m	38	19.2 (7.78)*‡Δ	38	10.42 (3.97)*#
MCC(mL)				
Baseline	40	277.7 (75.29)	40	289.2 (70.83)
At 3m	40	439 (55.22)*	40	439(41.24)*
At 6m	39	437.4 (55.36)*	40	438.2 (40.99)*
At 9m	38	350 (69.08)*‡Δ	38	430.5 (34.24)*#

*significant in intragrou
p comparison to "before intervention"
‡ significant in intragroup comparison to "3 months later"
Δ significant in intragroup comparison to "6 months later"
significant in intergroup comparison.
(Paired "t" test was the test of significance)
(MCC= Maximum Cystometric Capacity, N=Number of patients, SD=Standard Deviation, M=Month)

tric capacity and detrusor pressure improved significantly (p<0.001) in both groups. The mean values of urodynamic data at month 9 were significantly ameliorated (p<0.001) compared to data at months 3 and 6 in group I.

However, in group II, the mean volume at first desire and at strong desire were significantly ameliorated (p<0.001) compared to data at month 3 only (Table 4).

Side effects

Early postoperative hematuria was observed in 6 (4 women and 2 men) patients in group I and 9 (6 women and 3 men) patients in group II. During follow-up dysuria was observed in 6 (5 women and 1 male) and 15 (12 women and 3 men) patients in group I and II respectively. UTI was detected in 3 (2 women and 1 male) and 7 (5 women and 2 men) patients in group I and II respectively.

Table 4 - OABSS and QOL Changes.

Variables	BoNTA 100		BoNTA 200	
	N	Mean(SD)	N	Mean(SD)
OABSS				
Baseline	40	8.85 (2.166)	40	9.35 (1.994)
At 1m	40	2.85 (2.537)*	40	3.32 (2.092)*
At 3m	40	2.27 (2.391)*†	40	2.55 (2.417)*†
At 6m	39	2.28 (2.361)*†	40	2.37 (2.518)*†
At 9m	38	5.3 (2.11)*†‡Δ	38	2.6 (2.307)*†#
QoL				
Baseline	40	42.7 (8.58)	40	40.8 (6.82)
At 1m	40	83.6 (7.54)*	40	82.8 (7.60)*
At 3m	40	72.4 (16.45)*†	40	77.3 (11.67)*†
At 6m	39	73.4 (12.21)*†	40	77.3 (10.12)*†
At 9m	38	68.5 (7.57)*†Δ	38	77.1 (10.00)*†#

*significant in intragroup comparison to "before intervention"
†significant in comparison to "1 month later
‡significant in intragroup comparison to "3 months later"
Äsignificant in intragroup comparison to "6 months later"
#significant in intergroup comparison.
(Paired "t" test was the test of significance)
(N=Number of patients, SD=Standard Deviation, M=Month, OABSS=Over Active Bladder Symptom Score, QoL=Quality of Life)

DISCUSSION

Sacral neuromodulation or surgical bladder augmentation were the available options for treatment of IOAB, however they are highly invasive and have long term complications (10). A European consensus group gave a grade A recommendation for BoNTA use in IDO (4) and a recent systematic review suggested that its use for refractory OAB is well justified (11).

In relation to the aforementioned results, the current study showed significant improvement in all clinical symptoms (frequency, nocturia, urgency and UUI) after BoNTA treatment. We observed no significant difference between 100 U and 200 U at post injection at months 1, 3 and 6. The 200 U BoNTA dosage demonstrated consistent improvements till the end of the study and the significant difference between the study groups was observed at month 9 after injection. However, there was a significant amelioration at month 9 when compared to those at months 1, 3 and 6 in patients who received 100 U except for nocturia. The response rate and the incidence of side effects of BoNTA on IOAB are closely related to the dose (12).

Many investigators reduced the dose of BoNTA to 100 U and a satisfactory outcome can still be achieved for IDO due to the high incidence of side effects after BoNTA injections (7, 11, 13-15). The continence and the cure rate were respectively 22.9% and 15% by Nitti et al. (16), in the series by Visco et al. (17) the cure rate was 27%. In other studies detrusor injections of 200 U yielded long response duration of 12-15 months (12). Brubaker et al. (18) reported a mean duration of efficacy of 370 days of BoNTA 200 U.

The efficacy of a single injection of BoNTA toxin over 6 months and the literature supported 200 U as the dose most likely to provide this durability (19-22). Regarding the changes in the urodynamic parameters after BoNTA injection, we

found that the bladder capacity returned gradually, however the maximum effect on significant increase in cystometric capacity was observed at month 3 after treatment in both groups. Although BoNTA remained therapeutically effective up to 6 months, the effect reduced significantly in group I with time till the end of the study.

Doses ranging from 50 U to 300 U showed significantly greater improvement in symptoms of frequency, urgency, and UI as well as in urodynamic measures in the active-treatment arms with BoNTA doses of at least 100 U (18, 19, 23). Regarding the optimal BoNTA dose, one study suggested minimal added benefit above 150 U, (19) and a Class III study comparing 100 U vs. 150 U failed to demonstrate any differences between the two doses (14).

The number of adverse events was lower than the one observed by others. In this study, none of the patients developed urinary retention or significant elevation of post-void residual urine (PVR>100 mL) following injection. Many studies of BoNTA for IDO reported a 24-43% incidence of transient urinary retention requiring CIC and also reported a 32-72% incidence of a large PVR and difficulty in urination (24-28). Although a large PVR and chronic urinary retention remain obstacles for the wide use of BoNTA in treatment of refractory DO, no factors predicting these adverse effects have been found (7). Bauer et al. (29) have specifically looked at adverse events after injection of 100 U, 200 U and 500 U of BoNTA by using a patient questionnaire, but he concluded the higher doses of the toxin led to higher rates of adverse events.

We observed mild hematuria which was procedural related and resolved conservatively. In the series by Kuo et al. (22) the hematuria was up to 7.8% and 3.6% in the series by Chapple et al. (15). In the current study, dysuria was increased in group II. Previous studies also reported dysuria in 5.8% (15), 4% (30) of patients that received 100 U whereas; it was 33% for 200 U (31). On the other hand, many series did not notice this adverse effect (12, 14, 18, 20, 26, 27).

This study revealed more cases of UTI in group II; all cases received proper antibiotics and analgesics, which was comparable with others in which the rate of UTI ranged from 13% to 44% (14, 15, 18, 22, 23, 32) and this was related to clean intermittent self catheterization (CISC).

Our study is not without weaknesses; firstly, this study was done without a control arm. Secondly, it still represents a small number of patients. Finally, from the previous data we claim that further studies will be needed to confirm the effectiveness of 100 U and 200 U doses. They might also consider evaluating the efficacy and tolerability of repeated injections of BoNTA to optimize the risk–benefit ratio. This would help to introduce BoNTA as a treatment of choice in patients with refractory IOAB.

CONCLUSIONS

A single-injection procedure of 100 U or 200 U BoNTA is an effective and safe treatment for patients with IOAB who failed anticholinergic regimens. Following the procedure, OABSS and QoL were improved for 6 months; 100 U injections seemed to have comparable results with 200 U. At month 9 there was a significant difference towards 200 U with more incidence of adverse events.

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CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Sphingosine Kinase 1 urothelial expression is increased in patients with neurogenic detrusor overactivity

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ABSTRACT

Objectives: To evaluate the expression of sphingosine kinase 1 (SPK1) in the bladder wall in patients with neurogenic lower urinary tract dysfunction and its association with clinical, urodynamic and pathological features.
Materials and Methods: The expression of SPK1 was studied in bladder wall specimens obtained from cystectomy using immunohistochemistry in ten patients with spinal cord injury (n=8) or multiple sclerosis (n=2) with urodynamically proven neuropathic bladder dysfunction, and in controls (n=5). Inflammation and fibrosis were analysed with histological criteria and SPK1 expression was determined by individual immunohistochemical staining.
Results: Significant increased SPK1 urothelial immunoreactivity was shown in patients compared to control group (p=0.03). By contrast, SPK1 immunoreactivity in patients was significantly decreased in the sub-urothelium, muscles and nerves, p=0.02; 0.01 and 0.003, respectively. Patients with neurogenic detrusor overactivity (NDO) had higher SPK1 urothelium expression than those without any DO (p=0.04).
Conclusions: SPK1 is expressed in the human bladder wall, specifically the urothelium, in bladder specimens from patients with NDO. The role of SPK1 in the pathophysiology of NDO needs further elucidation.

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INTRODUCTION

Lower urinary tract dysfunction (LUTD) is common following neurological disorders such as spinal cord injury and multiple sclerosis. The commonest manifestation is an overactive bladder syndrome, characterized by urinary urgency, frequency and urge incontinence due to neurogenic detrusor overactivity (NDO). One pathophysiological hypothesis is an increase in afferent input from the bladder where nonadrenergic noncholinergic mechanisms become predominant (1). A shift from A delta-fibers to abnormal C-fibers activity is also observed. There is good evidence that the main transmitter contraction is ATP and that nitric oxid (NO) is responsible for the main part of inhibitory NANC responses (2). However, the mechanisms of altered excitability are not totally understood and the role of many others substances such as neuropeptides need to be fully established.

Sphingosine 1-phosphate (S1P) is a bioactive sphingolipid that is known to mediate di-

verse cellular mechanisms such as apoptosis and proliferation. These effects have been attributed to specific G protein-coupled receptors, namely $S1P_{1-5}$ (3). In addition to its role in tumour proliferation and immunity, S1P plays an essential role in smooth muscle (3). Sphingosine kinase (SPK) 1 is one of the two major enzyme isoforms which converts sphingosine to S1P via reversible phosphorylation. In rabbits it has been reported that S1P was involved in the regulation of detrusor contractions (4, 5). In an overactive bladder rat model, greater S1P expression associated with Rho-kinase expression was noted, suggesting a role for the S1P/SPK signalling pathway in this condition (6).

To date, bladder SPK1 expression has never been studied neither in people without any bladder disease nor in human with neuropathic bladder. Our aim was to study SPK1 bladder wall expression in the human neuropathic bladder dysfunction and to determine whether it was associated with a type of bladder dysfunction.

MATERIALS AND METHODS

Between September 2011 and June 2012 a total of 10 patients (6 females, 4 males) underwent total cystectomy and urinary diversion in our institution. The 10 patients suffered from neurogenic LUTD due to spinal cord injury (n=8) and multiple sclerosis (n=2). The indication of surgery was failure of conservative treatment resulting in refractory urinary incontinence, recurrent urinary tract infections and renal impairment and the inability to perform clean intermittent self-catheterisation (Table-1). Urinary diversion was through an ileal conduit in all cases. No postoperative complication was observed. All patients signed a consent form allowing us to do research in the bladder specimen and the local ethic committee approved the collection. Control bladder specimens (n=5) were obtained from cadaveric donors without any neurologic diseases or lower urinary tract symptoms (Table-2). All patients provided informed consent and the stu-

Table 1 - Clinical and urodynamic data of the patients group.

Patient	Clinical data							USP score			Urodynamic data	
	Sex	Age	Affection	Duration	Drainage	Treatment	Complications	Incontinence	OAB	Low stream	BC	DO
	(F/H)	(years)	(MS/SCI)	(years)	(IC/SC)	(AOT/IBT)	(OAS/RUI/RI)	(score/9)	(score/15)	(score/9)	(L/N)	(Y/N)
1	F	36	MS	39	IC	AOT	UI/RUTI	3	6	9	N	Y
2	H	55	SCI	16	SC	AOT/IBT	UI	5	15	9	L	N
3	F	68	SCI	15	SC	AOT/IBT	UI/RUI	4	0	2	N	Y
4	H	73	SCI	7	SC	AOT/IBT	RUTI/RI	3	5	7	L	N
5	H	42	SCI	14	SC	IBT	RUTI/RI	1	4	9	N	N
6	F	50	SCI	10	SC	AOT	UI/RUTI	9	20	3	N	N
7	F	42	SCI	13	SC	IBT	UI	1	2	9	N	Y
8	H	33	SCI	10	SC	AOT	UI/RI	9	13	0	N	Y
9	F	59	MS	23	SC	AOT/IBT	UI/RI	2	6	9	L	Y
10	F	47	SCI	20	IC	IBT	RI	0	5	9	L	N

MS = Multiple Sclerosis; **SCI =** Spinal Cord Injury; **IC =** Indwelling catheter; **SC =** Supra pubic catheter; **AOT =** Antimuscarinic Oral Therapy; **IBT =** Intravesical Botulinium Toxin Injection; **UI =** urinary incontinence; **RUTI =** Recurrent Urinary tract Infections; **RI =** Renal Impairment; **USP score [7] =** Urinary Symptom Profile score; **OAB =** Overactive bladder; **BC =** Bladder Compliance; **L/N =** Low/Normal; **DO =** Detrusor Overactivity

Table 2 - Clinical data of cadaveric donors.

Control	Sex	Clinical data		
	(F/H)	Age	Neurologic disease	Cause of death
		(years)	(Yes/No)	
1	F	48	No	Stroke
2	H	62	No	Acute Myocardial Infarction
3	F	55	No	Cardiac Insufficiency
4	H	79	No	Stroke
5	F	66	No	Acute Myocardial Infarction

dy was approved by the ethic committee and by the French transplantation agency (Agence de Biomédecine).

Preoperative evaluation

Before surgery, patients had a clinical and urodynamic evaluation. Lower urinary tract symptoms were assessed using the Urinary Symptoms Profile® questionnaire (7). Urodynamic evaluation was performed according to the ICS recommendations (8, 9).

Histological examination

From each bladder, 4 full-thickness bladder samples were harvested respectively from the dome, the two lateral faces and the trigone using a punch-biopsy device (Visipunch®, 8mm, Huot Instruments®, Menomonee Falls, WI, USA) and were fixed in 4% paraformaldehyde. Histological sections of 4µm were hematoxylin-eosin stained. Pathologist examination consisted in scoring lymphocytic and plasma cell inflammatory infiltration and fibrosis and according to severity was graded as moderate (+), mild (++) and severe (+++) respectively (10).

Immunochemistry

All sections were deparaffinised, hydrated, boiled with 10mmol/L of citrate buffer (pH 6) for 30 min, pretreated with 0.3% H2O2 for 5 min. Then, in moist chamber at room temperature the slices were pre-incubated in PBST (Phosphate Buffered Saline 0.1%Triton) for 3x2 min and incubated with the primary antibody (polyclonal anti-SpK1 Ab diluted 1:50, Abcam™, London, United Kingdom) for 2h. All sections were incubated 30 min in secondary antibodies coupled with Horse Radish Peroxydase (Dako EnVision™ FLEX HRP, Carpenteria, CA USA). Immuno-labelling was revealed by 3' di-amino benzidine. At the end, slices were incubated in 5min hematoxylin to show tissue morphology. Between each step sections were washed in Phosphate Buffered Saline Triton X100. Specimens were analysed with Axiophot® microscope with magnification 100 (Zeiss, Germany) by two independent observers in a "double person blind test" and they were scored using an arbitrary intensity scale: negative (0), faint/equivocal (+), moderate (++) or strong (+++) as previously used by Apostolidis (10). Percentage of each field was taken into consideration and mean value was calculated for each slide and afterwards for each case.

Statistical analysis

The two-sided Fisher's exact t-test was used to compare the distribution of qualitative variables. Student's t-test was used to compare means between groups. For immunochemistry studies, the relationship between urodynamic data and staining intensities was analysed by the two-sided Fisher's exact t-test. Statistical significance was set at a p value<0.05.

RESULTS

Five patients (50%) had NDO and 4 had low bladder compliance. Clinical and urodynamic data are summarized in Table-1.

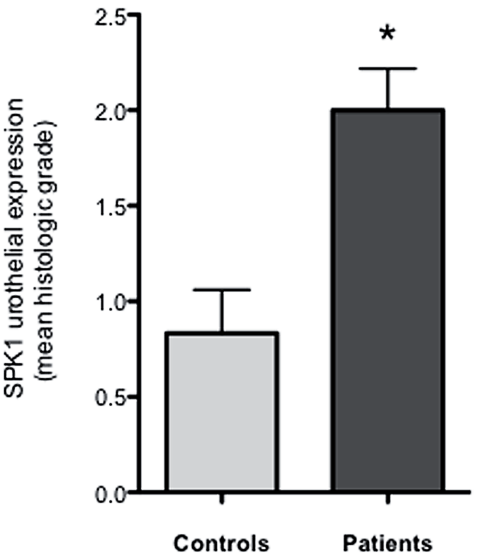
HISTOLOGICAL EXAMINATION

No specimen displayed malignancy. Inflammatory lymphocytic/plasma cell infiltration was observed in all patients specimen with predominance in the deep layers of the bladder wall. Mild or severe muscular infiltration was observed in three cases. A significant degree of fibrosis was identified in the patients compared to controls, $p<0.01$, predominantly in the detrusor layer. There were no significant differences in inflammation or fibrosis in the patient group regarding age, aetiology and bladder localization.

Immunochemistry

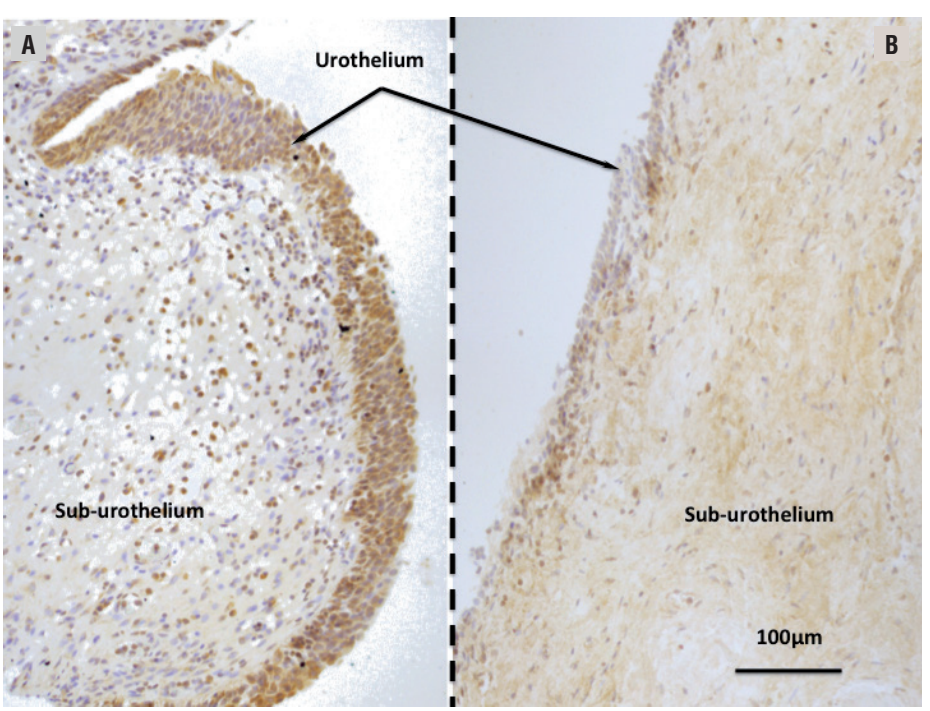
Significantly increased SPK1 urothelial immunoreactivity was noted in patients (Figures 1 and 2) as compared to controls ($p=0.03$). Whereas SPK1 immunoreactivity was significantly decreased in the sub-urothelium, muscles and nerves as compared to controls, $p=0.02$; 0.01 and 0.003,

Figure 2 - SPK1 urothelial expression.



Sphingosine Kinase 1 (SPK1) expression is significantly increased in the urothelium layer of patients as compared to controls, $p=0.03$.* $p\leq0.05$.

Figure 1 - Microscopic features of Sphingosine Kinase 1 expression.



Transverse sections after immunochemistry reactions (Dako EnVision™ FLEX HRP, Carpenteria, CA USA). Magnification X40; scale bar=100µm.
A) Patient with neuropathic bladder dysfunction. Significant increase in SPK1 expression is observed in the urothelium layer (arrow). **B)** Control bladder. Homogeneous expression of the Sphingosine Kinase 1 (SPK1).

respectively. SPK1 expression was homogeneous throughout the whole bladder in the control group (Figure-1). A significant increased in SPK1 immunoreactivity was identified in the urothelium layer of patients with DO, p=0.04 (Figure-3).

DISCUSSION

The purpose of this study was to characterize the expression pattern of SPK1 in neurogenic LUTD. To our knowledge, this is the first time SPK1 expression has been demonstrated in human bladder tissue from patients with neuropathic bladder dysfunction. Increased expression was demonstrated in the urothelium, higher in patients with NDO as compared to patients without NDO. We confirmed also the increase in bladder wall fibrosis of patients affected by NDO which is consistent with results previously reported by Comperat et al. (11).

Recently, animal studies have attempted to understand the role of the SPK1/S1P pathway in the lower urinary tract. Watterson (4) found that S1P could contract rabbit detrusor and suggested a role of dysregulation of SPK1/S1P signalling in overactive bladder. S1P induced contraction was dependent on stretch and intracellular calcium. It was of interest that S1P was supposed to regulate calcium channels in an S1P receptor-independent manner (4).

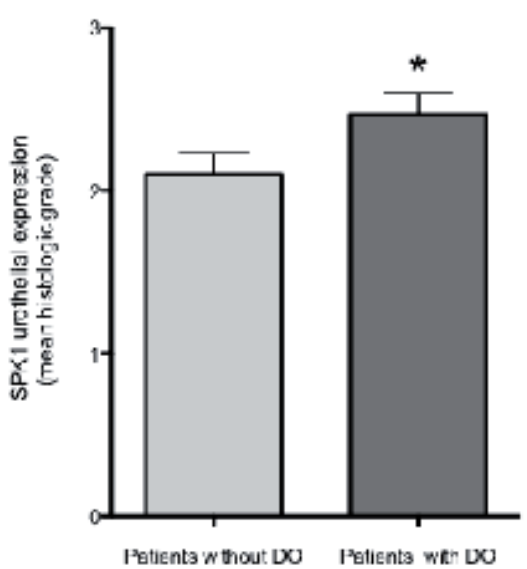
Sandhu et al. (12) demonstrated the presence of S1P receptors in the bladder wall of female rat and found regional heterogeneity in its expression. They suggested that the significance of expression variation in the lower urinary tract could have functional and clinical involvements. They proposed this pathway as a possible therapeutic target. They demonstrated that S1P mediated smooth muscle contraction but didn't hypothesize a mechanism of inducing contraction.

In a model of overactive bladder in rat, Aydin et al. (6) reported that S1P signalling pathway was significantly up-regulated in association with overexpression of Rho kinases and they hypothesised that S1P-induced bladder overactivity. These findings may suggest that S1P modulates detrusor contraction through a Rho-kinase signalling pathway in overactive bladder.

The link between S1P and NDO is still unclear. SPK activity is known to be stimulated by agonists of various G protein-coupled receptors as well as by depolarization-induced calcium entry (13). SPK activation may in turn activate calcium sensitizing mechanisms, such as Rho-kinase signalling pathway (6), which lead to increased myosin light chain phosphorylation and detrusor contraction. Interestingly, FTY720-phosphate, an agonist for all S1P receptors except $S1P_2$ induced distinct contraction properties (4). The link between S1P and detrusor contractions may involve others underlying mechanisms. S1P could act as a second messenger or interact with neurotransmitters or neuropeptides. In vascular muscles, S1P modulates muscle contraction either directly on S1P specific receptors or indirectly after being generated inside the cell (14).

Although no human data concerning SPK1 in the neuropathic bladder are available, a recent study (15) reported for the first time the presence of SPK1 and S1P receptors in the human bladder. mRNA profiling was performed on the cell lines of native human urothelium and demonstrated the

Figure 3 - SPK1 expression and detrusor overactivity.



Sphingosine Kinase 1 (SPK1) expression in bladder wall specimen of patients suffering from neuropathic bladder dysfunction. There was a significant increase in SPK1 immunoreactivity in the urothelium layer of patients suffering from detrusor overactivity (DO), p=0.04.* p≤0.05

expression of the two SPK isoforms SPK1, SPK2 and related receptors $S1P_{1-5}$. No data was available concerning regional distribution and patients with neurological disorders.

Recently the urothelium has emerged more as an integrator of sensory inputs and outputs in the bladder wall than a passive barrier (16, 17). In NDO, urothelial cells exhibit "neuron like" properties that allow them to respond to various stimuli. They are believed to release many substances including acetylcholine, ATP, nitric oxide, neural growth factor and prostaglandins that can affect smooth muscle, interstitial or immune cells but that can also modulate the activity of afferent nerves (18). Recently, non-adrenergic, non-cholinergic, non-prurinergic contractions of detrusor were found in the porcine urothelium with lamina propria (19). The neurotransmitter responsible for this is still unknown. As we found a significant increase in SPK1 immunoreactivity in the urothelium as compared to control group, a potential role of S1P in this pathway cannot be excluded.

In the same way, the significant increase in SPK1 immunoreactivity in the urothelium of patients with DO as compared to patients without DO suggests that S1P/SPK signalling may play a role in human detrusor overactivity. Further studies are needed with a larger sample to confirm these findings.

The understanding of the underlying mechanism of lower urinary tract dysfunction is crucial for effective management of patients with neuropathic bladder dysfunctions. The possible involvement of SPK1/S1P pathway in human bladder dysfunction and the pathogenesis of detrusor overactivity open up a potentially novel approach to managing neurogenic LUTD. Of late, drugs modulating the SPK1/S1P pathway have been studied as disease modifying treatment in multiple sclerosis, namely the monoclonal antibody (Sphingomab® (20) and an oral S1P receptor modulator, fingolimod (21), and it would be of clinical relevance to assess the impact these drugs may have on LUTD.

The main limitation of our study was the relatively small number of tissue samples that were studied. Whilst the nature of LUTD was established in the patient group, control tissue was obtained from cadaveric donors for whom no investigation assessing bladder dysfunction was done. Lastly, S1P expression was demonstrated only by immunohistochemistry and was not confirmed using mRNA profiling. Nevertheless, our study suggests that SPK1 is expressed in the human bladder wall, specifically the urothelium, in bladder specimens from patients with NDO and additional studies are needed to confirm the role of SPK1/S1P pathway in the model of overactive bladder and a larger study is required to confirm these results and to further understand the role of this pathway in the pathogenesis of detrusor overactivity.

We investigated for the first time in a prospective study SPK1 expression in the human bladder wall and in patients suffering from neuropathic bladder dysfunction. A significant increase in its expression in the urothelium was found in all patients and it was higher in patients with detrusor overactivity. These findings suggest that SPK/S1P pathway may be involved in the neurogenic pathological detrusor contraction mechanisms. Functional role of SPK1 is still to be confirmed. Further experiments with a larger number of patients are required to confirm highlight-underlying mechanisms. Moreover the expression in patients with idiopathic detrusor overactivity should be addressed and its level compared to patients with neurogenic detrusor overactivity.

CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Experimental use of a cellulosic biopolymer as a new material for suburethral sling in the treatment of stress urinary incontinence

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ABSTRACT

Purpose: To analyze the interaction between the cellulose exopolysaccharide (CEC) and urethral tissue when used as a pubovaginal sling.
Materials and Methods: Forty Wistar rats were divided into four groups. In groups A and B the cellulose exopolysaccharide (CEC) was implanted around the urethral tissue (bladder neck below the upper margin) and the rats were sacrificed at 30 and 90 days. Similar procedure was used in groups C and D using a polypropylene mesh. After sacrifice bladder and urethra were sent for histological analysis. The histological parameters (inflammatory reaction) by evaluated by quantitative analysis. For collagen deposition analysis it was used stereological method.
Results: The cellulose exopolysaccharide (CEC) was inert and well preserved at the implanted region at the time of examination. Morphologic alterations were not found at the CEC implant but some reactions of foreign body type were observed at the adjacent structures. In some areas a process of neovascular formation was observed. Stereological analysis at the suburethral area showed a significant difference in collagen presence in favor of CEC.
Conclusions: The CEC implant showed adequate results when used as a suburethral sling with good integration to the host tissue, preserving its architecture.

ARTICLE INFO

Key words:
Suburethral Slings; Urinary Incontinence; Cellulose; Biopolymers

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INTRODUCTION

Urinary incontinence affects millions of women in the World. It is estimated a prevalence ranging from 10 to 52% of the adult population (1).

Many surgical procedures have been proposed for the treatment of female SUI. Pubovaginal slings have shown good efficacy with satisfactory results in the long term follow-up (2). Autologous fascia has been widely used and became the treatment of choice for many women suffering from SUI (2, 3). The use of tension–free vaginal tape (TVT) started a new era of the use of heterologous materials which simplifies the procedure and decreases morbidity (4).

Several synthetic materials including polypropylene and polytetrafluorethylene (PTFE) have been used with this purpose despite the higher risk of infection and erosion (5, 6).

The cellulose exopolysaccharide has demonstrated effectiveness in different areas of surgery, acting as a conductor and inducing the healing process (7, 8).

In the present study we evaluated the interaction of the cellulose exopolysaccharide (CEC) with urethral tissue when used as a pubovaginal sling.

MATERIALS AND METHODS

Forty female Wistar rats with weight ranging from 215 to 297g were used in the present study. They were divided into 4 groups of 10. Two groups (A and B) were submitted to the implant of CEC that had 5cm in length and 0.3cm in width and groups C and D received the polypropylene mesh with the same dimensions. In both cases the surgical implantation occurred below the bladder neck with tension free fixation at the rectus muscle fascia (Figure-1).

Animals in subgroups A and C were sacrificed 4 weeks after the implant and animals in subgroups B and D after 12 weeks in order to evaluate tissue reaction and integration to the host. Bladder, urethra and abdominal musculature were removed as a single block keeping their anatomic relationship. The whole block was preserved in buffered 10% formalin for 15 days after which was processed for histological analysis.

Histological slides (5 microns) were processed using Haematoxilin-Eosin (HE) staining to evaluate inflammatory reaction. The following criteria were used for this purpose: (1) Absent: no inflammation or less than 5% at the analyzed area; (2) Slight: inflammatory reaction between 5 and 25%; (3) Moderate: Inflammatory reaction between 25 and 70%; (4) Intense: over 70% of the studied area. Collagen quantification was performed through stereological analysis and the slides were stained by Picrosirius red. Samples were analyzed by the same pathologist using 40X magnification.

Numeric variables were expressed by central tendency and dispersion. Kruskal-Wallis test was used to express continuous variables and Chi Square for categorical variables. The significance level to reject the nullity hypothesis was 5% ($p<-0.05$).

This research followed the rules of the experimental ethics code and the animal protecting laws practiced in Brazil. This study was approved by the Ethics Committee for animal study of the Institution.

RESULTS

No postoperative surgical complication was found in all animals. During sacrifice no atypical tissue reaction was observed as a consequence of the use of both materials. No fistula or abscess was found. Macroscopically, a good incorporation was observed (Figure-2).

Microscopically the subaponeurotic area showed intense inflammatory reaction in both groups with a significant presence of polymorphonuclear neutrophils in the analysis after 4 weeks. No atypical formation was observed.

The same analysis performed in animals sacrificed at 12 weeks showed a slight increase in collagen deposition at the subaponeurotic area in animals receiving CEC as compared to polypropylene mesh (25.90% vs 22.30), $p=0.21$ (Figure-3).

Stereological analysis at the suburethral area showed a significant difference of collagen presence in favor of CEC (58.60% vs 28.10), $p=0.003$.

Figure 1 - Surgical technique of implantation showing dissection of the urethra (A), positioning of the CEC at the suburethral space (B) and fixation to the abdominal wall (C).

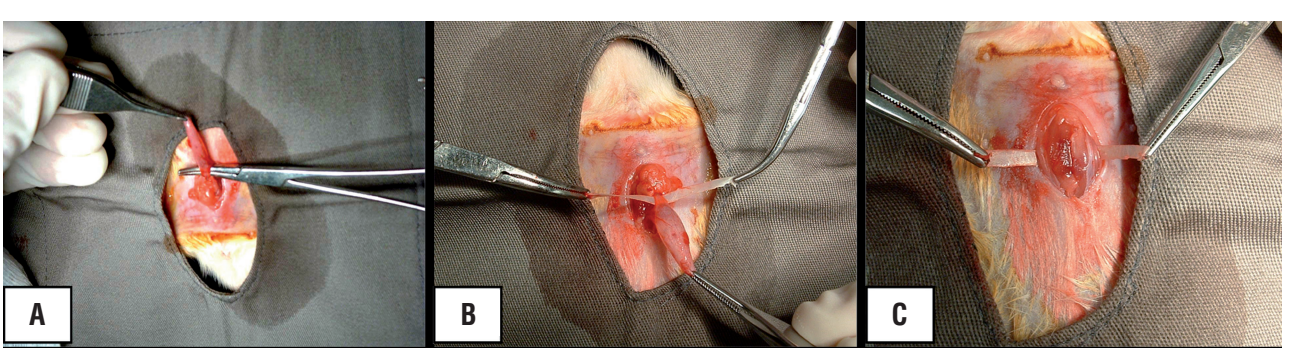


Figure 2 - Demonstration of polypropylene (A) and CEC (B) in situ at 120 days sacrifice. Adequate incorcoporation to the implanted area.

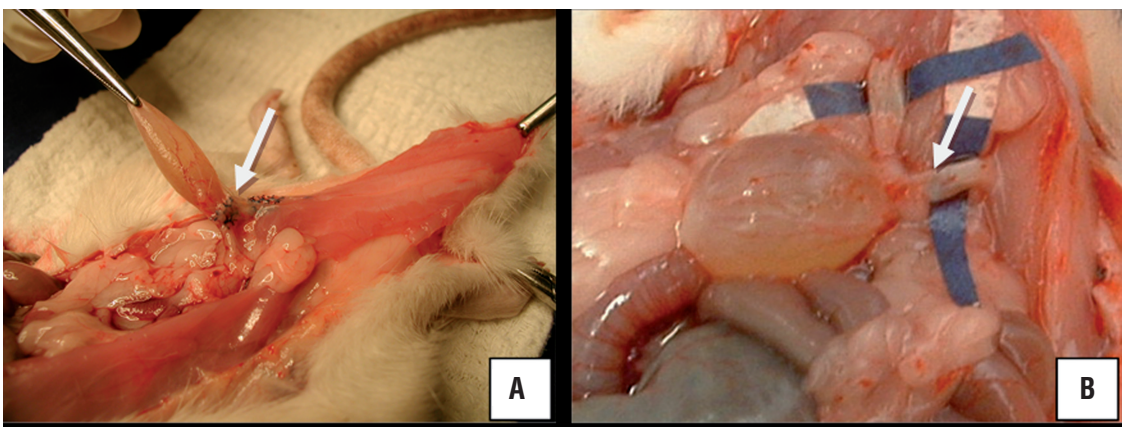
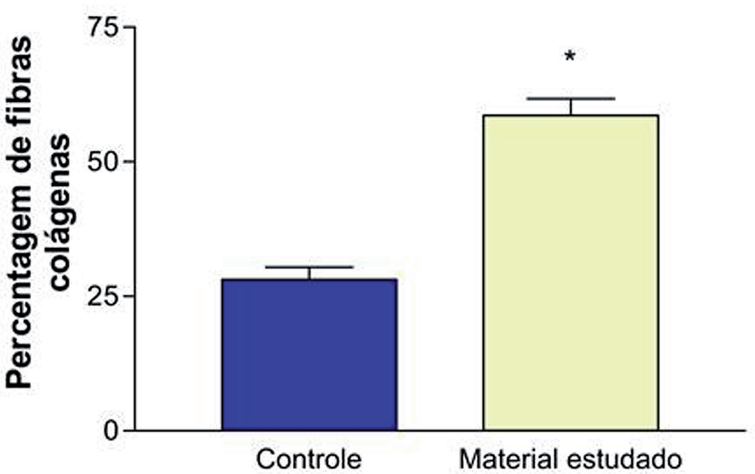


Figure 3 - Analysis of collagen deposition in the suburethral space. Comparison between the biomaterials.



*p<0.05.

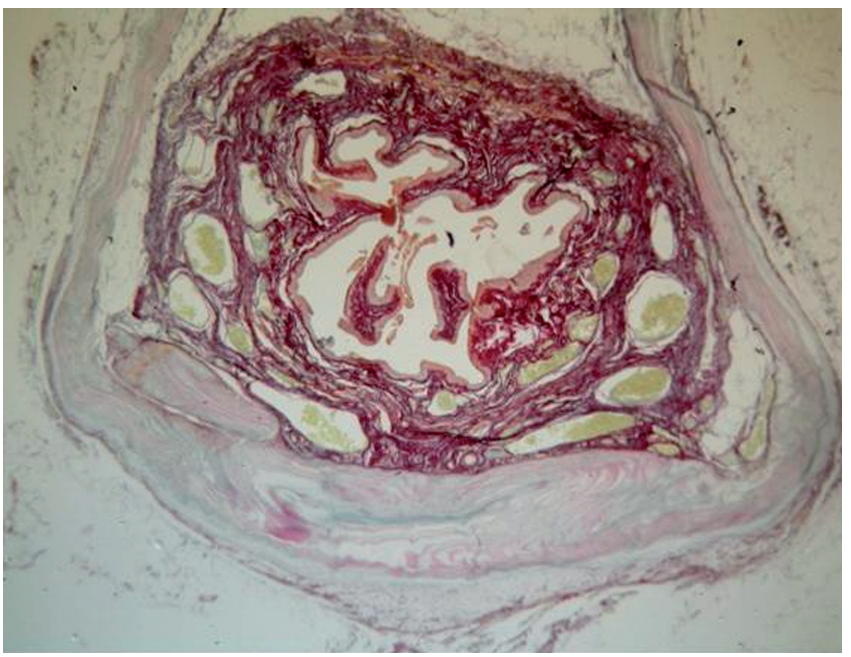
The inflammatory reaction induced by CEC sling showed an intense proliferation of polymorphonuclear cells even in the late phase. Some angiogenesis with discrete neovascularizaton penetrating the new material was found. No capsule formation was found around the CEC implant (Figure-4).

The qualitative analysis concerning inflammation and the degree of necrosis in groups B and D in both suburethral and subaponeurotic regions showed similar results. The polypropylene group showed less inflammatory reaction than CEC in the suburethral area.

DISCUSSION

The inflammatory reaction from a synthetic material in an animal model represents an important step in the evaluation of its future use in clinical trials. Several materials have been used as

Figure 4 - CEC Sling in the suburethral space (red arrow) (HE, 40X).



slings in the treatment of SUI and at the present polypropylene mesh is incorporated to various industrial mechanisms that are currently in clinical use (3, 5, 6, 9). The main reason to study a new material such as CEC is its physical properties and biocompatibility as well as the fact of being produced from a renewable material such as sugarcane sub product.

The cellulosic exopolysaccharide (CEC) has already been tested concerning cytotoxicity which has been evaluated through the adhesion index, nitric acid production and cellular viability of alveolar macrophages. These tests have been done in rats and no toxic reaction was found in the cell culture. There was also a high compatibility level in three cytotoxicity studies (10) which follow the recommendations of the Food and Drug Administration (FDA) that states that implantable medical devices and their constituents should not produce adverse reactions both locally and systemically, have no carcinogenic effect and produce no harm to the reproductive system and development (11).

CEC presents satisfactory physical properties such as elasticity, resistance to traction and flexibility allowing easy modulation in different shapes including the possibility of using as a sling. In an independent study in rats polypropylene mesh and CEC were implanted into the peritoneum and demonstrated that there was incorporation of both bio prosthesis without the presence of exudates and fistulas (12).

There is growing concern among surgeons using different types of industrial materials specially related to the risks of erosion, infection and biocompatibility status (5, 6, 13). In the early phase of the present study it was observed a high level of granulocytes infiltration as well as the presence of fibroblasts. These findings represent typical aspects of tissue repair involving the vascularization process. In this phase there is activation of the coagulation, platelets aggregation that involves its invasion by neutrophils, monocytes, macrophages and fibroblasts. This phenomenon is known as chemotaxis (13). The centripetal migration of endothelial cells in the area of the CEC implant interferes in the angiogenesis process justifying tissue remodeling and the integration of this new material in the suburethral area.

Microscopic analysis of the suburethral area showed a significantly elevated amount of collagen (30.5%) in the group that used CEC implant as compared to the control group. Erosion related to the use of the material was not found. A greater collagen deposition was observed in the suburethral region for both groups of materials when compared to subaponeurotic area.

This significant increase in collagen density is probably related to the interaction between cytokines and cells related to the healing process. Early research conducted to evaluate the biomechanics characteristics of CEC membrane demonstrated that after implantation there is an increase of its resistance to traction associated to the tissue integration. This phenomenon may represent a relevant point in favor of the increase of maintenance of continence rates when in clinical use (14).

In the present study a more intense inflammatory reaction was found at the suburethral area when compared to the control group in the 12 weeks group but this was not associated with urethral erosion.

Tissue reaction of polypropylene sling was studied in three different types of mesh which are different in relation to the structure and size of the pores (15). Other studies emphasize the role of these pores in allowing the filling with connective tissue, migration of immunocompetent cells and angiogenesis (16). Macroporous materials present greater molecular permeability and consequently allow quicker fibrinous fixation. They work as a biologic glue preventing the accumulation of secretions (2).

Histological analysis demonstrates a decrease in inflammatory reaction and fibrosis when a larger porous material is used as a sling. On the other hand lower porosity materials tend to facilitate capsule formation. This phenomenon did not occur with the CEC membrane.

More recently, small intestine submucosa (SIS) has been introduced as an option to be used as a sling in the treatment of SUI. This material is an acellular matrix that is produced from the intestinal submucosa of pigs. It is composed of collagen, growth factors, glycosaminoglycan and glycoprotein. The porosity is described as microscopic (17, 18).

Due to its composition SIS is infiltrated by the host cells that quickly proliferate and result in regeneration of local tissue in a well organized way. The authors concluded that there was complete absorption of the sling material due to the exceptional biocompatibility (18). Incontinence recurred in all patients probably due to the material absorption and loss of urethral support. Although no clinical study has proven this idea it is theoretically hypothesized that materials to be used with this purpose must stay in situ for a minimum of 12 weeks.

CEC was not affected by the degradation process and no acute complications such as abscess formation or extrusion or fistulas related to the implantation of this material. We believe that the presence of collagen coating the suburethral mucosa may be responsible for the preservation of this tissue architecture and viscoelastic characteristics (12, 14). This suburethral support probably reduces the risks of erosion which is one of the main complications associated with the use of synthetic slings.

It is important to emphasize that the objective of the present research was not to evaluate the functional properties of the CEC implant since no tension was applied over it. The absence of suburethral erosion and reduced inflammatory reaction as well as the tendency for incorporation in the long term as demonstrated by the angiogenesis process may be the most important features to be taken in account.

CONCLUSIONS

The CEC implant was shown to be appropriate when used as a suburethral sling in rats. Adequate integration to the host tissue and preservation of its architecture were the main findings of the study. The utilization of CEC may be considered as an ideal material to be used as a sling but the impact of its use in clinical practice still has to be tested.

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CONFLICT OF INTEREST

None declared.

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Contemporary Series of Robotic-Assisted Distal Ureteral Reconstruction Utilizing Side Docking Position

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ABSTRACT

Purpose: The robot-assisted approach to distal ureteral reconstruction is increasingly utilized. Traditionally, the robot is docked between the legs in lithotomy position resulting in limited bladder access for stent placement. We examined the use of side docking of the daVinci robot® to perform distal ureteral reconstruction.
Materials and Methods: A retrospective review of distal ureteral reconstruction (ureteral reimplantation and uretero-ureterostomy) executed robotically was performed at a single institution by a single surgeon. The daVinci robotic® Si surgical platform was positioned at the right side of the patient facing towards the head of the patient, i.e. side docking.
Results: A total of 14 cases were identified from 2011-2013. Nine patients underwent ureteral reimplantation for ureteral injury, two for vesicoureteral reflux, one for ureteral stricture, and one for megaureter. One patient had an uretero-ureterostomy for a distal stricture. Three patients required a Boari flap due to extensive ureteral injury. Mean operative time was 286 minutes (189-364), mean estimated blood loss was 40cc (10-200), and mean length of stay was 2.3 days (1-4). Follow-up renal ultrasound was available for review in 10/14 patients and revealed no long-term complications in any patient. Mean follow-up was 20.7 months (0.1-59.3).
Conclusion: Robot-assisted laparoscopic distal ureteral reconstruction is safe and effective. Side docking of the robot allows ready access to the perineum and acceptable placement of the robot to successfully complete ureteral repair.

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Video-Assisted Surgery; Ureter; Reconstructive Surgical Procedures

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INTRODUCTION

Laparoscopic techniques for ureteral reimplantation and reconstruction continue to grow. The technique and efficacy of the laparoscopic ureteral reimplantation has been well described (1-3). However, creating a non-refluxing ureteral reimplantation laparoscopically is technically very difficult, and has translated into poor adoption of the technique. With the introduction of the daVinci Surgical System® (Intuitive Surgical, Sunnyvale, CA) minimally invasive surgery has now allowed surgeons to accomplish increasingly complex procedures with a shorter learning curve and better efficacy (4).

Classically, the position of a robotic-assisted laparoscopic ureteral reimplantation is described by placing the patient in lithotomy position followed by steep Trendelenberg position and then the robot is docked between the patient's legs.

This position, however, results in limited access to the bladder for retrograde placement of a ureteral stent. Previously, side docking of the daVinci® robot has been described for various gynecologic surgeries (5, 6) as well as for performing a radical prostatectomy (7). We present an alternative docking position which simplifies surgical set-up, allows ready access to the bladder for stent placement and may ultimately lead to shorter operating time without compromising surgical technique.

MATERIALS AND METHODS

Retrospective chart review was performed on all patients of the senior author's who underwent robotic assisted laparoscopic ureteral reconstruction (i.e. ureteral reimplantation and uretero-ureterostomy) utilizing a side docking position.

Preoperatively, all patients were evaluated with retrograde pyelograms, except those with vesicoureteral reflux (VUR) who were imaged with voiding cystourethrograms. Preoperative management included ureteral stenting or nephrostomy tube placement for patients with ureteral injury or stricture, and observation or deflux in patients with VUR. Routine preoperative labs, including serum creatinine and urinalysis, were obtained in all patients.

All operations employed the daVinci robotic Si surgical platform® (Intuitive Surgical, Sunnyvale, CA), with the robot docked on the patient's right side parallel to the operative Table, e.g. "side docked" (Figures 1 and 2) (8). The patient was positioned in dorsal lithotomy position atop a memory foam pad to resist sliding, and legs were placed in yellow fin stirrups. The patient was then placed in a Trendelenberg position. The trocar placement did not differ significantly from traditional docking positions; we utilized an umbilical port for camera placement and the robotic ports were placed 8 to 10cm apart and triangulated about the camera port with adjustments made to avoid the anterior superior iliac spine.

Ureteral reimplant performed for VUR utilized the non-refluxing Lich Gregoire method (9). The ureter was identified and dissected towards the bladder until its attachment to the bladder was visualized. The detrusor muscle was divided from mucosa. A suture was used to advance the ureteral orifice caudally towards the bladder neck. The ureter was then tunneled atop the bladder mucosa and the muscle closed atop the ureter. Ureteral reimplant executed for ureteral injury, stricture, or

Figure 1 - daVinci robotic Si surgical platform port placement.

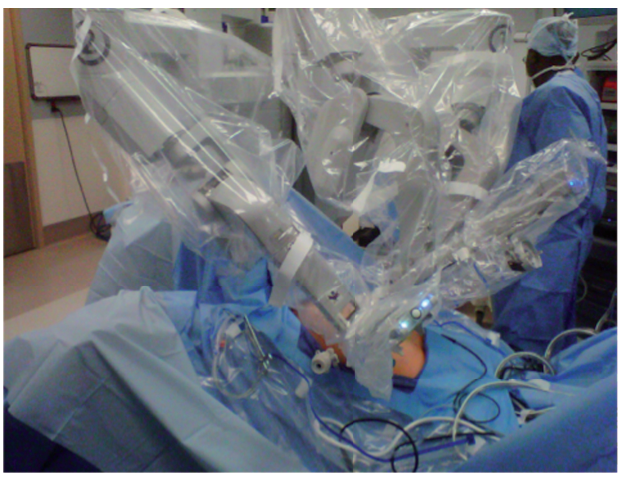
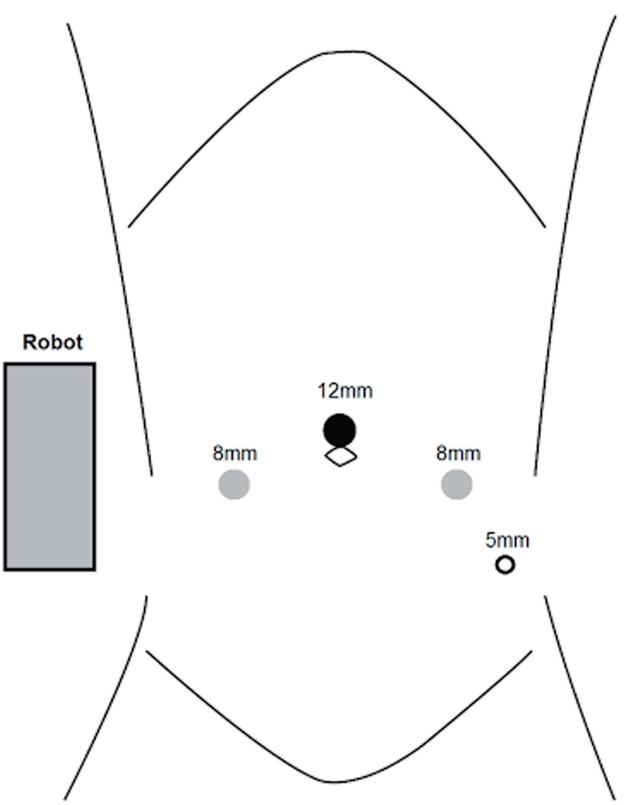


Figure 2 - Side docked position.



megaureter employed the Le Duc technique (10). The ureter was mobilized and introduced into the bladder through a short transmural channel in a nonrefluxing fashion. Distally, the ureteral end was widely spatulated and resulted in a distal ureteral plate that was fixed to the bladder mucosa, while the non-spatulated ureter remained unfixed. In all patients, ureteral stents and urethral Foley catheter were placed in a retrograde fashion during the procedure and Jackson-Pratt drains placed at the end of the operation.

Patients were postoperatively evaluated in the office setting approximately three to six weeks after the operation, with cystoscopy and stent removal. Additional follow-up with in-office renal ultrasound was scheduled at three months after surgery and yearly thereafter to assess the repair.

We collected the following demographic and procedural data from the electronic medical records of all patients: age, gender, body mass index (BMI), American Society of Anesthesiologists (ASA) score, estimated blood loss (EBL), indication for surgery, operative time, time from ureteral injury, laterality of the operation, and intraoperative complications. Operative time was defined as time from start of incision to cessation of anesthesia.

Early postoperative outcomes were also extracted from the electronic medical record, including hospital length of stay (LOS). Postoperative complications were defined using the Clavien grading system (11). Office notes were reviewed for results of in-office renal ultrasound. We assessed change in renal function by comparing preoperative and postoperative serum creatinine (sCr) using a paired-sample Student t test. Statistical significance was defined as $p<0.05$.

RESULTS

From March 2011 to September 2013 a total of 14 patients (13 female, 1 male) with a mean age of 39 years were identified and included in the study group. Indications for the procedure included ureteral injury during primary hysterectomy in 9 patients, vesicoureteral reflux in 2 patients, congenital stricture in 2 patients and megaureter in 1 patient. Demographic data is listed in Table-1.

Operative and postoperative data are listed in Tables 1 and 2. Mean operative time was 286 minutes (189-364 minutes) and mean EBL was 40.0cc (10-200cc). All the procedures were completed by the side-docking method without the need for re-docking. There was one intraoperative complication: a contralateral ureter was erroneously reimplanted and required reoperation and reimplantation of the correct ureter. All surgeries were completed without conversion to open or the need for re-docking. There was a single postoperative Clavien grade I complication (postoperative fever).

Mean length of stay was 2.3 days (1-4 days). Creatinine was available for analysis in 12 of 14 patients. The difference between preoperative and postoperative sCr was not statistically significant (p=68). Follow-up renal ultrasound was available for review in 10 out of 14 patients and demonstrated no evidence of complications in any patient.

DISCUSSION

Ureteral reconstruction can be accomplished using a variety of open procedures and has been described in the urologic literature with excellent long-term outcomes. However, open surgery is associated with more blood loss, postoperative pain, and longer lengths of hospital stay (2). The introduction of the daVinci robotic system® has changed the landscape of minimally invasive surgery. Despite the higher operating costs, longer setup, and loss of tactile feedback of the current robotic system, the benefits of a three-dimensional field of vision, increased degrees of freedom of movement, tremor elimination, and motion scaling make robotic ureteral reconstruction advantageous (12, 13).

Surgical literature has previously reported successful use of robotic side-docking for pelvic procedures, namely for obstetrics and gynecology operations (5, 6). Two urologic series of robotic side docking have been published in the literature, but their cohorts consisted of primarily urologic oncology cases and included only a single patient with ureteral reconstruction (8, 14). We present, to our knowledge, the first series of patients under-

Table 1 - Patient Demographic Data and Operative Data.

Patient	Age, years*	Side	Indication	Preop. Management	Procedure	OT	Postop. Complications	Postop. Imaging	FU
1	45	R	HI	NT	UR	288	None	N	4
2	34	R	MU	Observation	UR, MT	322	None	N	7
3	41	R	HI	Stent	UR, UL	364	None	Y	20
4	37	R	HI	NT	UR, UL	236	None	Y	6
5	22	L	VUR	Observation	UR	241	None	Y	59
6	21	R	VUR	Deflux	UR	224	None	Y	40
7	49	B	HI	NT	UR, UL	362	None	Y	24
8	61	R	HI	NT	UR	350	None	Y	44
9	47	R	HI	Stent	UR, BF	366	None	Y	26
10	28	L	Stricture	Stent	UU	189	None	Y	23
11	37	L	HI	Stent	UR, UL, BF	328	Fever	Y	12
12	64	L	Stricture	Stent	UR, UL, BF	251	None	Y	24
13	35	L	HI	NT	UR	235	None	N	0
14	25	R	HI	NT	UR	254	None	N	0

*Data include age (years); side of reconstruction (**R =** right; L = left; **B =** bilateral); indication for ureteral reconstruction (**HI =** Hysterectomy injury; **MU =** megaureter; **VUR =** vesicoureteral reflux), preoperative management (**NT =** nephrostomy tube), operative procedure (**UR =** ureteral reimplantation; **MR =** megaureter tapering; **UL =** ureterolysis; **UU =** ureteroureterostomy; **BF =** Boari Flap), operative time (**OT =** minutes), postoperative complications, postoperative imaging (**Y =** yes; **N =** No), and duration of follow-up (FU = months)

going robotic assisted ureteral reconstruction with a side docking position.

Our results suggest several important findings. First, our case series demonstrates that side docking of the robot is comparable in operative time to other studies using the conventional docking approach (15, 16). Specifically, our mean operative time was 286.4 minutes which is similar to the 221 minutes reported in the largest case series of conventionally docked robotic distal ureteral reconstructions (16). Of note, our operative times include the entire duration of surgery, not just robotic console time, and may account for some of the disparity between our operative time and the literature time. In addition, many of our patients had additional concurrent procedures performed (e.g. ureterolysis and Boari flap) and nearly all had previously undergone abdominal surgery with subsequent formation of adhesions, both of which prolong operative times. Our mean length of stay of 2.3 days was also similar to the literature means of 1.6 to 2.5 days using conventional docking, while our mean EBL of 40cc was also on par with means of 50cc to 171cc quoted in the literature (16, 17). Our single postoperative complication a Clavien I postoperative fever–and the absence of any long-term complications (as assessed by ultrasound and office evaluation) demonstrates the short and long-term safety of the repair. Additionally, the side-docking of the robot affords the safety advantage of requiring less abduction of the patient's legs, as the robot is no longer in that potential space. No patients in our series suffered from peroneal nerve injury or any musculoskeletal positioning complications. In patients with a history of hip surgery or muscle contractures, side--docking of the robot is an excellent, safe alternative to the traditional docking approach. Overall, our results suggest that the side-docking approach is safe, effective, and comparable to the conventional docking approach.

Robotic ureteral reconstruction with intracorporeal double J ureteral stent placement has been well described but poses several challenges (18, 19). First, confirming stent placement intracorporeally is often difficult and stent migration

Table 2 - Preoperative, Operative and Postoperative Data.

Patient Variables	
Age (years)	39.0±13.3
Female gender, n (%)	13(93)
BMI (Kg/m2)	26.9±7.8
ASA Score	2.1±0.3
Preop. sCr (mg/dL)	
Mean	0.9±0.2
Range	0.6-1.2
Operative Variables	
Mean Estimated blood loss (cc)	40 (10-200)
Mean Operative time (min)	286 (189-364)
Intraoperative complications, n (%)	1 (7.2)
Postoperative Variables	
Hospital stay (days)	2.3 (1.0-4.0)
Postop. Complications, n (%)	
Grade I-II	1 (7.2)
Grade III-V	0 (0)
Postop. Transfusion, n (%)	0 (0)
Postop. sCr nadir (mg/dL)	
Mean	0.9±0.2
Range	0.5-1.3
Mean time to stent removal (days)	49 (26-82)
Mean follow-up (months)	20.7 (0.1-59.3)

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

has been described as a complication (20). In our series, we had no stent migration, which may be attributed to the excellent visualization appreciated in typical ureteral stent placement. The direct access to the lithotomy position afforded by the side-docking position gives the assistant the opportunity to place a stent using a rigid rather than flexible cystoscope and therefore a better field of vision. The ability to easily place retrograde stents also allows for visual confirmation of both the patency of the ureter and a good curl of the distal end of the stent. Another challenge to conventionally docked robotic ureteral reconstruction is that intraoperative stent placement may be cumbersome with the robot blocking urethral access and requiring undocking of the robot or repositioning of the patient. Conversely, side docking of the robot allows easy cystoscopic access to the bladder for retrograde stent placement, especially when the ureter has been completely transected and the injury is managed via a nephrostomy tube.

There are several limitations to this study. First, its retrospective nature and relatively small sample size from a single surgeon introduce a possible selection bias. Nevertheless, this preliminary data may prompt other surgeons to adopt the side-docking approach to ureteral reconstruction and generate additional, larger studies of the approach. Second, we were unable to obtain the same surgeon's data for comparison with the conventional docking approach. Third, postoperative imaging was unavailable in 4/10 patients, making it harder to determine the true success rate of the operation. However, two of those four patients did receive in-office follow-up at 4 and 7 months postoperatively and neither had evidence of complications on evaluation. Moreover, 10/14 patients had extended follow-up with imaging and none had any long--term complications. Finally, this case series had a wrong site intraoperative complication. The error is attributed to the patient's prior abdominal surgery, which caused such extensive fibrosis that the contralateral ureter was shifted to the intended side and was mistaken for the right ureter. The patient's correct ureter was subsequently reimplanted with no short or long-term postoperative complications.

CONCLUSIONS

Side docking of the robot during robotic assisted laparoscopic ureteral reconstruction of the distal ureter confers some benefit over the conventional docking approach, as the surgeon has unrestricted, ready access to the perineum for retrograde stent placement without undocking the robot or repositioning the patient. This approach is also safe and effective in terms of operative time, length of stay, and EBL comparable to literature values of the conventional docking approach.

CONFLICT OF INTEREST

None declared.

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Serum interleukin -8 is not a reliable marker for prediction of vesicoureteral reflux in children with febrile urinary tract infection

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ABSTRACT

Objective: In view of the side effects of voiding cystourethrography (VCUG), identification of noninvasive markers predicting the presence of vesicoureteral reflux (VUR) is important. This study was conducted to determine the predictive value of serum interleukin-8 (IL-8) in diagnosis of VUR in children with first febrile urinary tract infection (UTI).

Materials and Methods: Eighty children with first febrile UTI were divided into two groups, with and without VUR, based on the results of VCUG .The sensitivity, specificity, positive and negative predictive value positive and negative likelihood ratio, and accuracy of IL-8 for prediction of VUR were investigated.

Results: Of the 80 children with febrile UTI, 30 (37.5%) had VUR. There was no significant difference between the children with and without VUR and also between low and high-grade VUR groups in terms of serum concentration of IL-8 (P>0.05). Based on ROC curve, the sensitivity, specificity, likelihood ratio positive, and accuracy of serum IL-8 was lower than those of erythrocyte sedimentation rate and C-reactive protein. Multivariate logistic regression analysis showed significant positive correlation only between erythrocyte sedimentation rate and VUR.

Conclusions: This study showed no significant difference between the children with and without VUR in terms of the serum concentration of IL-8. Therefore, it seems that serum IL-8 is not a reliable marker for prediction of VUR.

ARTICLE INFO

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Vesico-Ureteral Reflux; Interleukin-8; Urinary Tract Infections

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INTRODUCTION

Urinary tract infection (UTI) is a common disease in children. Its prevalence in male and female children is 1% and 3-8%, respectively (1). Urinary tract infection occurs in three forms: acute pyelonephritis, cystitis, and asymptomatic bacteriuria. Acute pyelonephritis is the most severe type of the disease. The lack of early diagnosis and appropriate treatment result in dangerous complications, such as renal scarring (2, 3). The prevalence of renal scarring is 18-49% (2, 4-7).

The identification of risk factors in UTI is very important. The vesicoureteral reflux (VUR) is

the most important risk factor of UTI. It corresponds to the retrograde flow of urine from the bladder to the ureter and, in some cases, to the pelvis and kidneys (2, 3). The prevalence of VUR in the first UTI is reported between 31.1-37.4% (8, 9). Unlike previous protocols, most current protocols advice towards a more selective use of voiding cystourethrography (VCUG) in children with first UTI, especially in children beyond infancy (10-12). Although VCUG can diagnose VUR, it is painful, invasive, and expensive and also iatrogenic UTI may ensue. Meanwhile, this method exposes children to radiation that may destroy gonads (13).

In view of the side effects of VCUG, lack of VUR in more than 50% of children with UTI, and spontaneous low-grade VUR recovery, researchers have been looking for cost-effective non-invasive predictors of VUR. Sun and et al. believed that serum procalcitonin is a suitable non-invasive marker for predicting VUR in children with UTI (14). We hypothesized that serum interleukin-8 is capable of predicting VUR in children with febrile UTI. Interleukin-8 is one of inflammatory cytokines that plays an important role in dealing with bacterial infections (15). Interleukin-8 was found to be elevated in the urine of children with infection or inflammation, so if a more intensified inflammatory response in children with VUR and UTI is regarded, then one could hypothesize a potential role of IL-8 not only locally (urothelium) but also at systematic level (15). The present study was conducted to determine the predictive value of serum IL-8 in diagnosis of VUR in children with the first febrile UTI.

MATERIALS AND METHODS

This prospective cross-sectional study examined 80 children less than 12 years old diagnosed with first febrile UTI, in Qazvin's Children's Hospital affiliated to Qazvin University of Medical Sciences, Iran, in 2012-2013. The sample sized was calculated based on P=88% (sensitivity for urine IL-8), 1-P=12%, d=0.08, α=0.05 and 1-α=0.95 (15) and using the following equation:

$$n = \frac{\left(Z_{1-\alpha/2}\right)^2 P(1-P)}{d^2}$$

The consecutive sampling was employed to achieve the required sample size.

The inclusion criteria for children with febrile UTI were as follows: 1-presence of clinical symptoms, such as fever, poor feeding, anorexia, vomiting, and restlessness during urination in infants; and fever, vomiting, abdominal and flank pain, dysuria, and frequency in children; 2-abnormal urinalysis (leukocyturia, a positive nitrite test, etc.); 3-positive urine culture (the presence of more than 105 microorganisms of one kind per cc of urine using midstream or clean catch method, the presence of more than 104 microorganisms per cc of urine using catheterization method, or the presence of even one organism in the urine sample using suprapubic method) (2, 3); 4-the first UTI; and 5-undergoing VCUG. The children with: 1-lack of fever; 2-experiencing UTI more than once; 3-using antibiotics before admission, 4-accompanying and underlying diseases; 5-failure to undergo VCUG, and 6-suffering structural abnormalities of the urinary system (such as UPJO, neurogenic bladder, etc.) except VUR were excluded. Prior to the beginning of the antibiotic treatment, serum samples were send to the laboratory in order to test white blood cell count, neutrophil count, platelet count, erythrocyte sedimentation rate, and C-reactive protein quantitative level. The tests were performed in the laboratory of the children's hospital. To measure serum IL-8, 4 cc of blood was drawn from patients' peripheral vein. The serum was removed after centrifuging samples at 3000 rpm and 4°C for 5 minutes. The serum was poured into acid-washed tubes and kept at--20°C until the test was performed. The serum IL-8 was measured using enzyme-linked immunosorbent assay (ELISA) method and AviBion Human IL-8 ELISA kit (Catalog no. IL08001, Orgenium Laboratories Business Unit, Orgenium Company, Finland). Based on the results of VCUG, the patients were divided into two groups: with VUR and without VUR. The diagnosis and grading of VUR were done according to International Study of Reflux in children (16). Grades 1 and 2 were regarded as low-grade VUR, and grades 3, 4, and

5 were regarded as high-grade VUR (17). Renal ultrasound was performed within the first 48 hours of admission. VCUG was performed at the end of the treatment when patients were discharged from the hospital. The 99mTc-dimercaptosuccinic renal scan was done in the first week of admission (3, 4). Ultrasound and VCUG were carried out by a radiologist, and renal DMSA scan was performed and interpreted by a nuclear medicine specialist. All patients were studied under similar conditions. Any report of hydronephrosis or hydroureteronephrosis without evidence of mechanical obstructions, such as UPJO, UVJO, and PUV, in the renal ultrasound, and any report of reduced uptake on the ^{99m}Tc-dimercaptosuccinic renal scan for pyelonephritic changes in the kidneys were considered as suspicion for VUR (2, 3). The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), likelihood ratio positive (LRP), likelihood ratio negative (LRN), and accuracy of the clinical, laboratory, and imaging findings were determined for diagnosis of VUR. The data were analyzed using chi-square test, t-test, nonparametric tests (Mann-Whitney test), and multivariate logistic regression through SPSS15 software. P values less than 0.05 were considered significant.

ETHICAL CONSIDERATION

The ethics committee of the Research Department in the Qazvin University of Medical Sciences (Project No.320) approved the study. All parents were provided information regarding the research method in simple language. The children were included in the study after their parents agreed and signed the informed consent form.

RESULTS

Of the 80 children with the first febrile UTI, 10 (12.5%) and 70 (77.5%) children were male and female, respectively. Minimum and maximum age of the children were respectively 3 months and 132 months with median±IQR of 18.5±61.75 months. The most frequent symptoms were fever (100%), dysuria (77.5%) and frequency (77.1%). The most frequent microorganism grown in the urine culture was E-coli (91%). Of the 80 children with febrile UTI, 30 (37.5%) had VUR. Ratio of males/females in VUR and without VUR patients were 4/26 and 6/44, respectively (P=0.86). The minimum, maximum and median±IQR of age in the VUR and without VUR patients were 6, 132, 27±73 months and 3, 132, and 17.5±50.5 months, respectively (P=0.21).

The differences between the with and without VUR groups regarding clinical, laboratory and imaging findings are shown in Table-1. No significant difference was observed between low and high-grade VUR groups in terms of clinical, laboratory, and imaging variables (P>0.05). There was no significant difference between with and without VUR groups and also, low and high-grade VUR groups in terms of serum concentration of IL-8 (P>0.05) (Table-1). Based on cut-point values determined using ROC curve, sensitivity, specificity, LRP, and accuracy of serum IL-8 was lower than those of erythrocyte sedimentation rate and C-reactive protein.

Maximum sensitivity, likelihood ratio positive, and accuracy were related to ESR≥31mm/h, and maximum specificity was related to CRP≥43mg/dL (Table-2). Sensitivity, specificity, likelihood ratio positive, and accuracy of serum IL-8≥3.8pg/mL were 70, 32, 1.02, and 46, respectively (Table-2, Figure-1). Multivariate logistic regression analysis of VUR as the dependent variable and WBC count, platelet count, neutrophil counts, ESR, CRP, IL-8, ^{99m}Tc-dimercaptosuccinic renal scan and other variables as independent variables revealed a significant positive correlation only between ESR and VUR (95% CI: 1.06-1.54, Beta=0.25, Odds Ratio=1.28, P=0.008).

DISCUSSION

This study showed that serum IL-8 is not a reliable marker for prediction of VUR. The importance of diagnosing VUR is definitely clear for preventing renal damage in children (2, 3). Different methods are used to identify VUR, including VCUG, direct radionuclide cystography, radionuclide cystography, voiding uro-sonography and magnetic resonance voiding cystography (18, 19). VCUG is considered the gold standard investiga-

Table 1 - Comparison of clinical, laboratory and imaging findings between children with and without VUR.

Variables	VUR positive (n=30)	VUR negative (n=50)	P
Fever ≥ 38 0C	25(83.3) [c]	42(84)	0.93
Abdominal pain	15(50) [c]	8(16)	0.001
Flank pain	11(36.7) [c]	10(20)	0.1
Vomiting	16(53.3) [c]	29(58)	0.68
Frequency	26(86.7) [c]	31(62)	0.018
Urinary incontinence	17(56.7) [c]	10(20)	0.001
Dysuria	18(60) [c]	44(88)	0,004
Urgency	16(53.3) [c]	36(72)	0.09
Anorexia	11(36.7) [c]	26(52)	0.18
Irritability on micturation	1(3.3) [c]	0(0)	0.19
Neutrophil count (/ mm^3)	71.9±8.6 [a]	66.7±11.8	0.04
Platelet count (/ mm^3)	368433±82718 [a]	344955±91376	0.26
ESR (mm/h)	39.7±13.8 [a]	23.3±9.3	0.01
CRP (mg/dL)	66.8±16.2 [a]	48.3±21.5	0.01
Urine leukocyte/hpf	24.5(78.2) [b]	22(56.5)	0.71
Urine RBC/hpf	7(9) [b]	12(7.5)	0.058
Urine nitrite positive	22(73.3) [c]	41(82)	0.35
Urine leukocyte esterase positive	10(33.3) [c]	15(30)	0.75
Urine leukocyte cast	19(63.3) [c]	27(54)	0.41
Urine hyaline cast ≥ 1/LPF	10(33.3) [c]	13(26)	0.48
Urine RBC cast	3(10) [c]	3(6)	0.51
Ecoli/Other bacteria	25(83.3) [c]	48(96)	0.052
US findings suggestive of VUR	26(86.7) [c]	25(50)	0.001
DMSA findings suggestive of VUR	27(90) [c]	20(40)	0.01
IL8 (pg/mL)	12.1(122) [b]	33.5(84.6)	0.38

[a] Mean±SD (T-test); [b] Median±IQR (Mann-Whitney test); [c] Chi-square test

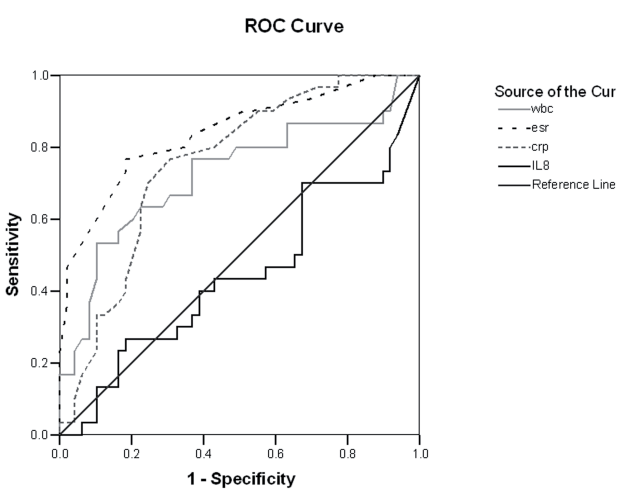
tion for VUR. Unfortunately, this technique has drawbacks. It requires urethral catheterization, which causes pain, risk of infection, and the use of radiation (20).

Due to the complications of VCUG, researchers have been looking for a noninvasive serum marker for predicting VUR (13). The noninvasive serum markers that have been studied are procalcitonin and acute phase reactants (14, 17). According to literature review and as far as our knowledge is concerned the present study is the first one that has surveyed the value of serum IL-8 in

Table 2 - Sensitivity, Specificity, P.P.V and N.P.V Of variables according ROC Curve.

Variables	Sensitivity (%)(95%CL)	Specificity (%)(95%CL)	PPV (%)(95%CL)	NPV (%)(95%CL)	LRP	LRN	Accuracy %
Serum interleukin 8≥8pg/mL	70(53-86)	32(19-44)	38(25-51)	64(45-82)	1.02	0.93	46
WBC count≥14800/mm^3	76(61-91)	64(49-76)	56(40-71)	81(69-93)	2.08	0.36	68
Neutrophil count≥64.5%/mm^3	80(65-94)	39(25-52)	44(31-57)	76(59-92)	1.3	0.51	54
Platelet count≥359000/mm^3	60(42-77)	49(34-63)	44(29-59)	64(48-80)	1.17	0.81	53
ESR≥31mm/h	76(61-91)	82(71-92)	72(56-87)	85(75-95)	4.2	0.28	80
CRP≥43mgdL	90(79-100)	43(28-55)	48(35-61)	87(74-100)	1.55	0.23	60
Urine leukocyte/≥23hpf	57(38-76)	59(43-73)	47(29-64)	68(53-83)	1.39	0.72	58
Urine RBC≥6/hpf	56(32-80)	24(3-43)	41(20-61)	36(7-64)	0.73	1.85	39

Figure 1 - ROC curve for specificity and sensitivity of WBC and CRP and ESR and IL8 measurements. Area under ROC curve for WBC was 0.725 (95% CI 0.601-0.849 P=0.001, for ESR 0.840, 95%CI 0.747-0.934 P=0.0001, for CRP 0.755 95%CI 0.648-0.862 P=0.0001 and for IL-8 0.443 95%CI 0.307-0.579 P=0.396).



prediction of VUR in children with febrile urinary tract infection.

Sun and et al. reported that serum procalcitonin is useful for diagnosing acute pyelonephritis and predicting dilating VUR in young children with a first febrile urinary tract infection. They mentioned that a voiding cystourethrography is indicated only in children with high procalcitonin values (≥1.0ng/mL) and/or abnormalities found on a ultrasonography (14). These results were confirmed in another study (21). Leroy and et al. reported that serum procalcitonin is a sensitive and validated predictor strongly associated with VUR≥3, regardless of the presence of early renal parenchymal involvement in children with the first UTI (21).

Soylu and et al. reported that serum CRP>50mg/l seems to be a potentially useful predictor of VUR and high-grade VUR (17). A few studies have been conducted about the role of urinary IL-8 in prediction of VUR, but the results are contradictory (22-23). In our study, sensitivity, specificity, LRP, and accuracy of serum IL-8 were lower than those of other markers especially erythrocyte sedimentation rate and C-reactive protein. In addition, there was a significant positive correlation only between ESR and VUR.

These findings mean that serum IL-8 is not a good predictor of VUR in children with children with first UTI. The reason may be that IL-8 probably acts mainly locally and not systematically during acute UTI even in more intensified inflammatory status, thus its serum levels are not significantly different. According to previous studies, IL-8 seems to be important at younger ages and the wide age--range of the present study might have affected the results (22, 23). Also, inclusion of a much smaller number of boys may be a potential confounding factor of the results. Interleukin-8 is a chemokine produced by macrophages and other cell types such

as epithelial cells and endothelial cells in response to inflammatory processes (24). A trace amount of this cytokine exists in healthy people's urine (22, 23). Our limitation was lack of measurement of other cytokines such as IL-6.

CONCLUSIONS

This study showed no significant difference between the children with and without VUR in terms of serum concentration of IL-8. Therefore, it seems that serum IL-8 is not a reliable marker for prediction of VUR.

ABBREVIATIONS

UTI = Urinary tract infection
VUR = Vesicoureteral reflux
VCUG = Voiding cystourethrography

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CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Outcomes of Prostate Biopsy in Men with Hypogonadism Prior or During Testosterone Replacement Therapy

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ABSTRACT

Introduction: The relationship between Testosterone Replacement Therapy (TRT) and prostate cancer remains controversial. Most TRT studies show no change in prostate specific antigen (PSA) but some men do have PSA rise or develop an abnormal digital rectal exam (aDRE). Our objective was to examine the biopsy results of men with symptomatic hypogonadism before or during therapy.
Materials and Methods: Data was extracted from our medical record on men with hypogonadism who had a prostate biopsy within the past 4 years done by 3 Urologists with guideline driven practice patterns.
Results: 96 men were identified. Mean age at biopsy was 63 (range 40-85) and median PSA was 3.78ng/dL (0.5-662). Of the 61 men not on TRT, median PSA was 4.34 (0.5 to 662) and mean total testosterone 254 (191-341). There were 29 (47.5%) prostate cancers found (6 Gleason score 6, 13 Gleason score 7, 10 Gleason score 8 or 9). Of the 35 men on TRT, median PSA was 3.27 (0.5 to 13.7). The %PSA increase ranged from 2 to 251% (mean 93.5%). Mean total testosterone was 383 (146-792). Of the 14 men treated < 2 years, none had cancer. Of the 21 men treated 2 or more years 5 had cancer (2 Gleason score 6, 3 Gleason score 7).
Conclusions: Men with hypogonadism and a clinical indication for biopsy often have prostate cancer, many high grade. No men with an initial PSA rise on TRT had cancer. Men on long term TRT should be monitored with PSA and DRE per guidelines.

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Hypogonadism; Testosterone; Biopsy

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INTRODUCTION

The interaction between Testosterone Replacement Therapy (TRT), hypogonadism and prostate cancer remains controversial. While prostate cancer has remained a contraindication in the package insert of TRT formulations, an emerging body of evidence suggests that TRT does not increase the risk of developing prostate cancer (1), is safe following therapy for prostate cancer (2) and may be safe during active surveillance of prostate cancer (3). Current guidelines recommend measuring testosterone prior to starting TRT and age appropriate monitoring of Prostate Specific Antigen (PSA) and digital rectal exam before and during therapy (4). Anecdotal evidence so far in North America is that these guideline recommendations are often not followed (5).

In studies of TRT, mean PSA usually remains stable (6) or rises slightly (7). Nevertheless, individual patients may have a significant jump in PSA after starting TRT which is concerning for prostate cancer and often

prompts a prostate biopsy. We have not found any published studies to date that address how often these patients are found to actually have prostate cancer in this clinical scenario.

AIM

To study the results of prostate biopsy in men with a diagnosis of hypogonadism, comparing the outcomes both prior to and after starting TRT, with an emphasis on men whose PSA rapidly rises following initiation of therapy.

MATERIALS AND METHODS

Under an Institutional Review Board approved protocol we retrospectively extracted data from the Cleveland Clinic electronic medical record of patients with a prostate biopsy done between 2010 to 2014 with a concurrent clinical diagnosis of hypogonadism (CPT 257.2). We focused on patients cared for by three urologists specialized in the evaluation and treatment of men with low testosterone and who all follow common clinical guidelines for diagnosis, treatment and monitoring (4). Initially 125 men were identified. Following individual chart review 29 men were excluded for reasons such as prior prostate cancer, incorrect diagnosis or missing data. This left 96 patients for analysis. Data collected included age, initial PSA, prior PSA, initial morning total testosterone, testosterone after therapy (most recent value prior to biopsy), duration of therapy and pathology of biopsy including Gleason score. Men on testosterone therapy received a variety of agents including injections, topical gels and implantable subcutaneous pellets. All patients had a minimum of 12 cores taken for pathology and graded according to the latest Gleason score. The study period predates our use of MRI-fusion biopsy.

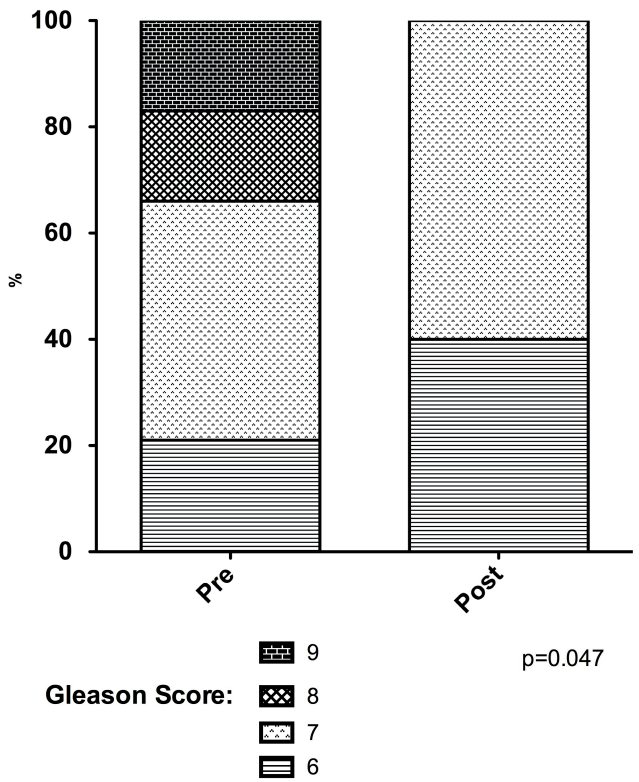
Data was analyzed using Prism 5.0 for Mac (GraphPad). Continuous variables were compared by t test or ANOVA when parametric and Mann--Whitney or Kruskal-Wallis test when non-parametric. Categorical outcomes were compared by the Chi squared test with Fischer correction. All tests were double sided. Statistical significance was set at $P<0.05$.

RESULTS

Overall 96 men were identified. Mean age at time of biopsy was 63±9 years (range 40-85) and median PSA was 3.78ng/dL (0.5-662). There were 61 men with low testosterone who had not yet started TRT who were biopsied before starting therapy due to elevated PSA or abnormal DRE. In this group mean age was 64 years, median PSA 4.34 (0.5 to 662) and mean total testosterone 276ng/dL (191-341). There were 9 men with PSA<2.5 whose indication for biopsy was an abnormal DRE. Of 61 biopsies in men pre TRT, 29 (47.5%) had prostate cancer. Of these cancers there were six Gleason score 6 tumors, thirteen Gleason score 7, five Gleason score 8 and five Gleason score 9. Two men had metastatic disease at presentation. There was no significant difference in testosterone between men with a normal biopsy and those with cancer (257±12.9 vs 250.0±14.9, p=0.72), whether measured in aggregate or separating Gleason score 7 or above (total testosterone 258.6±8.2). By contrast, the PSA was significantly lower in those with a normal biopsy vs cancer (median 3.4 vs 5.0, p<0.0001 by Mann-Whitney) driven by high PSA values in those with high grade disease (Gleason score 7 or higher median PSA 5.12, p=0.0002 by Kruskal-Wallis with Dunn Multiple Comparison test). For the 32 biopsies without cancer, 11 had prostatic intraepithelial neoplasia and 9 had some degree of parenchymal inflammation.

Of the 35 men on TRT, mean age was 60 years and median PSA was 3.27ng/mL (range 0.5 to 13.7). Nine had PSA<2.5 but were biopsied due to an abnormal DRE. The % increase PSA compared to the prior value ranged from 2 to 251% (mean 93.5%). Mean total testosterone was 383ng/dL (146-792) which was significantly higher than the pre TRT group (P=0.047) (Figure-1). Of the 14 men treated for less than 2 years, none had cancer. Of the 21 men treated 2 or more years there were 5 cancers (two Gleason score 6 and three Gleason score 7) (Figure-2). There was no difference between those men on TRT with a normal biopsy vs cancer for testosterone (median 328 vs 388 p=0.58) or PSA (median 3.27 vs 2.76 p=0.81). For the 24 biopsies without cancer, 8 had prostatic intraepithelial neoplasia and 9 had some degree of parenchymal inflammation.

Figure 1 - Comparison of Total Gleason Score Distribution in Patients Prior to or Receiving Testosterone Replacement Therapy.



DISCUSSION

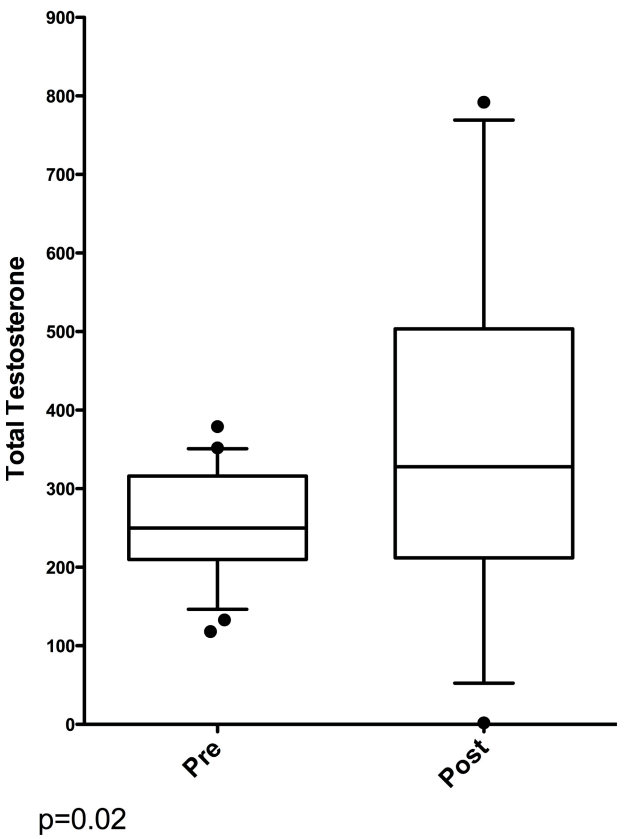
Much current controversy surrounds TRT in men, particularly related to risk of development and progression of prostate cancer. While most prostate cancer cells require testosterone for growth, emerging evidence supports a testosterone "saturation hypothesis" which states that cells require a relatively low concentration of testosterone for maximal receptor engagement and higher exposure does not further promote growth (8). Indeed, some studies have implicated low serum testosterone as a risk factor for the development of prostate cancer, particularly high grade tumors (9). Furthermore, PSA levels either don't rise or rise modestly during long term TRT (6). Indeed, published guidelines do recommend screening men for prostate cancer prior to initiating TRT and then monitoring PSA and DRE during therapy (4). Surprisingly, in one study over half of men on TRT did not have a testosterone or PSA prior to starting therapy and did not have monitoring while on therapy (5).

The genesis of this study was an observation that some men starting TRT had an early sharp increase in PSA but that all biopsies done in response to this rise were negative. When collecting data from our Men's Health registry, we restricted the patients to the practice of three urologists of the staff with a specialty interest in TRT who closely follow treatment guidelines, specifically checking PSA and DRE in age appropriate men before and after therapy. Our findings have clinical implication for patients considering therapy and for those on treatment.

There were 61 men who had low T and had a prostate biopsy due to an abnormal DRE or elevated PSA during workup prior to starting TRT. There were 29 cancers and 10 were Gleeson 8 or 9. An association between low testosterone and high grade prostate cancer has previously been reported (10). Furthermore, low testosterone in men with prostate cancer is associated with higher stage and risk of extraprostatic involvement (11). This finding is troubling in light of the high number of patients who do not have a DRE or serum PSA measured prior to starting therapy (5). Prior to TRT this is a higher risk population and if cancer is not discovered before therapy, the testosterone will likely be blamed when the cancer is subsequently discovered, likely at a high stage and grade.

There were 14 men treated with TRT for less than 2 years who had a biopsy for clinical indication, and all these biopsies were negative for cancer, despite several patients having PSA levels jump by 100 to 250%. Clinical trials of TRT typically have reported no increase in mean PSA over time (6) but the mean can hide individual variation. Coward et al. showed PSA to be stable at 1 year intervals up to 5 years and the earliest patient found to have prostate cancer had been treated for 22 months (1). Gerstenbluth et al. found 6 of 54 patients on TRT whose PSA elevated above 4.0ng/

Figure 2 - Box Plot of Testosterone Levels Before and After Testosterone Replacement Therapy.



mL and 1 patient had a positive biopsy for prostate cancer (7). The reason for this benign jump in PSA in unclear. Indeed one study showed that over 6 months of TRT, there was little change in prostatic testosterone levels or gene expression (12). None of our patients was symptomatic and they did not have category IV prostatitis (13) on their biopsies in proportions that differ from our typical biopsied patients. Based on these limited findings, we wouldn't recommend against a biopsy in a TRT patient with early PSA rise however they may be candidates for other tests that could stratify their risk (eg. PCA3, multiparametric magnetic resonance imaging (14).

There were 21 men on TRT for longer than 2 years who were biopsied and 5 had cancer, although none were greater than Gleason 7. Other long term studies have found men on TRT to develop prostate cancer at a rate similar to those without therapy (15). Furthermore, men who develop prostate cancer while receiving TRT do not appear to have worse outcomes (16). This does emphasize the necessity to follow treatment guidelines and continue age appropriate prostate cancer screening when on TRT.

The primary limitations of this study include: its retrospective nature, lack of exact standardization for timing of tests and interventions, short term follow-up and the relatively small numbers in each group.

In conclusion, we examined the results of prostate biopsy in patients with low testosterone before or after TRT. Patients prior to TRT were at significant risk for aggressive prostate cancer, emphasizing the need for age appropriate screening prior to initiating therapy. None of the men on TRT with an early PSA rise had prostate cancer but prostate cancer did develop in some men after 2 years of therapy. This further supports the majority of evidence that 1 TRT does not cause or promote prostate cancer and 2 safe use of TRT requires regular monitoring with PSA and DRE (when age appropriate).

CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Prospective comparison of ligation and bipolar cautery technique in non-scalpel vasectomy

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ABSTRACT

Objectives: There is no trial comparing bipolar cautery and ligation for occlusion of vas in non-scalpel vasectomy. This study aimed to compare the effectiveness of these vasectomy occlusion techniques.
Materials and Methods: Between January 2002-June 2009, patients were allocated in alternate order. We recruited 100 cases in cautery group and 100 cases in ligation group. Non-scalpel approach was performed during vasectomy and fascial interposition was performed in all cases. First semen analysis was done 3 months after vasectomy. Vasectomy success was defined as azoospermia or non-motile sperm lower than 100.000/mL.
Results: Four patients from the cautery group were switched to the ligation group due to technical problem of cautery device. Thus, data of 96 patients as cautery group and 104 patients as ligation group were evaluated. After vasectomy, semen analyses were obtained from 59 of 96 (61.5%) patients in cautery group and to 66 of 104 (63.5%) patients in ligation group. There was no statistical significant difference between the two groups in terms of the success of vasectomy (p=0.863).
Conclusion: Although bipolar cautery technique is safe, effective and feasible in non--scalpel vasectomy, it has no superiority to ligation. There was no statistically significant difference in terms of the success and complications between the two groups.

ARTICLE INFO

Key words:
Cautery; Ligation; Vasectomy

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INTRODUCTION

Vasectomy is a popular and effective family planning method today. Vasectomy has two main steps: exposing the vas out of the scrotum and occluding the vas. Conventional, non-scalpel, and percutaneous methods are used for the isolation of the vas (1, 2). The non-scalpel vasectomy technique, which is commonly used for the isolation of the vas, was described in 1974 in China by Dr. Li Shunqiang (3).

There are various methods to occlude the vas when performing a vasectomy, such as the division and excision of a segment, the ligation of the vas with metal clips or suture materials, cauterization of the mucosa of the vas lumen, fascial interposition, and a folding back of the divided vas. A fascial interposition (FI) is the only vasectomy occlusion that was well evaluated in a randomized trial. (4) Sokal et al. (5) demonstrated that adding an FI to the ligation with the suture material and the excision of a 1cm segment significantly reduced the failures by about half based on the semen analysis, from 12.7% to 5.9%.

In the United States, the cautery is the most commonly used method for the occlusion

of the vas (6). However, in low-income countries, the ligation and excision is the most widely used occlusion technique (7). Comparative studies suggested that intraluminal cauterization of the ends of the vas, is more effective than ligation (8-10). However, there is no randomized trial comparing these two approaches, yet (4).

Intraluminal cauterization was the main technique of the trials when an electro-cautery or thermal cautery was used (7, 11, 12). There is no prospective comparative trial with a bipolar cautery and ligation for the occlusion of the vas. Our study aimed to compare the effectiveness of these techniques when both are combined with FI.

MATERIALS AND METHODS

From January 2002 to June 2009, patients were divided and given a number in order of their applications. Odd numbers were given to patients who were included in the ligation group, and even numbers were given to patients who were included in the cautery group. The study was planned with 100 cases in each group. The information about vasectomies was given to the couples before the operation, and their informed consent was obtained. Patients who chose the vasectomy as a family planning method, who agreed to participate in the study, and who did not have a history of a vasectomy, scrotal, inguinal, or pelvic surgeries were included in the study.

The procedure was performed by three experienced surgeons in the Urology Department of Tepecik Research and Education Hospital, a specialized urology clinic. After a local anesthesia was given, the isolation of the vas was performed using the non-scalpel approach (3, 13). In the cautery group, approximately 1cm segment of the vas was excised, and the extremities of both ends of the divided vas were cauterized using a bipolar cautery. In this technique, unlike the classic intraluminal cauterization, only the mucosal mouth of the lumen was sandwiched and fulgurated/cauterized between the two tips of the bipolar cautery from different sides until the lumen was closed. Bipolar cautery was not inserted into the lumen of the vas. In the ligation group approximately 1cm segment of the vas was excised and both ends of the vas were ligated using a silk 3/0. FI was performed on the prostatic end of the vas in all of the patients in both groups (3, 13). Patients were evaluated for complications one week after vasectomy. The first semen analysis was done three months after vasectomy. Vasectomy success was defined as azoospermia or presence of non-motile sperm lower than 100.000/mL (12, 14). Semen analysis was repeated one month later in the patients with motile sperm. If motile sperm was still detected in a repeated semen analysis, these cases were classified as an unsuccessful vasectomy (Figure-1).

The study was approved by the Local Ethics Committee. The chi-squared test was used to assess the differences between the study groups. P value <0.05 was considered significant.

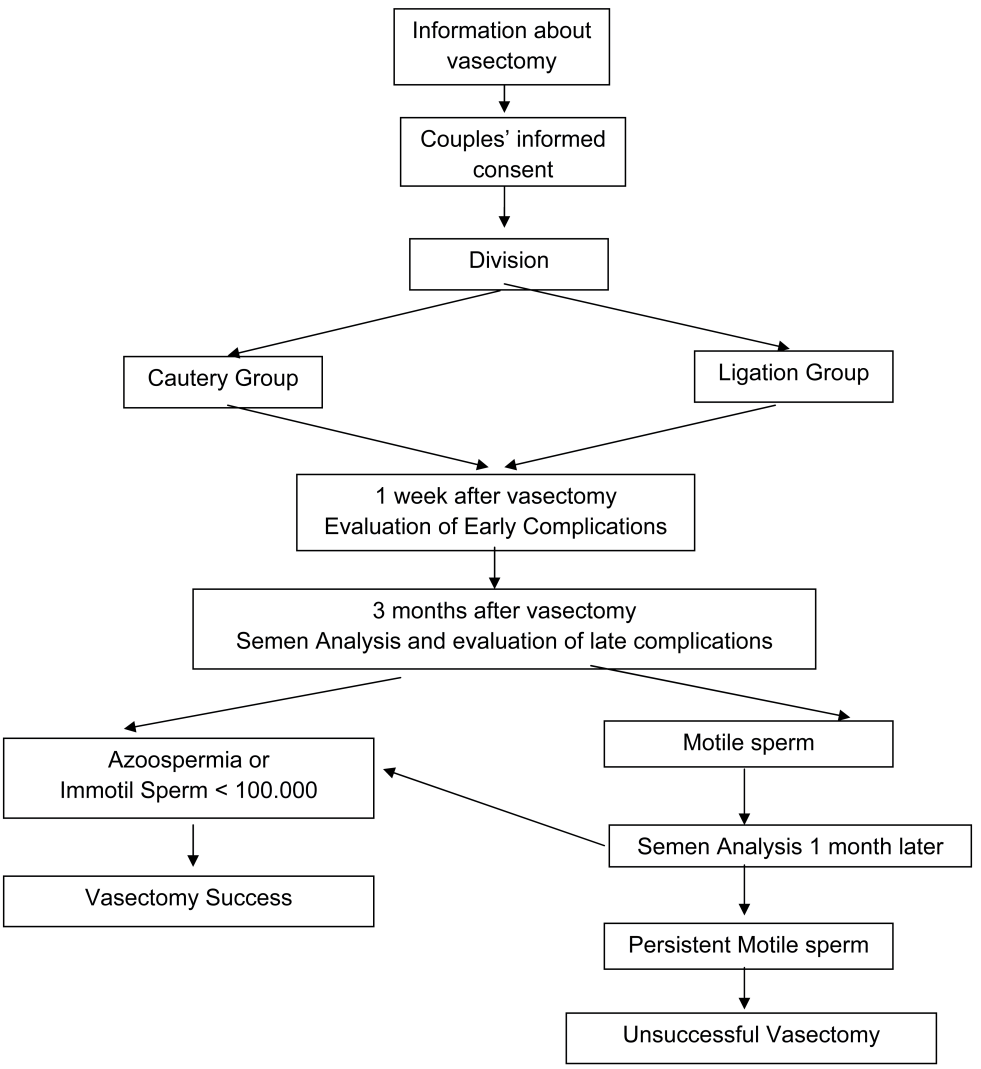
RESULTS

Four patients from the cautery group were switched to the ligation group due to a malfunction of the cautery device. Thus, the data of 96 patients in the bipolar cautery group and 104 patients in the ligation group were evaluated. The characteristics of the patients, including age, duration of marriage, number of children, and education level were similar in both groups (Table-1). Vasectomy was performed without any problem in all of the patients.

After vasectomy, semen analyses were obtained from 59 of the 96 (61.5%) patients in the cautery group and from 66 of the 104 (63.5%) patients in the ligation group. The proportion of patients with successful occlusions was similar in the cautery (55/59, 93.2%) and the ligation (61/66, 92.4%) groups (p=0.863; Table-2). All of the patients in the cautery group who were considered successful were azoospermic. However, four patients had rare non-motile sperm in the ligation group (Table-2). The proportion of patients with presumed recanalization was similar in both groups (7.8% vs. 7.6%, Table-2).

We phoned the 75 patients who did not return for semen analysis after vasectomy and reached 38 patients (21 from cautery group and 17 from ligation group). No pregnancies were reported. Considering this additional information, the

Figure 1 - Study Diagram.



success rate could be estimated as 95% (76/80) in the cautery group and 93.9% (78/83) in the ligation group (p=0.76; Table-3).

Intraoperative complications were not observed in the bipolar cautery group. However, a vasovagal reflex occurred in one patient in the ligation group. Postoperative hematomas were observed in three (3.1%) cases in the cautery group, one with epididymitis requiring antibiotics and one requiring drainage, and none were observed in the ligation group (p=0.07). In the ligation group, no surgical complications were observed, but three patients had psychological problems associated with the vasectomy and emotional distress was observed in these patients (2.8%).

DISCUSSION

Very few studies that address vasectomy occlusion techniques are prospective and even fewer studies are randomized or quasi-randomized (4, 10, 12). The review of the failure rates for the intraluminal cautery technique with FI ranged from 0% to 0.55% (12). According to the American Urological Association (AUA) (12) and the European Association of Urology (EAU)

Table 1 - Characteristics of patients.

Characteristics (Mean ± SD)	Cautery Group	Ligation Group	p
N	96	104	
Age (range)	40.6 ± 5.7 (27-58)	40.8 ± 5.8 (25-55)	0.806
Years of marriage (range)	15.3 ± 5.6 (2-27)	16.0 ± 5.5 (4-32)	0.373
Number of children (range)	2.5 ± 0.9 (1-6)	2.5 ± 0.9 (1-7)	0.999
Educational level n (%)			0.937
No education	0 (0)	1 (1.0)	
Primary	64 (66.7)	68 (65.4)	
High school	22 (22.9)	24 (23.1)	
University	10 (10.4)	11 (10.6)	

SD = Standard Deviation

Table 2 - Semen analysis results.

Group	N	Azoospermia or Immotil Sperm<100.000	Motil Sperm
Cautery	59	55 (93.2%)	4 (6.8%)
Ligation	66	61 (92.4%)	5 (7.6%)
Total	125	116 (92.8%)	9 (7.2%)

Chi-square test p:0.863

Table 3 - If avoiding pregnancy is accepted as vasectomy success.

Group	Azoospermia or Immotil Sperm<100.000	Pregnancy (-)
Cautery	55 (93.2%)	76 (95%)
Ligation	61 (92.4%)	78 (93.9%)

Chi-square test p:0.76

(14) vasectomy guidelines, the most effective vas occlusion technique is the intraluminal mucosal cauterization with FI; however, without FI is also likely to be consistently effective. In our study, we used a bipolar cautery and occluded only the mucosal mouth of the lumen with cauterization instead of intraluminal cauterization. To our knowledge, this is the first prospective trial for a bipolar cautery with FI in a non-scalpel vasectomy. The occlusive failure rate (presumed recanalization) of 6.8% in our study was much higher than those reported in the other studies about intraluminal cautery. In the bipolar technique, unlike the classic intraluminal mucosal cautery method, only the mucosal mouth of the vas was occluded until it seems to be closed. We considered that the results were inadequate because of the insufficient necrosis and subsequent occlusion. The occlusive failure of over 1% is considered unacceptable according to the AUA guidelines (12).

Failure rates for the ligation technique with FI ranged from 0% to 5.85% (12). The only randomized controlled trial that evaluated this technique was reported by Sokal et al. (5). Their reported a failure rate of 5.85%. Our failure rate was also higher in our study (7.6%) in the ligation group.

In our study, vasectomy success was not statistically or significantly different between the study groups. However, our study lacked the statistical power to observe a small difference between the groups. If the bipolar cautery technique with FI had been as effective as the other cautery technique reported in the literature (under 1%), the difference with the ligation group (7.6%) would have been highly significant.

Of all the patients, 75 (37 (38.5%) from the cautery group and 38 (36.5%) from the ligation group) patients did not return for semen analysis after vasectomy. Thomas et al. evaluated the compliance of 1,892 patients after vasectomy and reported that 34% of patients did not return after the procedure (15). Sheynkin et al. also evaluated the compliance of 214 vasectomy-performed patients and detected that 46.2% of the patients did not return for the semen analysis (16). They reported that the rate of noncompliance was independently higher in men with four or more children, smokers, and those with a lower education level (16). These results are similar to our study. We phoned these 75 patients and reached 38 patients (21 from the cautery group and 17 from the ligation group). The main factor of noncompliance was the religious and cultural effect of masturbation. Pregnancy was not detected in any of these patients. If pregnancy was accepted as a vasectomy success, the failure rate of our study would be a little lower (Table-3). In this case, the failure rate (6.1%) in the ligation group of our study would be similar to the literature (5).

In regard to complications, there was no statistically significant difference between the two groups in our study, and the rate of postoperative surgical complications in the cautery group (3.1%) could be attributed to chance or to a lack of statistical power. However, all of the hematomas were related to the vas isolation and not to the occlusion technique. Overall, the rate of hematomas (1.5%, 3/200) in our study is in the range of 1%-2%, which is considered acceptable by the AUA (12).

Although the bipolar cautery technique combined with FI appears to be as safe as the ligation with FI as an occlusion vasectomy technique, the failure rate was much higher than the intraluminal mucosal cautery techniques. In light of these results, we no longer perform bipolar cautery in our clinic and we are considering alternative occlusion techniques to the ligation and FI to improve our occlusion effectiveness.

CONFLICT OF INTEREST

None declared.

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Comparison of Cajal-like cells in pelvis and proximal ureter of kidney with and without hydronephrosis

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ABSTRACT

Objectives: To evaluate effects of Cajal-like cells on human renal pelvis and proximal ureter on peristalsis.
Materials and Methods: 63 patients submitted to nephrectomy due to atrophic non--functional kidney associated with hydroureteronephrosis were included as study group and 30 cases with nephrectomy due to other reasons were included as control group. Samples from renal pelvis and proximal ureters were obtained and sections of 5µ form paraffin blocks of these samples were prepared; layers of lamina propria and muscularis mucosa were examined by immune-histochemistry using CD117 in order to determine count and distribution of Cajal-like cells.
Results: During immune-histochemical examinations of sections, obtained from renal pelvis and proximal ureter of hydronephrotic kidneys by CD117, Cajal-like cells number determined in lamina propria and muscularis propria was statistically significantly lower compared to control group ($p<0.001$). Distribution of Cajal-like cells in renal pelvis and proximal tubulus was similar under examination by light microscope, and also both groups were not different from each other regarding staining intensity of Cajal-like cells by c-kit.
Conclusion: Significantly reduced number of Cajal-like cells in study group compared to control group, shows that these cells may have a key role in regulation of peristalsis at level of renal pelvis and proximal ureter in urinary system.

ARTICLE INFO

Key words:
Interstitial Cells of Cajal; Hydronephrosis; Ureter

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INTRODUCTION

Cajal's interstitial cells have been identified by Ramon Cajal about 120 years ago as primitive intestinal neurons (1). Majority of studies about Cajal cells were conducted in gastrointestinal system. These cells were found to be localized in general within muscle layers and neighbored to nerve plexus. In several studies, Cajal cells were showed to be pacemaker cells controlling peristalsis in gastrointestinal system (GIS) (2). Reduction in number of these cells or anomalies in their distribution are considered to play a role in etiology of certain diseases associated with motility disorder of GIS (3).

Cajal-like cells (CLC) were demonstrated in urinary system firstly in 1999 (4). During animal studies, these cells were determined in rat vas deferens, rat ureter, guinea-pig prostate, guinea-pig urinary bladder and rabbit urethra. In humans,

CLC were demonstrated in ureteropelvic junction (UPJ), renal pelvis, ureter, vesicouretral junction, urinary bladder and urethra (5-9). During their study, Solari and colleagues determined reduced number of these cells at congenital UPJ obstruction and stablish a role in the etiology of congenital UPJ obstruction (5). In another study, these cells were also suggested to be responsible for motility in rat vas deferens (6). Kuzgunbay et al. showed that CLC were found in rat ureter subjected to unilateral distal ureter obstruction, and these cells appeared to be increased during earlier phase of obstruction and then decreased during late phase. Consequently the conclusion was that these cells could be cells regulating motility of ureter (10).

In this study, count and distribution of CLC in renal pelvis and proximal ureter of patients who had undergone nephrectomy due to non-functional kidney associated with hydroureteronephrosis were compared to that of patients submitted to nephrectomy due to some other reasons and without hydroureteronephrosis. Our aim was to show whether CLC are reduced or not in renal pelvis and ureter of completely non-functional kidneys compared to control group. If CLC are significantly reduced in non-functional hydronephrotic kidneys, this would show that CLC are potentially among cells controlling peristalsis in pelvis and ureter.

MATERIALS AND METHODS

Between January 2000 to February 2010, 63 patients with nephrectomy due to non-functional kidney associated with hydroureteronephrosis were included as study group and 30 patients with nephrectomy due to other reasons were included as control group at Başkent University Ankara central and Adana Teaching and Research Hospital. The patients without hydronephrosis whose collecting tubules system were intact with renal function and without systemic metastasis of renal tumors were chosen for control group. The study group was entirely composed of patients who developed hydroureteronephrosis due to ureteral obstruction and stone resulting in atrophic non-functional kidney. Out of the paraffin blocks of the samples obtained from kidney pelvis and proximal ureters, 5µ sections were prepared and the sections were put on slides with polylysine, and in order to determine count and distribution of CLC, their lamina propria and muscularis mucosa layers were examined immune-histochemically using CD117.

As mast cells are also positively stained by CD 117, these cells were discriminated from CLC by using toluidine blue before histopathological examination of CLC. Sections of 5µ were transferred on slides and they were kept within solution of toluidine blue (0.5 g toluidine and 100 mL distilled water, pH3) for an incubation period of 15 minutes following de-paraffinisation process, then they were rinsed by tap water, dried and kept within xylene for 5 minutes. Samples on slides were covered by lamella and examined under light microscope. Mast cells showed granular meta-chromatic staining.

Orange-brown staining was accepted as positive due to chromogen used in microscopic valuation of samples. Five serial sections were examined for each patient. Number of Cajal cells with CD117 positive was counted on lamina propria and muscularis propria of samples of renal pelvis and ureter at 10 HPF (x400) area and the mean was determined. Cells with round nucleus and cytoplasm with intensely granular staining were considered to be mast cells and cells with fusiform cytoplasm and nucleus were considered as CLC.

Statistical analysis

Data analysis was done by SPSS for Windows 11.5. Shapiro Wilk test was used to determine whether distribution of continuous variables was close to normal or not. Descriptive analysis included: mean±standard deviation for age (minimum-maximum); median for cells count (minimum-maximum); and subject number and (%) for nominal variables. Student t test was used to investigate the significance between groups for mean age; and Mann Whitney test was used to investigate the significance of median cell count. Similarity of gender distribution was analyzed by Pearson χ-square test. Results were accepted statistically significant for $p<0.05$.

RESULTS

The mean age was 43.5 (2-72 years) for 63 patients (31 women, 32 men) with nephrectomy due to atrophic hydronephrotic kidney. The mean age was 58.6 (38-82 years) for 30 patients (12 women, 18 men) with nephrectomy due to renal tumor and without hydronephrosis. At microscopic examination, CLC were brown due to chromogen used for immunohistochemical staining. These cells stained by anti c-kit showed radial branched extensions. The distribution of CLC number according to groups is shown on Table-1.

Cajal-like cells count determined on lamina propria and muscularis propria obtained from sections of hydronephrotic renal pelvis by immunohistochemical CD117 examination was statistically significantly reduced compared to control group (Figure-1A). Distribution of CLC in proximal ureter and renal pelvis of both study and control groups were similar under light microscopy and also staining intensity of CLC by c-kit was not different between both groups.

Cajal-like cells determined on renal pelvis lamina propria and muscularis propria in study group and control group are shown in Figure-2.

Table 1 - Number of Cajal-like cells on lamina propria and muscularis propria of renal pelvis and proximal ureter according to groups.

	Cajal-like cell numbers		
	Control Group	Study Group	P
Renal pelvis lamina propria	32 (26-42)	22 (14-28)	<0.001
Renal pelvis muscularis propria	42 (34-64)	26 (15-36)	<0.001
Proximal ureter lamina propria	24 (20-26)	12 (9-20)	<0.001
Proximal ureter muscularis propria	29 (25-32)	17 (10-23)	<0.001

Data are shown as median (minimum-maximum), Mann Whitney U test.

Figure 1 - A) Cajal-like cells count at renal pelvis lamina propria and muscularis propria according to groups. B) Cajal's cell count at proximal ureter lamina propria and muscularis propria according to groups.

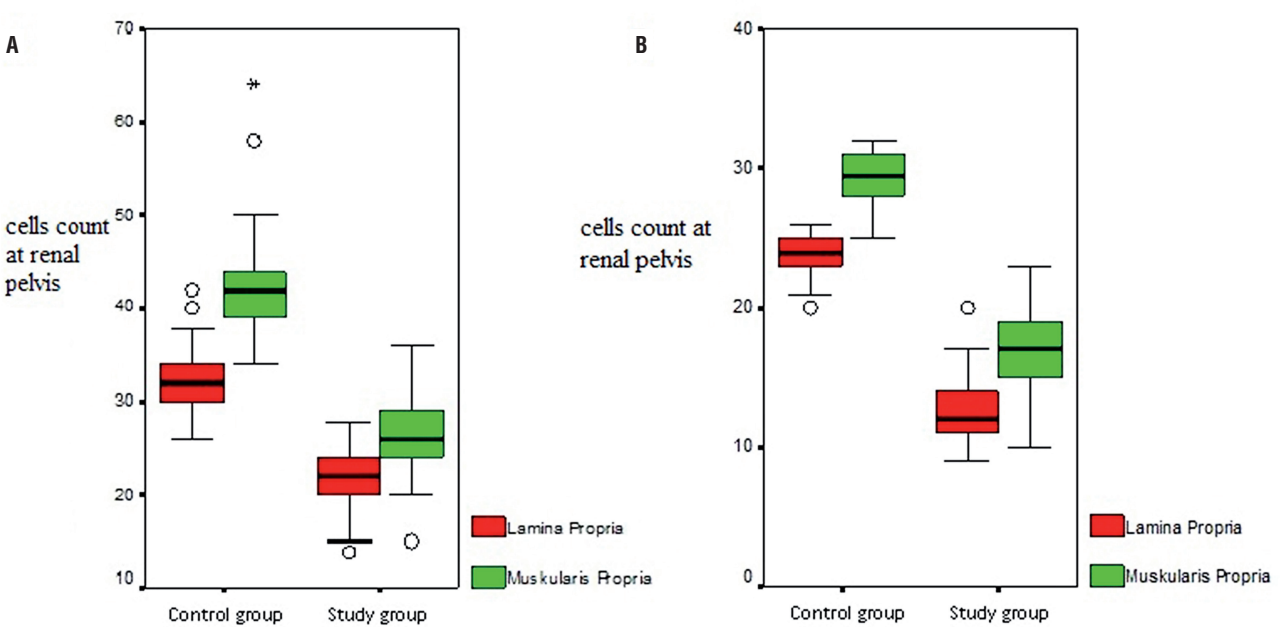
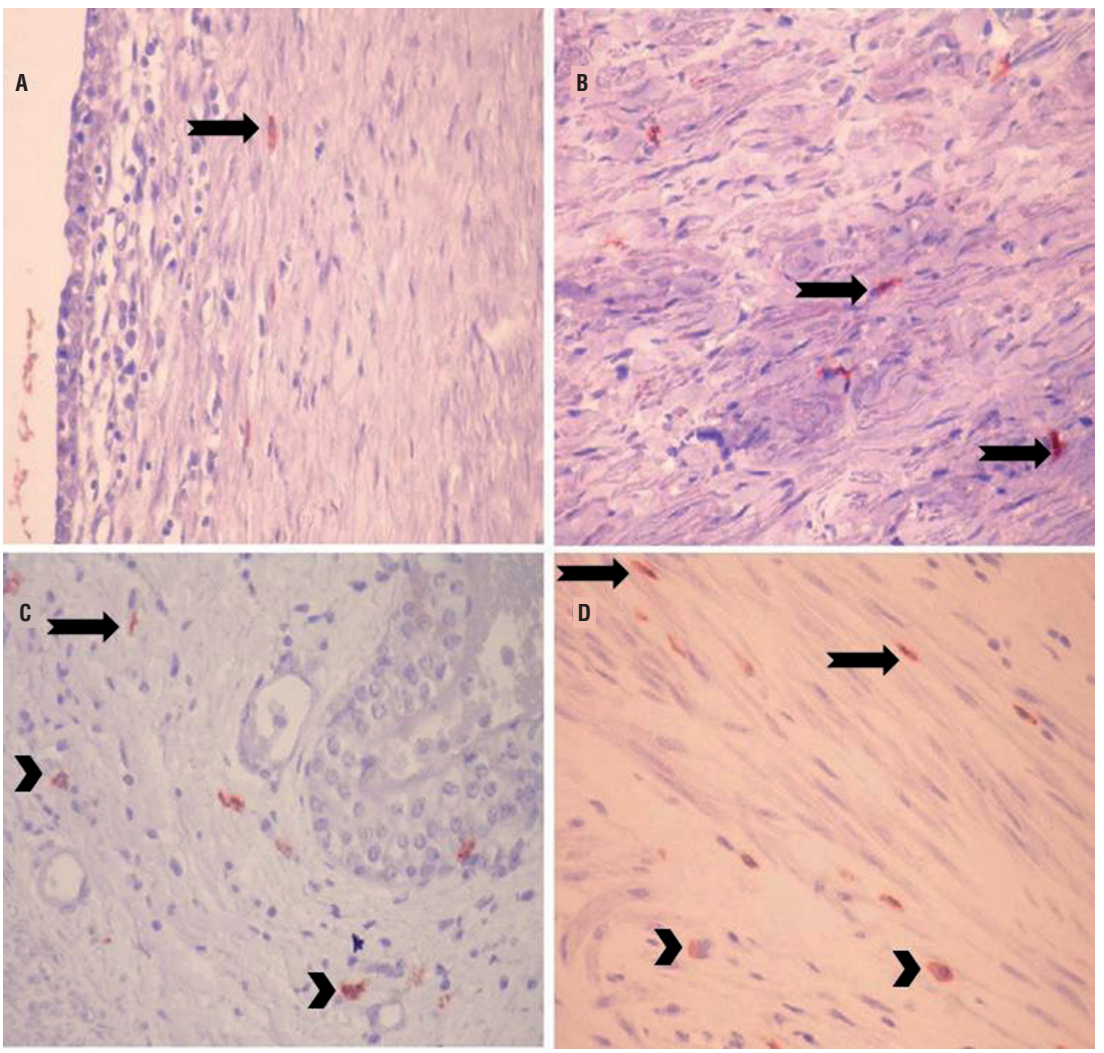


Figure 2 - Cajal-like cells on renal pelvis lamina propria layer of study group (a and b) and control group (c and d) (CD117; x400) (Arrows: Cajal cells, arrowheads: mast cells).



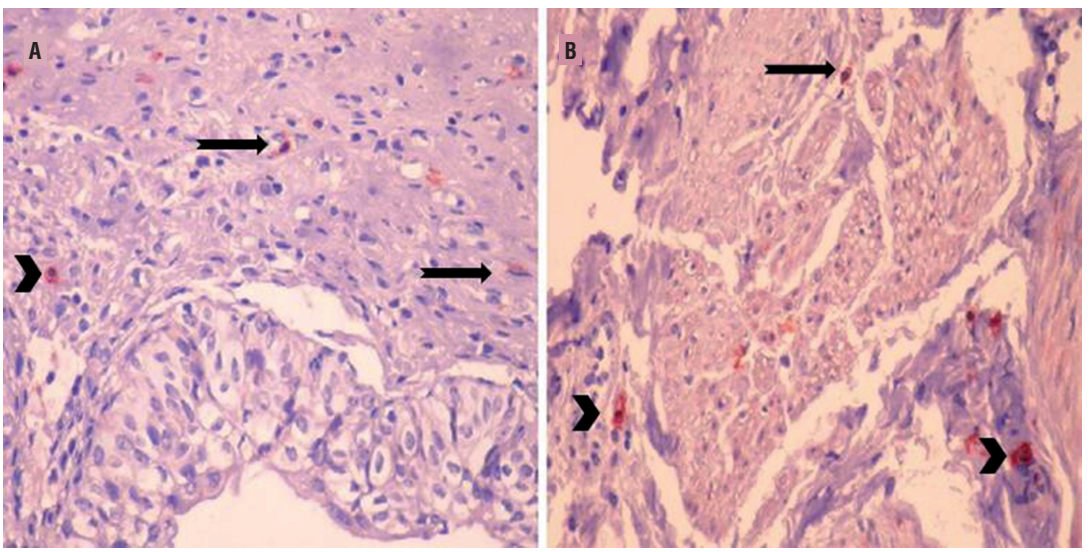
Cajal-like cells count determined on both lamina propria and muscularis propria of sections obtained from proximal ureter was statistically significantly reduced compared to control group (Figure-1B). Cajal-like cells determined on proximal ureter lamina propria and muscularis propria in study group are shown in Figure-3.

DISCUSSION

In this study based on results showing that CLC play a role in regulation of urinary system motility as in gastrointestinal system, significant reduction in number of Cajal cells with CD117 positive staining was shown in layers of lamina propria and muscularis propria of renal pelvis and proximal ureter of patients submitted to nephrectomy due to hydroureteronephrosis. This result indicates that interstitial CLC are also closely associated with peristalsis in urinary system.

Cajal-like cells have been firstly identified as primitive intestinal neurons in 1893, and then they have been associated with neurons and intestinal smooth muscle cells (1). Several studies

Figure 3 - Cajal-like cells on proximal ureter lamina propria layer of study group (a and b) and mast cells (a) (CD117; x400) (Arrows: Cajal cells, arrowheads: mast cells).



showed that these cells are pacemaker cells localized between nerve ending and smooth muscle cells in GIS and responsible of transmitting slow electrical waves necessary for peristaltic movement (2). Additionally, reduced c-kit immune-reactivity and difference in CLC distribution were observed during certain gastrointestinal disorders associated with impaired peristaltic movement such as Hirschsprung's disease, infantile hypertrophic pyloric stenosis, slow-passage constipation (11-13).

In the studies carried out during following years, interstitial CLC were detected in all organs of the urinary system and connection between them and urinary system disorders were tried to be revealed (5-10). Studies on CLC in urinary system suggest that these cells have pacemaker activity especially in tubular organs and that they may induce peristalsis. Although interstitial CLC have been shown in urinary system in the last 13 years, number of studies on these cells in both human and experimental animals is recently increasing. Majority of cases included in these studies are patients with anatomical or functional obstruction on various regions of urinary system or with experimentally induced obstruction. Demonstration of statistically reduced number of these cells at proximal of obstruction and at obstructed segment in gastrointestinal system, especially in cases with intestinal obstruction, compared to control groups, suggests that CLC could also have similar properties within urinary system.

In a study carried out in recent years in order to understand vesicoureteral reflux mechanisms and structural changes they induce in the urinary system, 32 patients with vesicoureteral reflux at varying degrees were compared with 8 control cases regarding presence of intramural c-kit positivity cell and it was determined that collagen stroma replaced smooth muscle bundles and these was remarkable reduced in interstitial cells. It was accepted that interstitial CLC provided automatic rhythm and ureteral peristaltism coordination in ureters and a relation was thought to exist between the loss of interstitial CLC and vesicoureteral reflux and impaired active ureteral valve mechanisms (14). Another study conducted in patients with primary obstructive mega-ureter determined that distribution of interstitial cells stained positively by c-kit was normal in longitudinal and circular muscle layers at dilated segments localized on proximal part of obstruction, but it showed also that there was remarkable

smooth muscle hypoplasia in obstructed segment and number of interstitial cells was reduced or no interstitial cells were present. Absence of CLC in longitudinal muscle layer is explained by the absence of c-kit positive embryological muscle cells precursors (15).

Solari and colleagues determined that human UPJ contained many c-kit positive Cajal cells and their number was very low in obstruction of ureteropelvic junction compared to control group or they were completely absent in UPJ obstruction. In that study, it was claimed that CLC were responsible of ureter peristalsis (5). Kuzgunbay et al. created experimental obstruction in distal ureter of a total of 175 rats and they investigated changes in number and morphology of CLC in UPJ after obstruction. A statistically significant increase was determined in mean number of CLC in study group compared to control group and that these cells decreased during late phase. Authors suggested that the cause of increased number of CLC following first days of obstruction was the differentiation of CLC precursors in response to increased peristaltic activity during early phase. However, the cause of discontinuation of this increase in spite of reduced peristalsis during late phase was associated with prolonged neuromodulator role of CLC in chronic obstruction (10). In our study, statistically significant reduction in number of interstitial CLC stained positively by CD117 was determined at renal pelvis and proximal ureter in patients with nephrectomy due to hydronephrosis compared to control group. In the study of Kuzgunbay and colleagues, CLC number was evaluated after maximal 90 days following obstruction. In our study, changes in CLC number that occurred during years following obstruction were investigated. In the light of the data from these both studies, the study of Kuzgunbay and colleagues may be considered as acute and sub-acute period and our study may be considered as chronic period. It may be concluded that there is an increase of CLC number associated with increased peristaltic activity due to obstruction during early period and also reduction of CLC number associated with loss of peristalsis during chronic period.

Kuvel and colleagues conducted a study to investigate underlying basic histopathology at intrinsic UPJ obstruction and to associate this with surgical treatment results, and they examined the obstructed segment of 32 cases with intrinsic UPJ obstruction; the segment of UPJ with chronic obstruction in 15 cases with nephrectomy due to chronic obstruction associated with lithiasis, tumor and reflux; and lastly they examined also normal ureteropelvic junction segment of 30 patients submitted to nephrectomy due to renal tumor or trauma; then they evaluated CLC in these segments. Cells positive with c-kit for immunohistochemical staining of CD117 monoclonal antibody were generally spread and in rare lining and they were not in regular interaction. These cells were observed to have morphological aspects of CLC cited in literature and were readily discriminated from mast cells showing positive staining. When distribution and density of C-kit positive Cajal cells alterations at intrinsic UPJ obstruction segments were compared to normal ureter segments and chronically obstructed ureter segments, intrinsic UPJ obstruction segments were similar except proximally to surgical area limit and no statistically significant difference was present. However, c-kit positive cells were higher at proximal and distal edge margins of intrinsic UPJ segments compared to obstruction area. According to these results, authors suggested that CLC are responsible of intermediate mechanisms having a role between pacemaker cells and innervations in state of a direct role in pathogenesis of intrinsic UPJ obstructions (16).

When we go over the studies that Solari and Kuvel related to CLC in UPJ junction obstruction, the fact that these cells decrease in cases with UPJ obstruction compared to control group stands out. This result may be interpreted as these cells don't function in functionally or anatomically obstructed UPJ. In our study, proximal ureter and renal pelvic segments without any peristaltic function were evaluated and our findings and results of studies conducted in patients with UPJ obstruction are similar, since in our study number of CLC in muscularis propria and lamina propria of non-functional pelvis and proximal ureter are significantly lower. Therefore according to these results, CLC could be reduced in ureter with loss of motility and their function could also be diminished.

In recent years, studies about localization, number, function of CLC in urinary system and their role in pathophysiology of urinary system disorders are increasing in parallel to advances in pathological and immunohistochemical diagnostic tools. In electron microscopy trials, morphological alterations of CLC in obstructed urinary system may be revealed and if necessary, in association with electrophysiological trials, most of unknown ultrastructural changes of these cells may be demonstrated.

CONCLUSIONS

Neurophysiological trials in association with better understanding of association of structural aspects and peristalsis of CLC in urinary system, will lead to better understanding of function of these cells in urinary system and the development of new treatment modalities for urinary system pathologies related with peristalsis.

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CONFLICT OF INTEREST

None declared

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ORIGINAL ARTICLE

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Protective effect of hydrogen sulfide on renal injury in the experimental unilateral ureteral obstruction

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ABSTRACT

Introduction/Objective: Ureteral obstruction is a common pathology and causes kidney fibrosis and dysfunction at late period. In this present study, we investigated the antifibrotic and antiinflammatory effects of hydrogen sulfide on kidney damage after unilateral ureteral obstruction (UUO) in rats.
Materials and Methods: 24 rats were divided into four groups. Group 1 was control, group 2 was sham, group 3 included rats with UUO and group 4 rats with UUO which were given sodium hydrogen sulfide (NaHS)-exogenous donor of hydrogen sulfide (intraperitoneally 56µmoL/kg/day). After 14 days, rats were killed and their kidneys were taken and blood analysis was performed. Tubular necrosis, mononuclear cell infiltration and interstitial fibrosis were determined histopathologically in a part of the kidneys; nitric oxide (NO), malondialdehyde (MDA) and reduced glutathione (GSH) levels were determined in the other part of the kidneys. Urea-creatinine levels were investigated by blood analysis. Statistical analyses were made by the Chi-square test and one-way analysis of variance (ANOVA).
Results: There was no significantly difference for urea-creatinine levels among groups. Pathologically, there was serious tubular necrosis and fibrosis in group 3 and there was significantly decreasing of tubular necrosis and fibrosis in group 4 ($p<0.005$). Also, there was significantly increase of NO and MDA levels and decrease of GSH levels in group 3 compared to other groups ($p<0.005$).
Conclusions: hydrogen sulfide prevents kidney damage with antioxidant and antiinflammatory effect.

ARTICLE INFO

Key words:
Ureteral Obstruction; Hydrogen Sulfide; Nephrogenic Fibrosing Dermopathy; Oxidative Stress

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INTRODUCTION

Obstructive nephropathy is a common cause of renal insufficiency in children and adults. Decreases in renal blood flow and glomerular filtration occur after obstruction. Increased hydrostatic pressure causes damage to the tubule-interstitial compartment of the kidney (1). Apoptosis in tubular cells, capillary rarefaction, and interstitial cell inflammatory infiltration can be observed. The ensuing progressive fibrosis results in loss of parenchyma (2, 3). The obstruction can occur at any level of the urinary tract. The most common cause of obstruction in adults is urolithiasis, while

obstructive nephropathy in children is mostly congenital (4).

Unilateral ureteral obstruction (UUO) is a well-established model known to imitate the process of obstructive nephropathy in a simple, accelerated and species-independent manner (5). In recent years, recovery of renal morphology following the relief of unilateral ureteral obstruction (UUO) has been examined in neonatal rats. Interestingly, it has been demonstrated that progressive tubule-interstitial and glomerular damage persisted in the obstructed and contralateral kidney and a decrease in glomerular filtration rate (GFR), and an increase in proteinuria occurred at the end of 1 year after relief of UUO (5, 6). Reactive oxygen species (ROS) are a recently recognized mechanism in the pathogenesis of UUO in experimental studies (7). Increased lipid peroxidation has been reported in renal cortexes of UUO animals. It has been shown that oxidative stress in UUO contributes to the development of tubulo-interstitial lesions and renal fibrosis. Various factors with complex cellular and molecular interactions have also been proposed as possible causes that lead to tubulo-interstitial lesions and renal fibrosis (8). Consequently, new therapy approaches are needed to prevent progression of renal injury along with surgical intervention. Therefore, concomitant treatment with an antifibrotic agent at the time of relief of UUO may prevent deterioration of renal function due to fibrosis. As previously reported, one of these agents may be hidrogen sulfide (H_2S). For decades, hydrogen sulfide (H_2S) has been known as a toxic gas, and, together with nitric oxide (NO) and carbon monoxide (CO), it is currently recognized as an endogenous gaseous physiological molecule (9). H_2S is synthesized from cysteine by two pyridoxal-5′-phosphate dependent enzymes, cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE), and a pyridoxal-5′-phosphate-independent enzyme, 3-mercaptpyruvate sulfurtransferase (3-MST), in most mammalian tissues, including the kidney (10, 11). Progression of fibrosis is associated with oxidative stress, inflammatory response, vascular tone, and intracellular signaling pathways. Recent studies in human and animal have demonstrated involvement of H_2S in those factors in various diseases, including atherosclerosis, ischemia and reperfusion (I/R) injury, hypertension, and end-stage renal disease (ESRD) (10, 11). In a previous study, H_2S supplementation was associated with the suppressions of oxidative stress, inflammation and nitrosative stress (12).

Because of these effects of H_2S, in this study we investigated the role of H_2S in renal damage due to UUO. We used an exogenous donor of hidrogen sulfide-sodium hidrogen sulfide. We evaluated the antifibrotic, antinflammatory and antioxidative effects of H_2S in rat kidneys.

MATERIALS AND METHODS

Drugs and Animals

Male Wistar albino rats (200-250 g) were housed in clean plastic cages in a temperature and humidity-controlled facility with a constant 12 h light/dark cycle with free access to food and water. The use of animals and the experimental protocol were approved by the Institutional Animal Care and Use Committee and animals were treated in accordance with the Guide for the Care and Use of Laboratory Animals of Research Council. Like previous study, sodium hydrogen sulfide (NaHS)--exogenous donor of H_2S (Merck, Schuchardt, OHG, 85662 HOHENBRUNN, Germany), was administered intraperitoneally 56μmoL/kg/day for 14 days (13).

Experimental design

One week after acclimatization, UUO was induced. Briefly after induction of general anesthesia by intraperitoneal injection of thiopental (100mg/kg), the abdominal cavity was exposed via midline incision and the left ureter was ligated at 2 points with 4-0 silk. The sham-operated rats had their ureters manipulated but not ligated. All rats were given amikacin sulfate (6mg/kg, intramuscularly route) before operation (14).

After a quarantine period of 7 days, 24 rats were randomly divided into four groups, each consisting of six animals as follows: Rats in group 1 were control; Rats in group 2 were submitted to sham operation; Rats in group 3 underwent unilateral ureteral ligation and received no treatment; Rats in group 4 were subjected to unilateral ureteral ligation and received NaHS (intraperitone-

ally 56µmoL/kg/day) for 14 days. At this time, no animals showed symptoms of pyonephrosis and no one died because of pyonephrosis. So, we did not have to replace any animals. After 15 days, rats were killed and their kidneys were taken and blood analysis was performed. Tubular necrosis, mononuclear cell infiltration and interstitial fibrosis scoring were determined histopathologically in a part of kidneys; nitric oxide (NO), malondialdehyde (MDA) and reduced glutathione (GSH) levels were determined in the other part of the kidneys. Urea and creatinine levels were investigated by blood analysis.

Biochemical Assays

Twenty four hours after the administration of the last doses of NaHS, on 15th day, rats were anesthetized by intraperitoneal injection of ketamine and sacrificed. Renal cortical tissues were separated into two parts for biochemical analysis and light microscopic examination. Blood samples were also taken by cardiac puncture to assess the serum levels of urea and creatinine concentrations. In frozen tissues biochemically malondialdehyde (MDA), end product of lipid peroxidation, reduced glutathion (GSH), nonenzymatic antioxidant, and total nitrite, a stable product of nitric oxide (NO), were evaluated as a means of oxidative stress. Renal impairment was assessed by serum urea and creatinine levels, as well as by the kidney histology. Serum urea and creatinine levels were determined with an autoanalyzer (Syncron LX20, Ireland) by using commercial Becman Coulter diagnostic kits. Kidney tissue (300mg) was homogenized in icecold tamponade containing 150mM KCL for determination of MDA. MDA levels were assayed for products of lipid peroxidation. MDA referred to as thiobarbituric acid reactive substance, was measured with thiobarbituric acid at 532nm using a spectrofluorometer, as described previously. GSH was determined by the spectrophotometric method, which was based on the use of Ellman's reagent. Total nitrite (NOx) was quantified by the Griess reaction after incubating the supernatant with Escherichia coli nitrate reductase to convert NO_3 to NO_2. Griess reagent (1mL 1% sulfanilamide, 0.1% naphtyl-ethylenediamine hydrochloride, and 2.5% phosphoric acid; Sigma Chemical Co., St. Louis, MO, USA) was then added to 1mL of supernatant. The absorbance was read at 545 nm after a 30-min incubation. The absorbance was compared with the standard graph of $NaNO_2$, obtained from the reduction of $NaNO_3$ (1-100mmoL/L). The accuracy of the assay was checked in two ways; the inter- and intraassay coefficients of variation were 7.52 and 4.61%, respectively. To check conversion of nitrate to nitrite (recovery rate), known amounts of nitrate were added to control plasma samples; these samples were deproteinized and reduced as above.

Histopathological Examinations

Histopathological evaluation of the kidney tissues was done. Paraffin embedded specimens were cut into 6µm thickness and stained with Hematoxylin-Eosin stain for light microscopic examination using a conventional protocol (Olympus, BH-2, Tokyo, Japan). A semi-quantitative evaluation of renal tissues was accomplished by scoring the degree of severity according to previously published criteria (15). All sections of kidney samples were examined for tubular necrosis. Briefly, a minimum of 50 proximal tubules associated with 50 glomeruli were examined for each slide and an average score was obtained. Severity of lesion was graded from 0 to 3 according to the percentage of tubular involvement. Slides were examined and assigned for severity of changes using scores on scale in which (0) denotes no change; grade (1) changes affecting <25% tubular damage (mild); grade (2) changes affecting 25-50% of tubules (moderate); grade (3) changes affecting >50% of tubules (severe). Histopathological evaluation was performed on left kidney tissues. Paraffin-embedded specimens were cut into 5mm thick sections and stained with Hematoxylin & Eosin and Masson's trichrome for examination under the light microscope (BH-2; Olympus, Tokyo, Japan). To evaluate leukocyte infiltration, the widening of interstitial spaces with focal leukocyte infiltration was assessed in five randomly chosen sections prepared from each kidney sample. For each section, the average number of leukocytes per $0.28mm^2$ was calculated from these leukocyte-infiltrated foci using a high-power microscopic field (X400). To estimate the grade of interstitial fibro-

sis, the interstitial area that was stained green with Masson's trichrome was evaluated as a percentage of the total examined area in five randomly chosen sections prepared from each kidney sample using an image analyzer (Leica; Leica Micros Imaging Solutions, Cambridge, UK). For each section, interstitial space widening with focal leukocyte infiltration and interstitial fibrosis was assessed in high-power fields (X400) to quantify the results. The Banff classification of kidney pathology was used for scoring the degree of mononuclear cell infiltration and interstitial fibrosis. The score was graded from 0 to 3, depending on the severity of histological characteristics (16).

Statistical analysis

Results of all groups are shown as mean values ± standard deviation (SD). Statistical analyses of the histopathologic evaluation of the groups were carried out by the Chi-square test and biochemical data were analyzed by the one--way analysis of variance (ANOVA). The significance between two groups was determined by the Dunnett's multiple comparison test, and P<0.05 was accepted as statistically significant value.

RESULTS

Biochemical Variables in Plasma and Tissue

There was no significantly difference for urea-creatinine levels between groups (Table-1). Tissue MDA levels significantly increased in group 3 compared with groups 1, 2, and 4 (p<0.05). Rats with NaHS administration (group 4) showed reduced levels of lipid peroxidation as measured by MDA levels (Table-2). UUO also induced a significant increase in the tissue NO levels that have been prevented by NaHS (Table-2). The unilateral ureteral ligation was accompanied by a marked reduction in GSH level in the kidney tissues of rats (p<0.05), and treatment with NaHS partially elevated the GSH levels (Table-2).

Histopathologic Examinations Results

Histopathologic examination of kidney showed no pathologic findings in control group (Figure-1a). In rats with UUO, there were mild and

Table 1 - Effects of UUO alone and its combination with NaHS on plasma urea, creatinine levels in rats.

Parameters	Control (Group 1)	Sham (Grup 2)	UO (Grup 3)	UO+NaHS (Grup 4)
Urea (mg/dL)	29±7.4	28.7±8.1	30.2±9.5	29.9±10.3
Creatinine (mg/dL)	0.38±0.3	0.36±0.1	0.42±0.2	0.40±0.1

Values are expressed as mean ± SD for six rats in each group.

Table 2 - Effect of H_2S on the Levels of Malondialdehyde, Glutathione and Nitric Oxide in Each Rat Group.

Parameters	Control (Grup 1)	Sham (Grup 2)	UO (Grup 3)	UO+NaHS (Grup 4)
NO (nmoL/g wet tissue)	24.83±3.95	24.63±3.28	38.90±6.82[a]	27.03±3.61[b]
MDA (nmoL/g wet tissue)	2.75±0.19	2.84±0.29	4.17±0.79[c]	3.07±0.45[d]
GSH (mg/g wet tissue)	2.25±0.10	2.25±0.04	1.20±0.73[e]	2.06±0.15[f]

Values are expressed as mean ± SD for six rats in each group
[a,c,e] significantly difference from control group (p=0.02, p=0.005, p<0.001).
[b,d,f] significantly difference from UO group (p=0.006, p=0.015, p<0.001).
NO = nitric oxide, **MDA =** malondialdehyde, **GSH =** reduced glutathione

severe tubular necrosis in the proximal tubules compared with control and sham groups (Figures 1b and 1c). In rats treated with UUO+NaHS, despite the presence of mild tubular degeneration and tubular necrosis, the findings were less severe, and glomeruli maintained a better morphology when compared with UUO group (Figure-1d). Histopathologic examination was normal in rats with only sham operation (group 2) and in rats with no operation (group 1). Severe leukocyte infiltration was observed in the periglomerular and peritubular interstitium of the kidneys of the rats in group 3 with UUO (Figures 2a and 2b). Quantitative analysis of the focal leukocyte infiltration area in the interstitium showed that leukocyte infiltration was significantly reduced in rats that received UUO+NaHS (group 4) (Figure-2c). UUO caused a significant interstitial fibrosis in rats that received no treatment (group 3), and the percentage area of interstitial fibrosis in the kidney of rats with UUO that received no treatment was significantly greater than that of rats with UUO that received NaHS (group 4) (Figures 3a, 3b and 3c). These changes are summarized in Table-3.

DISCUSSION

Obstructive uropathy, caused by prevention of urine flow, results in permanent renal damage and loss of renal function. The obstruction can occur at any level of the urinary tract. The most common cause of obstruction in adults is urolithiasis, while obstructive nephropathy in children is mostly congenital (4). Acute obstruction of the ureter rapidly triggers a cascade of events in the kidneys. First, renal blood flow and

Figure 1 - A) normal tubulus and glomerules in kidney kortex H&Ex200 (control group). B) normal tubulus and glomerules in kidney kortex H&Ex200 (sham group). C) severe tubules total necrosis, tutbular degeneration and epithelial vacuolization in proximal tubules H&Ex200 (UUO group). D) mild epithelial vacuolization in the proximal tubules and normal glomerules H&Ex200 (UUO+NaHS treated group).

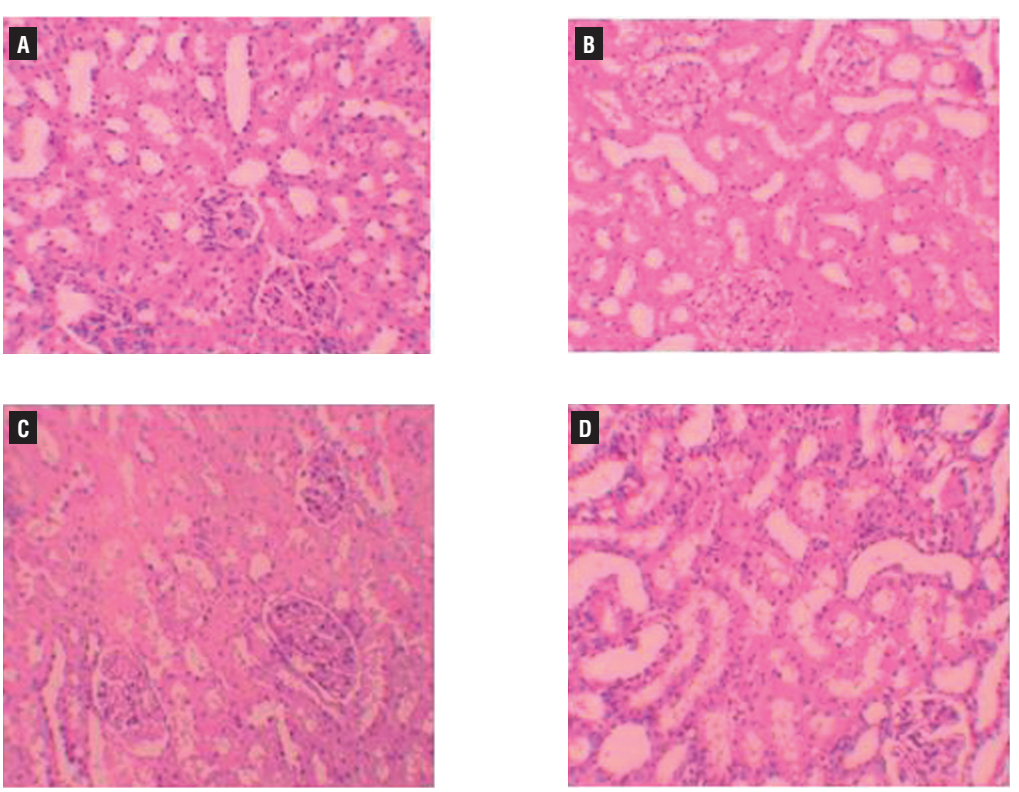
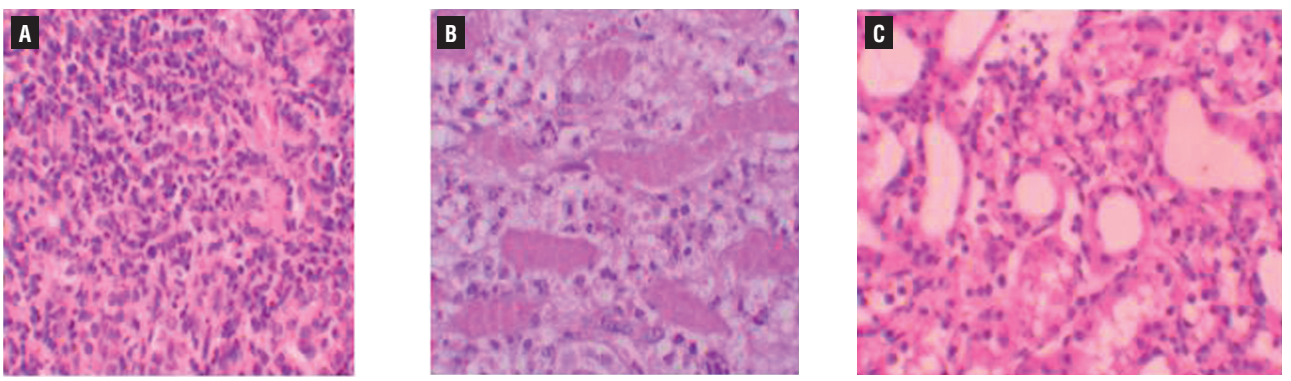


Figure 2 - A) severe mononuklear leukocyte infiltration in the cortex of UUO group (hematoxylin & eosin x400) B) leukocyte infiltration was observed in the peritubular interstitium of the UUO (hematoxylin & eosin x400); c) leukocyte infiltration was reduced in the NaHS-treated group (hematoxylin & eosin x400).



the glomerular filtration rate drop. Within a few days, hydronephrosis starts to develop, followed by interstitial inflammatory infiltration, apoptosis, and necrosis.

The pathogenesis of renal fibrosis caused by UUO involves infiltration of the kidney by inflammatory cells including monocytes, activation and possible transformation of intrinsic renal cells, and interactions between infiltrating and resident cells. Reactive oxygen species (ROS) are a recently recognized mechanism in the pathogenesis of UUO in experimental studies (17). So we decided to measure the MDA, GSH, and nitric oxide (NO) content, as a means of oxidative stress. In our study confirmed through a quantitative survey the protective role of H_2S on renal tissue damage after the induction of UUO in rats. Our results showed that the obstructed kidney had significantly higher tissue MDA, NO levels, and lower GSH levels along with more fibrosis. Our findings corroborate those of earlier studies demonstrating that an enhanced endogenous oxidative stress has a major role in the severity of UUO-induced acute renal failure (18, 19). On the other hand, H_2S reduced the severity of injury, depressed the concentration of these cytokines and increased the antioxidative capacity.

Endogenous H_2S has been proposed as a novel cytoprotective mediator (20), and there is growing evidence of direct and indirect antioxidant effects of H_2S. In cell culture experiments, H_2S/HS^- generated from NaHS has been shown to

Figure 3 - A) normal kidney morphology in a sham group, group (masson & trichrome x200). b) severe fibrosis was observed in the peritubular interstitium of the UUO, group (masson & trichrome x400). c) mild fibrosis was reduced in the NaHS-treated group (masson & trichrome x400).

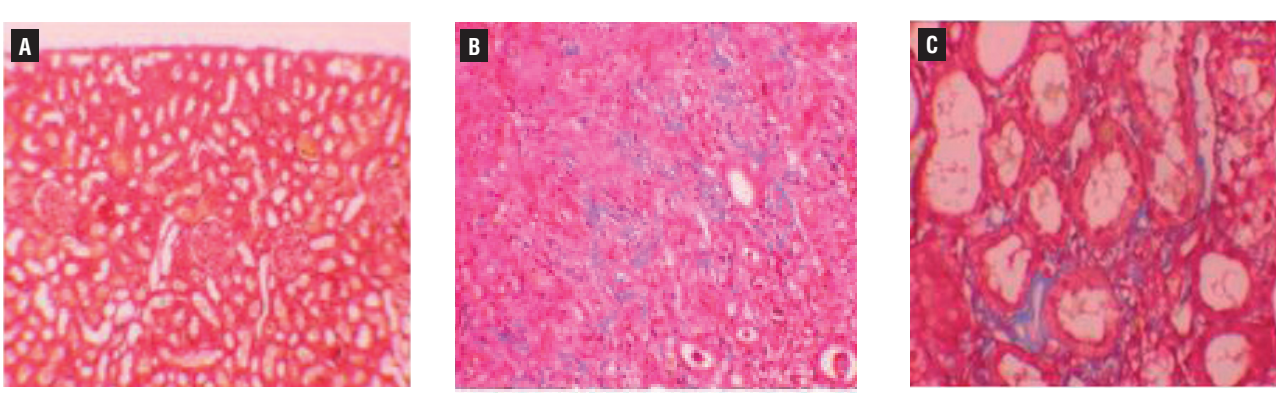


Table 3 - Semiquantitative analysis of tubular necrosis, interstitial fibrosis, mononuclear cell infiltration in control, Sham, UO, and UO+H_2S treated rats.

	Tubular necrosis					Interstitial fibrosis				Mononuclear cell infiltration			
	n	0	1	2	3	0	1	2	3	0	1	2	3
Control	6	6	0	0	0	6	0	0	0	5	1	0	0
Sham	6	6	0	0	0	6	0	0	0	5	1	0	0
UO[a]	6	0	0	4	2	0	1	3	2	0	1	3	2
UO+NaHS[b]	6	1	3	2	0	2	3	1	0	1	1	4	0

Score 0: no degeneration, 1: mild degeneration, 2: moderate degeneration, and 3: severe degeneration
[a] Statistical significant difference from the Sham group
[b] Statistical significant difference from the UO group and $P < 0.05$.

'scavenge' detrimental pro-inflammatory oxidants, such as H_2O_2 (21), ClO^- (22), superoxide, $ONOO^-$ and NO, inhibit cell death induced by these mediators as well as prevent oxidative modification of intracellular proteins (22) and LDL (low-density lipoprotein) (23). In neuronal cells, NaHS inhibited cell death induced by β-amyloid, mediated at least in part via antioxidant effects (24) and up-regulating intracellular glutathione synthesis through increasing cysteine uptake and elevating γ-glutamylcysteine synthetase activity. NaHS is also reported to degrade lipid peroxides (24), inhibit the expression and activity of NADPH oxidase and up-regulate thioredoxin-1 expression in vascular endothelial cells (25). Increased hepatic GSH synthesis and decreased lipid peroxidation are also observed with NaHS treatment in a murine hepatic ischaemia/reperfusion injury model (26).

Increased lipid peroxidation (LPO) has been reported in renal cortexes by the induction of excessive ROS in renal ischemic reperfusion (27). MDA is the product in the LPO process and is widely used as a reliable marker of tissue damage. In the present study, we found increased MDA levels in UUO group and as protective effect of H_2S lower MDA levels in group determined by UUO+NaHS. The GSH antioxidant system is considered the most notable cellular protective mechanism. GSH has a very important role in protecting against oxygen free radical damage by providing reducing equivalents for several enzymes, as well as scavenging hydroxyl radicals and singlet oxygen. Its depletion is a common consequence of increased formation of ROS like UUO- induced nephrotoxicity. In group given UUO+NaHS, we found increased GSH levels. However, our study have shown that H_2S effects NO levels protectively similar to some previous studies with different antioxidant agents (28). H_2S can inhibit NO production and NF-kappaB activation in LPS-stimulated macrophages through a mechanism that involves the action of HO-1/CO (29). Because of that, in our study we found decreased NO levels in UUO+NaHS group compared to UUO group. These findings strongly indicate that H_2S is important in protecting the kidney from UUO-induced injury through improvement in oxidant status.

In this study, the histopathologic examination of kidneys showed severe and extensive damage in UUO rats which have tubular necrosis and edema. This could be due to the formation of highly reactive radicals as a consequence of oxidative stress caused by UUO. The kidneys of the control group showed normal histological features, but the UUO group revealed more extensive and marked tubular necrosis. On the other hand, the tubules from rats of the UUO+NaHS group were nearly normal in histological appearance except for a slight desquamation and atrophy of the tubular epithelial cells. Similar changes were also reported by some studies who demonstrated structural changes in renal tissue of gentamicin-treated animals and its reversal by various agents (30).

In conclusion, the results reported here indicate that H_2S exerts a preventive effect on UUO-induced kidney damage in rats by reducing oxidative stress. At present, hydronephrosis is an urological condition that needs a quick surgical treatment. But, surgery pre-operative phase might take longer than we planned. So, we can use NaHS treatment to prevent the kidney damage in clinics until the surgical treatment. One limitation in our study was that the underlying molecular mechanisms that are responsible for the positive effects of NaHS are yet to be determined. We therefore propose that NaHS supplementation therapy can be used for kidney protection in patients with UUO, such as with ureteral stones. Hovewer, further animal and clinical studies are needed to confirm our suggestion.

CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Bladder response to acute sacral neuromodulation while treating rats in different phases of complete spinal cord injury: a preliminary study

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ABSTRACT

Background: Compared to conventional therapies, sacral neuromodulation (SNM) may offer an alternative, non-destructive treatment for SCI patients with bladder dysfunction. Understanding bladder response to SNM treatment for SCI in different phases may yield new insights for innovative use of this promising technique.
Materials and Methods: Female Sprague-Dawley rats were used in this study to examine the effects of acute SNM on bladder reflex in complete SCI rats. All rats were anesthetized and set up for continuous saline infusion. Acute SNM treatment was implemented for about 6 hours for each rat. Cystometric parameters, including time between contractions, contraction duration, bladder peak pressure, and number of uninhibited contractions, were analyzed and compared within rats before and after SNM treatment.
Results: For the spinally transected rats during early phase (less than two weeks post spinalization), the time between contractions and contraction duration both increased after SNM treatments, yet the increased amplitude was about or less than 20%. For the spinally transected rats with a longer days survival (about two to four weeks post spinalization), the time between contractions and contraction duration substantially increased after SNM treatment and the changes for their average values were more than 90%. For the spinally transected rats with a much longer days survival (more than five weeks post spinalization), the time between contractions and contraction duration increased after SNM treatments, yet the magnitude of changes were less than 30%.
Conclusion: The present study suggested that the significant effectiveness of SNM for complete SCI played its role after the spinal shock phase and prior to the development of detrusor overactivity. It indicated that the time point of SNM treatment is necessary to be paid attention.

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INTRODUCTION

Supra-sacral lesions to the spinal cord nearly always lead to serious disruption of lower urinary tract function (LUTD). Previous reports showed electrical stimulation has emerged as a valuable minimally invasive treatment option for patients with LUTD in whom conservative treatments have failed. The locations of stimulation used in patients with supra-sacral spinal cord injury (SCI) or disease have been reported in a number of ways, including the bladder wall (1), the pudendal or dorsal genital nerve (2, 3), the conus medullaris (4), the tibial nerve (5), the sacral an-

terior roots (6), or the mixed sacral nerves (7, 8). In practice, only the latter two sites have demonstrated clinical significance. However, stimulation of the sacral anterior roots always combined with posterior sacral rhizotomy can prevent many suitable patients from accepting this therapy, although cystometry and clinical examination should show that rhizotomy is effective to suppress reflex incontinence.

Sacral neuromodulation (SNM) may be an alternative solution to sacral deafferentiation, which involves stimulation of sacral afferent pathways rather than cutting them to suppress reflex incontinence (8-10). From early application of SNM until now continuous research is carried out to improve this therapy and to determine the exact mechanism of action. The efficacy of SNM for treatment of LUTD probably relies on spinal and supra-spinal reflex arcs (11). This assumption is supported by the observation that SNM is not effective in patients with complete or nearly complete SCI (12, 13). Recently, Sievert and colleagues's investigation indicated that early SNM in patients with complete spinal cord injury during spinal shock (ie, the bladder arreflexia phase) prevented detrusor overactivity and urinary incontinence (14). Prevention of LUTD before irreversible effects occur is a convincing concept and the findings reported by Sievert and colleagues are exciting. Their research emphasized the significance of the time point of SNM.

Taking into account that SNM is minimally invasive and completely reversible, it is of great interest whether this treatment option is valuable for neurogenic LUTD following complete SCI before resorting to more invasive procedures. Our previous study (15) have substantiated that SNM could offer an alternative, non-destructive treatment for complete SCI animal with bladder dysfunction about three weeks post-spinalization to resemble the condition of urinary bladder hyperreflexia. Further, it is necessary to examine the effect of SNM implementation during different phases after spinalization.

The goal of this study is to investigate the effects of acute SNM on the bladder responses in model rats with complete spinal cord lesion after different days of model surgery.

MATERIAL AND METHODS

Animal Model of SCI

All animal care and experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committees of Shanghai University for Science and Technology. Experiments were performed on female Sprague-Dawley (250g--300g) rats. In order to create an urodynamic pattern similar to humans with supra-sacral SCI, T9-T10 level were considered by researchers to be the spinal transection site for the experiment rats (16).

Under general anesthesia with chloral hydrate (400mg/kg), the rat underwent complete spinal cord transection by a micro-scissor after laminectomy at the T9-T10 level. The rat's body temperature was maintained at 37°C during and after the surgery using a heating blanket until it woke up. To ensure complete disconnection of spinal fibers, the open cavity separating the two ends was filled with hemostatic gel foam. The muscle and skin were then sutured. The animal was returned to its cage after full recovery from anesthesia. Upon awakening from anesthesia, animals were given buprenorphine (0.1mL/100g body weight) subcutaneously for pain control. Postoperatively, rats were housed in shallow cages with high absorbent bedding and had access to food and water ad libitum. Penicillin (15-20mg/kg sc) was administered once daily during the feeding. The bladder was manually expressed twice daily (Crede's maneuver).

Cystometric Studies

The spinally transected rats (7, 12, 15, 18, 20, 27, 36 and 42 days survival, respectively) were anesthetized with chloral hydrate (400mg/kg).

Cystometric study was performed using transvesical catheter implanted into the bladder dome. Transvesical ways, although more invasive than a transurethral way, prevent the impossibility of the cystometric recording because the external urethral sphincter closes tightly following complete SCI. Therefore, the leaking urine was not detected in this study since the external urethral sphincter closes tightly. Before cystometric study, the bladders were manually expressed using Crede's maneuver. After a midline abdominal incision, the urinary bladder was exposed and a polyethylene tube (PE-60, 1.0mmID

and 1.5mm OD) was inserted into the dome of the bladder. The free end of the implanted catheter was connected via a T-stopcock to a pressure transducer (MLT0380/D, ADInstruments Pty Ltd, Sydney, Australia) for monitoring bladder pressure and an infusion pump for infusion saline. The tube was secured with a purse-string suture and the incisions were closed in layers.

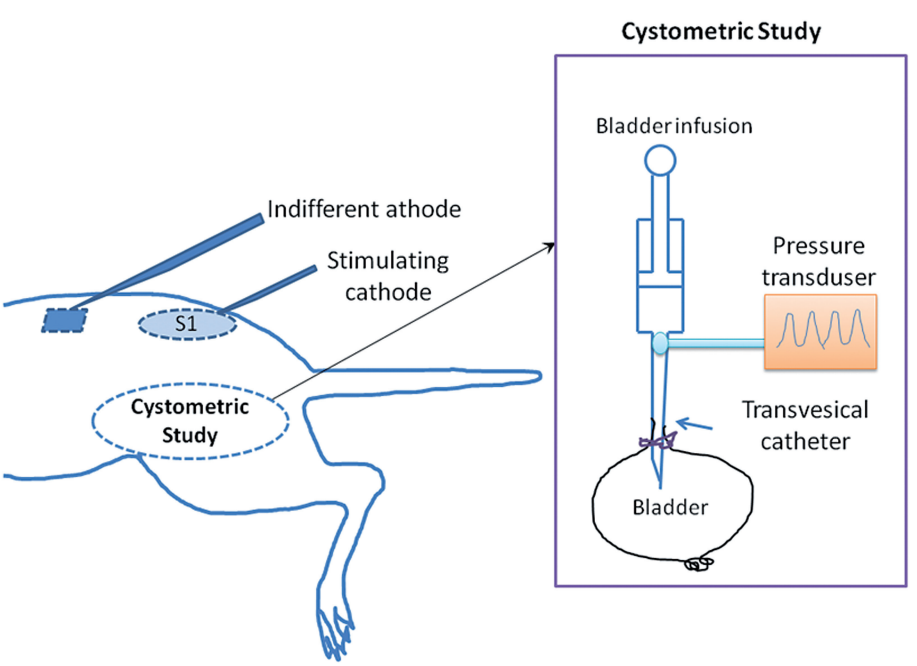
During surgery, animal body temperature was maintained at 37°C using a heating blanket. Room temperature saline solution was infused continuously into the bladder with catheter. The intravesical pressure signal was stored using a biological signal collecting and processing system (PowerLab 4/26, AD Instruments Pty Ltd, Sydney, Australia). Urodynamic characteristics of bladder contractions were investigated in this study. Several parameters were calculated based on urodynamic signal, including bladder contraction duration, bladder contraction cycle period (the time interval between two continuous contractions), peak bladder pressure and the number of uninhibited contraction. The cystometric parameters were recorded before and after SNM treatment. The number of uninhibited contractions was also recorded. The experimental setup is shown in Figure-1.

SNM Treatment

Unilateral sacral foramen electrode has been the gold standard for SNM (17). Indeed, there is no evidence that bilateral simultaneous stimulation has any added benefits to unilateral stimulation (18). Unilateral sacral segmental stimulation with an electrode at the level of the sacral foramen S1 was accepted and performed by most researchers in the rat with SNM experiment (19, 20). In the experiment with acute SNM, the time of SNM treatment was not the same between experiments, however, a short or a long treatment time was considered to be inefficient or lead to increased mortality in experimental animals. Therefore, in this study the unilateral S1 roots of rat were electrically stimulated for 6 hours using stainless steel electrodes inserted into the S1 foramina.

The stimuli used in the experiments were monophasic negative pulses with frequency of 20Hz, pulse duration of 0.1ms, train duration of 30 sec,

Figure 1 - Schematic diagram of the experimental set up on the rats for recording bladder activity with SNM treatment. A PE tube was inserted into the bladder dome, which was in turn connected via a three-way stopcock to an infusion pump for filling with saline, and to a pressure transducer for monitoring bladder pressure. The cathode electrode was inserted into the S1 foramina, and the anode was placed under the skin of the back.



and train period of 80 sec (Figure-2). The stimulation amplitude was adjusted to 80% of the value that induced a visible tail tremor (about 1.5-4.0 V, which is variable for individual rat). Before and after stimulation, the rats underwent continuous urodynamic recording with saline infusion at the rate of 0.1mL/min. During the period of experiment study, analgesic depth was assessed continuously by the eyelash reflex and the paw retraction on moderate pinching. Anesthesia was maintained with low dose of chloral hydrate (100mg/kg).

Data Analysis

In the study, 20 values of the cystometric parameters were applied to analysis. Therefore, 20 cycles of bladder response for each rat before and after SNM treatment were recorded. The intravesical pressure was recorded and analyzed by two different persons, and the latter was blinded to the rat's conditions. The results are expressed as mean and standard deviation (±SD). The significance of differences before and after SNM treatment was compared. A $P<0.05$ was considered statistically significant. The statistical analyses were run in MATLAB software (Math Works Inc., MA, USA).

RESULTS

SCI rats in different phases were treated with SNM. Cystometric parameters before and after SNM treatment, including contraction cycle period, contraction duration, bladder peak pressure, and number of uninhibited contractions, are presented in Table-1. The SCI rats were analyzed individually with bladder infusion because the condition of rats and the parameters used in electrical stimulation are not identical, and it is also necessary to evaluate the effect of the therapeutic neuromodulation for each rat. For the spinally transected rats during early phase less than two weeks post spinalization, i.e. within 7 and 12 days survival in the present study, the time between contractions and contraction duration both increa-

Figure 2 - The stimuli used in the experiments. The pulses were monophasic negative pulses with frequency of 20 Hz, pulse duration of 0.1ms, train duration of 30 sec, and train period of 80 sec. The stimulation amplitude was about 1.5-4.0 V, which is variable for individual rats.

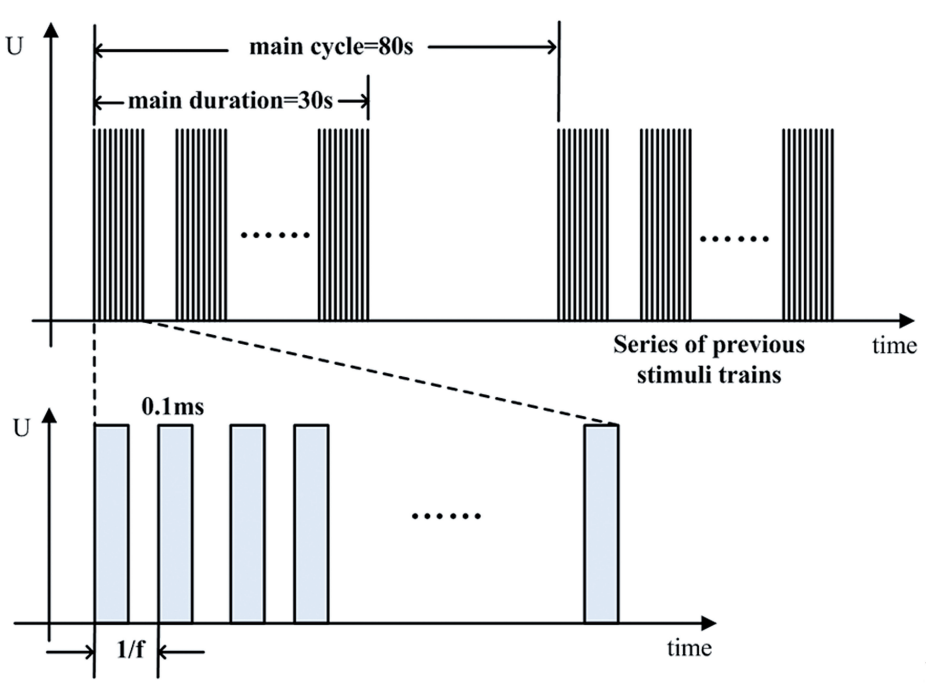


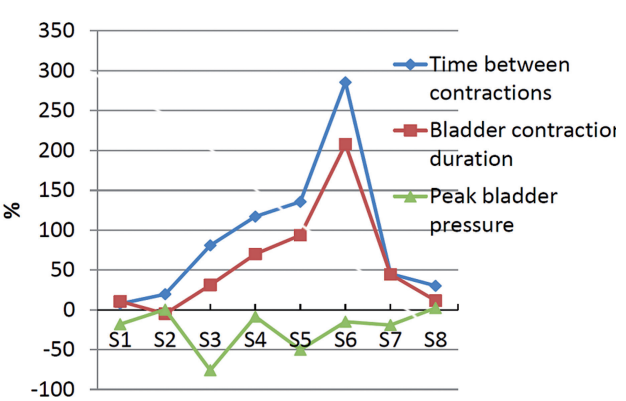
Table 1 - Measurement from the SCI rat 7, 12, 15, 18, 20, 27, 36 and 42 days post surgery before and after SNM treatment under saline infusion with 0.1mL/min. bSNM: before Sacral Neuromodulation; aSNM: after Sacral Neuromodulation.

Rat	Conditions	Time	Time between contractions (s)	Bladder contraction duration (s)	Peak bladder pressure(cm H_2O)	No. of uninhibited contraction
S1	7 days post surgery	bSNM	68.5±50.5	49.5±43.6	3.7±1.3	0
		aSNM	73.8±36.7	54.8±22.7	3.0±0.4	0
S2	12days post surgery	bSNM	48.6±35.5	39.4±32.8	3.9±1.7	0
		aSNM	58.2±47.0	37.5±14.6	3.9±1.8	0
S3	15 days post surgery	bSNM	20.9±9.3	19.7±8.7	11.3±6.7	0
		aSNM	37.9±39.4	25.8±25.1	2.7±1.3	0
S4	18 days post surgery	bSNM	79.1±53.0	35.0±17.3	3.4±1.4	0
		aSNM	171.7±156.9	59.5±38.6	3.1±1.5	0
S5	20 days post surgery	bSNM	62.0±18.1	30.3±4.5	7.7±5.5	2
		aSNM	146.2±137.3*	58.6±19.8*	3.9±1.4	0
S6	27 days post surgery	bSNM	24.9±6.5	22.5±5.9	7.0±3.2	1
		aSNM	96.2±31.7*	69.31±23.88*	6.0±2.7	0
S7	36 days post surgery	bSNM	52.2±29.7	28.3±20.1	11.8±5.2	10
		aSNM	75.8±31.6	40.9±31.6	9.6±7.0	8
S8	42 days post surgery	bSNM	40.4±16.5	33.7±10.3	20.9±13.9	17
		aSNM	52.6±43.2	37.6±26.0	21.4±14.6	12

*P<0.05 vs bSNM

sed after SNM treatments (Table-1), yet the increased amplitude was about or less than 20% (Figure-3). The peak bladder pressure decreased or had little changes. For the spinally transected rats with longer survival days (about two to four weeks post spinalization, i.e. with 15, 18, 20 and 27 days survival in the present study), the time between contractions and contraction duration showed substantial increases after SNM treatment (Figure-3). Especially, the parameters of time between contractions and contraction duration for SCI rats with 20 and 27 days survival dramatically increased (P<0.05, Table-1) and the changes for their average value were more than 90% (Figure-3). For the spinally transected rats with more than five weeks post spinalization, i.e. 36 and 42 days survival in the present study, the time between contractions and contraction duration increased after SNM treatments (Table-1), yet the magnitude of changes were about or less than 45% (Figure-3). The parameter of peak bladder pressure decreased or changed little after SNM treatment (Figure-3). The uninhibited contraction appeared during the late phase of SCI, and its number decreased after the SNM treatment (Table-1). Due to the high mortality rate associated with lower

Figure 3 - Changes of average values of parameters after SNM treatment. The parameters, i.e. time between contractions and bladder contraction duration, for the SCI rat in the late phase of spinal shock or shortly after the spinal shock phase present a substantial increase.



urinary tract complications and decubitus ulcers infection, the details of the parameters for SCI rats with a very long survival days have not been included.

DISCUSSION

The control of the lower urinary tract is a complex, multilevel process that involves both the peripheral and central nervous systems (21). The lesion of spinal cord produces LUTD by eliminating the brain mechanisms. SNM has become a well--established and widely accepted treatment modality in recent years for LUTD (22), whereas SNM has been attempted without success in complete SCI patients (13). However, a recent study (14) in which bilateral SNM was initiated early during the recovery period from complete thoracic spinal cord injury prevented the development of neurogenic detrusor overactivity and urinary incontinence. It is assumed that the effectiveness of the SNM treatment to some extent was determined by the dynamic neurologic process and reorganization or neuroplasticity that might occur after a spinal lesion, emphasizing the significance of the time point of SNM treatment. In the present study, SNM was treated on completely spinalized rats to understand its effectiveness via urodynamic parameters during different SCI phases.

With SCI the normal connections between the sacral cord and the supraspinal circuits that control urine storage and release are disrupted. Patients or animals in the early phase of SCI, e.g. spinal shock phase, will typically present with overflow incontinence due to detrusor failure or severe bladder outlet obstruction (15). Experimental studies in rats have shown that, soon after SCI, bulbospinal pathways are damaged and this disrupts the control of sympathetic preganglionic neurons (23). In the present study, SNM treated during spinal shock phase (rats S1, S2, S3 and S4) didn't achieve a significant result. However, it is worth mentioning that during the late phase of spinal shock phase (rat S4) the treatment of SNM improved the bladder function including significant promotions of time between contractions and contraction duration. This phenomenon seems to imply the neuromodulation of the electricity current initially play its part in the late spinal shock phase during which the reflex neurogenic bladder and autonomic hyperreflexia appears (24). During late spinal shock phase, electrical stimulation influences spinal cord plasticity and performance including promoting synaptic maturation and refinement of neural circuits (24).

After the spinal shock phase, detrusor overactivity develops (15). This overactivity is mediated by a spinal micturition reflex that emerges in response to a reorganization of synaptic connections in the spinal cord (25). During this phase, C-fiber bladder afferents proliferate within the urothelium and become sensitive to bladder distention. These changes lead to the emergence of a new C-fiber--mediated voiding reflex, which is strongly involved in detrusor hyperreflexia (26). In the present and our previous study (2), the significant effectiveness of SNM is observed during this phase of SCI. Sievert and colleagues indicate that early SNM may preserve nerve plasticity, such that C-fibres remain silent, detrusor overactivity is avoided, and sympathetic preganglionic neuron activation in the thoracolumbar cord is suppressed, supporting detrusor contractility (14). The precise mode of action of SNM is still unclear but it has been hypothesized that the electrical current modulates reflex pathways involved in the filling and evacuation phase of the micturition cycle (27). However, supra-spinal pathways seem to be involved as well (28). Studies in animals indicate that dysfunction of the lower urinary tract after SCI is dependent in part on plasticity of bladder afferent pathways as well as reorganization of synaptic connections in the spinal cord. Bladder afferent nerves are critical for sending signals of bladder fullness and discomfort to the brain and for initiating the micturition reflex. Over a period of several weeks following cord injury, major neuroplasticity appears within bladder afferent circuitry. Researchers conclude that one of mechanisms of SNM treatment is to influence the spinal neuronal circuits via the activation of afferent nerve fibers causing inhibition of the voiding reflex at a spinal and/or supraspinal level (29). The effectiveness of SNM from the clearly evident improvement in urodynamic parameters in the present results indicate that SNM prevent the reorganization of afferent nerve fibers, e.g. bladder C-fiber afferents that contributes to detrusor overactivity. Also, development of detrusor overactivity is related to the interrupted regulatory mechanism between the lower urinary tract and midbrain for urine

storage and voiding (30), suggesting the SNM works through the remaining intact sympathetic trunk extending to the brain (bypassing the SCI).

In the results from the present study, the efficacy of SNM substantially weakened about five weeks post spinalization although the number of uninhibited contraction decreased due to SNM probably. Also in clinical practice, the biggest obstacle to the acceptance of SNM is its potential to fail over time. The reasons for these failures are not clear, but the natural plasticity of the nervous system, leading to reactivation of pathological reflex arcs (31), was considered one of possible explanations.

CONCLUSION

Electrical stimulation has been investigated for many years for the purpose of restoring function to the neurogenic bladder. SNM for treating functional voiding dysfunction has become established in urology. SNM was originally not considered an option for neurogenic LUTD, and SNM has been attempted without success in complete chronic SCI patients. The present study indicated that the time point of SNM treatment is necessary to be paid attention. Although SNM seems to be a promising therapy for neurogenic disease, further studies and long-term results with an extended cohort of complete SCI patients are yet to be obtained.

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CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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The evaluation of pulmonary function and blood gas analysis in patients submitted to laparoscopic versus open nephrectomy

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ABSTRACT

Background: The aim of this study was to assess the early postoperative pulmonary function and arterial blood gases in patients who have undergone open versus laparoscopic nephrectomy.
Materials and Methods: Forty patients were randomly assigned to undergo laparoscopic (LN, n=20) or open nephrectomy (ON, n=20). Pulmonary function tests including forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC), forced expiratory volume at 25% (FEF_{25}), forced expiratory volume at 50% (FEF_{50}), forced expiratory volume at 25% to 75% (FEF_{25-75}), forced expiratory volume in 1 second (FIV1) and peak expiratory flow (PEF) were performed one day before the operation and on the postoperative day 1. The arterial blood gas analysis (pH, pCO_2, pO_2, SaO_2) was made at breathing room preoperatively, in the recovery phase and on postoperative day 1.
Results: All spirometric variables decreased after both open and laparoscopic nephrectomy on postoperative day 1. FEV_1, FVC, FEF_{25} and FEF_{25-75} values decreased on postoperative day 1 (39.7%, 37.4%, 27.7%, 51.8% respectively) in the open surgery group and they were significantly lower in laparoscopic group (29.9%, 32.5%, 23.2%, 44.5% respectively). There were no significant differences in FEF_{50}, PEF and FIV_1 between the groups. The SaO_2 and pO_2 values also decreased in both groups. During early recovery, pH decreased while pCO_2 increased significantly but they returned to preoperative values on postoperative day 1 in both groups.
Conclusion: Laparoscopic nephrectomy is better than open nephrectomy considering pulmonary functions.

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INTRODUCTION

Pulmonary complications like hypoxemia, atelectasis and pneumonia are the main causes of morbidity after abdominal surgery (1, 2). Upper abdominal surgeries are more associated with pulmonary impairment, which seems to be related with diaphragmatic dysfunction (3, 4). Laparoscopic surgery has been the method of choice over open techniques with numerous advantages such as reduced perioperative bleeding, less post--operative pain, less injury to the abdominal wall muscles and may have less effect on pulmonary functions (5, 6).

Like other laparoscopic surgeries, laparoscopic nephrectomy offers multiple advantages over open nephrectomy including reduced postoperative morbidity and pain, rapid recovery, shor-

ter hospital stays and faster discharge (7, 8). Moreover, significant reduction in the probability of adverse events and improvement of perioperative safety outcomes were observed in laparoscopic compared open nephrectomy with two observational patient safety studies, respectively (8, 9).

In literature, numerous studies compare pulmonary functions among various open and laparoscopic upper abdominal surgery techniques involving cholecystectomy (4, 5, 10), gastric bypass (6), colectomy (11), and esophagogastric surgery (3) but we were not able to find any investigations related to nephrectomy. In the present study under general anesthesia with isoflurane and remifentanil and providing a good postoperative analgesia, we examined and compared impairment of pulmonary functions after laparoscopic and open nephrectomy using spirometric tests and arterial blood gas analysis. The aim of this study was to assess the early postoperative pulmonary functions and to show any superiority for laparoscopic over open nephrectomy.

MATERIALS AND METHODS

The Ministry of Health, Turkey, General Directorate of Pharmaceuticals and Pharmacy ethics board approved this study. The study included American Society of Anesthesiology (ASA) I-II and III risk groups of forty 27-65 years old patients with renal cell carcinoma with no history of lung disease and with normal lung function tests. After the patients gave an informed consent, they were randomized in one of two groups using a computer generated table of random numbers, open (ON, n=20) or laparoscopic (LN, n=20) nephrectomy. To evaluate pulmonary function, forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC), forced expiratory volume at 25% (FEF25), forced expiratory volume at 50% ($FEF5_0$), forced expiratory volume at 25% to 75% (FEF_{25-75}), forced expiratory volume in 1 second (FIV_1) and peak expiratory flow (PEF) as determined by spirometry and arterial blood gas analysis (pH, $PaCO_2$, PaO_2, SaO_2) were used. Pulmonary function tests of the patients were performed in standing posture, at rest before and on first day after the operation with a mobile hand spirometer (Spirobank G, MIR, Via del Maggiolino 12500155 ROME). Arterial blood gases were analyzed during rest and 1 hour and 24 hours after the operation. Standard general anesthesia consisting of inhalation anesthesia with isoflurane and adding remifentanil infusion was administered to all patients.

Analgesic protocol was the same for all patients and early postoperative analgesia was provided using intravenous 20mg tenoxicam and 0.25mg kg^{-1}meperidine. Within the first 24 hours, postoperative pain was assessed using a visual analogue scale (VAS, 0=no pain, 10=maximum pain) and whenever score was >3 meperidine (1mg kg^{-1} intramuscular) was administered. Pulmonary function tests were not performed unless VAS score was under 3.

Maximum intra-abdominal pressure was limited to 14mmHg with CO_2 insufflation in the LN group. Abdominal incision was performed for ON operations. The same anesthesia and surgeon team performed all procedures.

Transperitoneal laparoscopic nephrectomy was performed with descending technique (also called Tunc technique). The patients were maintained in the lateral decubitus position where they were in a semi-oblique position with an angle of 70º with the bed. For nephrectomy, only three trocars were used. The first trocar was placed 3-5cm superior to the umbilicus and 3-4cm lateral to midline. The second trocar was 10-15cm lateral to the umbilicus. The third trocar was 3-5cm superior to the first trocar and 1cm lateral. These locations can change according to the operation side. Open radical nephrectomy was performed with a flank incision. At either method colon was moved away from the kidney, the renal hilum was identified and dissected. First renal artery and then renal vein were clamped and cut. Proximal 1/3 ureter was dissected, clamped and cut. Nephrectomy specimen contained the adrenal gland in case of upper pole tumors. In the laparoscopic nephrectomy group, the specimen was removed with an endobag. The duration of surgery was defined as the time interval from skin incision in the open nephrectomy group or first trocar placement in the laparoscopic group to closure in minutes.

Data analysis was performed using SPSS 17.0 statistical program and summarized as mean±SD, n or %. The Kolmogorov-Smirnov test

was used to show a normal distribution of the data. Demographic data, duration of operation and anesthesia, VAS and pulmonary function tests were compared using Student t test between groups. Intra-group comparison of continuous data was evaluated with repeated measures of variance test and when a significant difference was present, a Bonferroni correction was made. Paired sample t test was used to compare intra-group differences in the results of pulmonary function tests and VAS. Gender and ASA were compared using chi-squared or Fisher's exact chi-squared tests. P values less than 0.05 were considered statistically significant.

RESULTS

There were no significant differences between the groups regarding age, sex, weight, height and ASA classification (Table-1). Duration of surgery varied from 147.21min to 98.0min ($p<0.05$) for ON and LN, respectively, with duration of anesthesia also being shorter for the LN group.

Preoperative and postoperative first day mean pulmonary function test results are listed in Table-2. In both groups, a decrease in all spirometric variables was detected on the postoperative day 1. A decrease of 39.7%, 37.4%, 27.7%, 51.8% in Group ON and 29.9%, 32.5%, 23.2%, 44.5% in Group LN was detected for FEV_1, FVC, FEF_{25} and FEF_{25-75}, respectively, when compared to preoperative values. The difference was significantly lower in Group LN ($p<0.05$). PEF and FIV_1 showed the same tendency in both groups without a significant difference between the two groups.

Blood gas data are presented in Table-3. There were no statistically significant differences between the two groups for all parameters of blood gas analysis. There was a significant difference ($p<0.05$) in pH, which decreased, and pCO_2, which decreased, in the first hour following the operation; however, they both returned to the preoperative levels on postoperative first day. A significant increased ($p<0.05$) in SaO_2 and pO_2 values was documented on postoperative day 1 in both groups.

The VAS pain scores were similar in both groups half an hour, an hour and 24 hours after the operation (Table-4).

DISCUSSION

Upper abdominal surgeries have been known to have more pronounced effect on pulmonary functions than lower abdominal surgeries,

Table 1 - Patient characteristics and duration features (mean±standard deviation (min-max).

	Laparoscopic Nephrectomy (n=20)	Open Nephrectomy (n=20)	p
ASA (I/II/III)	3/12/5	5/13/2	0.401
Gender (M/F)	11/9	11/9	1.000
Age (year)	55.15±9.73 (29-65)	51.10±13.65 (27-65)	0.287
Weight (kg)	74.00±10.98 (55-96)	69.60±11.55 (52-96)	0.224
Height (cm)	165.80±9.50 (150-186)	162.63±9.45 (145-178)	0.303
Duration of operation (min)	98.00±24.98 (60-133)	147.21±36.35* (88-195)	<0.0001
Duration of anesthesia (min)	111.90±25.27 (70-150)	158.74±36.82* (100-207)	<0.0001

ASA = American Society of Anesthesiologists
*p<0.05 in comparison with laparoscopic group

Table 2 - Spirometric data as percentages of individual predictive values comparing laparoscopic and open nep hrectomy [mean (standard deviation)].

		Laparoscopic Nephrectomy (n=20)	Open Nephrectomy (n=20)	p
FEV_1 (%)	**Preoperative**	87.1 (27.7)	79.5 (27.3)	0.362
	Postoperative 1st day	57.2 (27.4)†	39.8 (17.7)*†	0.025
FVC (%)	**Preoperative**	91.2 (23.3)	80.2 (28.9)	0.192
	Postoperative 1st day	58.7 (27.5)†	42.8 (19.0)*†	0.043
FEF_{25} (%)	**Preoperative**	65.4 (25.8)	54.0 (26.7)	0.177
	Postoperative 1st day	42.2 (22.0)†	26.3 (14.9)*†	0.012
FEF_{50} (%)	**Preoperative**	69.5 (23.5)	57.1 (27.1)	0.135
	Postoperative 1st day	42.3 (23.1)†	34.0 (27.7)†	0.324
FEF_{25-50} (%)	**Preoperative**	92.8 (36.0)	84.1 (35.7)	0.452
	Postoperative 1st day	48.3 (24.1)†	32.3 (19.9)*†	0.027
PEF (%)	**Preoperative**	70.2 (24.6)	57.2 (26.2)	0.118
	Postoperative 1st day	51.0 (24.5)†	38.2 (23.9)†	0.112
FIV_1 (%)	**Preoperative**	94.6 (27.8)	82.0 (36.2)	0.233
	Postoperative 1st day	43.8 (15.8)†	52.3 (24.0)†	0.236

FEV_1 = forced expiratory volume in 1 second; **FVC =** forced vital capacity; **FEF_{25},** = forced expiratory volume at 25%; **FEF_{50} =** forced expiratory volume at 50%; **FEF_{25-75} =** forced expiratory volume at 25% to 75%; **FIV_1 =** forced expiratory volume in 1 second; **PEF =** peak expiratory flow
*** =** $p<0.05$ in comparison with laparoscopic group
† = $p<0.05$ in comparison with preoperative values

Table 3 - Values of arterial blood gas analysis comparing laparoscopic and open nephrectomy [mean (standard deviation)].

		Laparoscopic Nephrectomy (n=20)	Open Nephrectomy (n=20)	p
$PaCO_2$	**Preoperative**	32.69 (4.50)	32.73 (4.21)	0.978
	Postoperative 1st hour	36.68 (4.08)†	35.30 (5.25)†	0.357
	Postoperative 1st day	32.82 (3.93)	32.02 (3.72)	0.523
PaO_2	**Preoperative**	73.89 (8.18)	77.15 (7.04)	0.185
	Postoperative 1st hour	85.02 (34.41)	94.65 (41.61)	0.430
	Postoperative 1st day	68.25 (8.90)†	69.70 (8.84)†	0.617
SaO_2	**Preoperative**	95.35 (1.53)	95.59 (1.95)	0.668
	Postoperative 1st hour	95.43 (2.60)	94.98 (2.90)	0.608
	Postoperative 1st day	94.17 (1.91)†	93.27 (3.59)†	0.343
pH	**Preoperative**	7.42 (0.03)	7.43 (0.02)	0.290
	Postoperative 1st hour	7.36 (0.04)†	7.36 (0.06)†	0.776
	Postoperative 1st day	7.42 (0.03)	7.42 (0.03)	0.622

† = $p<0.05$ in comparison with preoperative values

Table 4 - VAS scores [mean±standard deviation (min-max)].

	Laparoscopic Nephrectomy (n=20)	Open Nephrectomy (n=20)	p
Postoperative 30th minute	3.80±1.28 (0-5)	3.80±0.70 (3-5)	1.000
Postoperative 1st hour	2.85±1.22† (0-5)	2.95±0.69† (2-4)	0.752
Postoperative 1st day	1.25±0.91† (0-2)	1.30±0.80† (0-2)	0.855

VAS = Visual analogue scale
† = p<0.05 in comparison with preoperative values

mostly attributed to postoperative pain and diaphragm dysfunction (4, 12). Laparoscopic techniques seem to cause less damage to abdominal wall and less postoperative pain, this is why less pulmonary complications are considered (5, 6). Comparison of pulmonary functions in laparoscopic versus open abdominal surgeries have been well studied (3-6, 10-15). So, we hypothesized that improved postoperative pulmonary function described after various laparoscopic surgeries could be expected similarly to benefit patients undergoing nephrectomy. Our findings for laparoscopic and open nephrectomy are in line with other data. We similarly showed that considerable changes in pulmonary function occurred after both laparoscopic and open nephrectomy but these were less pronounced after LN than ON.

In patients undergoing abdominal surgery, besides maintaining basic principles of general anesthesia, there should be a good relaxation of abdominal wall tonus, and suppression of sympathetic and hemodynamic reflexes, and perfect postoperative analgesia should be provided (16). General anesthesia itself has an important role in impairment of pulmonary functions due to disturbance of gas exchange (17). This effect of general anesthesia lasts short and mostly return to baseline values within a day (5, 10). However, independent from the effects of general anesthesia, upper abdominal surgery has more pronounced and long-lasting effects on pulmonary functions that are characterized in restrictive patterns (15).

Moreover, as operation and anesthesia durations last longer, the decline in pulmonary function test parameters gets greater. Osman et al. (10) in their comparative study observed a rapid decrease in FEV_1 if anesthesia lasted longer than 60 minutes and in that study, anesthesia lasted 102 minutes in open cholecystectomy and 57 minutes in laparoscopic cholecystectomy group. In the present study, mean duration of anesthesia was noted as 111.90 minutes for the laparoscopic group and 158.74 minutes for the open group, which are statistically significant. Even though the above mentioned authors suggested that laparoscopy has more advantages as it provides shorter duration of anesthesia, longer anesthesia duration of open nephrectomy group in our study may represent a limitation to the study.

In the present study comparing early pulmonary functions in laparoscopic versus open nephrectomy, in line with results of other upper abdominal surgery studies, considerable changes were noted after both LN and ON, although these were less pronounced after ON than those after LN. FEV_1 and FVC were more suppressed in patients undergoing open nephrectomy ($p<0.05$). FEV_1 and FVC decreased by 49.93% and 46.63%, respectively, in ON and 34.32% and 35.63%, respectively, in LN, when compared to their preoperative values ($p<0.05$). Similar changes occurred in FEF_{25} and FEF_{25-75}. There were no significant differences concerning FEF_{50}, PEF and FIV_1. Crema et al. (3) compared pulmonary dysfunction in patients who underwent open and laparoscopic esophagogastric surgery and demonstrated a 25.2% and 27.6% reduction in open and a 13.7% and 15.7% reduction in laparoscopic group for FEV_1 and FVC,

respectively, on postoperative second day. Hasukic et al. (4) evaluated pulmonary functions in 60 patients who underwent either laparoscopic or open cholecystectomy and concluded that pulmonary functions were better preserved after laparoscopic resection. Shauer et al. (15) also demonstrated less impairment of postoperative pulmonary function after laparoscopic cholecystectomy than conventional open cholecystectomy. On postoperative first day, they found a decrease of 79% and 76% after open, and 49% and 44% after laparoscopic group in FVC and FEV_1, respectively, when compared to their preoperative values. Nguyen et al. (6) also found less postoperative suppression of pulmonary function with laparoscopic gastric bypass than open gastric bypass. The diversity of reduction rate for spirometric parameters in literature can be explained by the variability of surgical methodologies, the site and size of incisions, and the severity of diaphragmatic dysfunctions.

There are two possible approaches for laparoscopic nephrectomy, transperitoneal or retroperitoneal. While both have individual benefits and disadvantages, anesthesia related main difference is bilateral intraperitoneal versus unilateral retroperitoneal CO_2 insufflation. El-tohamy SA. et al. (18) demonstrated markedly higher $PaCO_2$ at the same insufflation pressure and significantly greater peak airway pressure in transperitoneal compared to retroperitoneal CO_2 insufflations maintaining the same minute ventilation. The authors concluded that ventilatory, hemodynamic and cerebral implications were more pronounced in transperitoneal nephrectomy than in retroperitoneal nephrectomy. Similarly, in an experimental study (19), to maintain normocapnea, significantly greater peak airway pressures were needed to administer an adequate tidal volume in intraperitoneally versus retroperitoneally CO_2 insufflated animals. These findings are in accordance with a previous study that suggested a less pulmonary impairment with retroperitoneal technique (20). Even though retroperitoneal approach was associated with less pulmonary disturbance, the selection of the approach depended on the surgeon team in the present study and they preferred transperitoneal approach based on their experience. Anyway, both transperitoneal and retroperitoneal approaches were found excellent with no significant difference between the groups for postoperative complications in a comparative study and the approach to be selected was at the discretion of the surgeon.

In previous studies comparing laparoscopic open techniques, there is diversity in the results of blood gas analysis. We found no statistically significant differences between the two groups in all parameters of blood gas analysis. pH decreased at immediate recovery in both groups with an increase in pCO_2 but all returned to preoperative levels on postoperative day 1. SaO_2 and pO_2 values reduced on postoperative day 1 when compared to preoperative values, however, this reduction was not significant between laparoscopic and open groups. Similarly, Hasukic et al. (4) found no significant changes in arterial pO_2, pCO_2 and pH at 24 h between laparoscopic and open cholecystectomy patients. On the contrary, Karayiannakis et al. (13) and Mimica et al. (14) reported better oxygenation in laparoscopic cholecystectomy than in open surgery. Despite this variability, laparoscopy is considered to deteriorate blood gas values to a lesser degree.

The incision used in open nephrectomy is painful and can be the reason for altered ventilation in those patients. In order to avoid bias, we used a strict protocol for the standardization of anesthesia management, care and analgesic use. Notably, the protocol of the present study mandated the maintenance of VAS<3 especially when the pulmonary function tests were performed. However, only early, first 24 hours and postoperative impairment of pulmonary functions were compared in the current study and it is difficult to identify the reasons of deterioration of pulmonary functions. Another limitation in our study is the lack of recording the amount of analgesics consumed and moreover the VAS pain scores were evaluated only at rest but scores might differ between the groups during coughing and mobilization.

CONCLUSIONS

Early postoperative pulmonary functions are found to be less impaired in the laparoscopic nephrectomy group than in the open nephrectomy

group, but the effects on arterial blood gases were similar in both groups and no clinically relevant differences were found. In the light of these findings, we concluded that laparoscopic nephrectomy would be superior to open nephrectomy considering pulmonary functions.

CONFLICT OF INTEREST

None declared.

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Robot-Assisted Extended Pelvic Lymph Nodes Dissection for Prostate Cancer: Personal Surgical Technique and Outcomes

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ABSTRACT

Objective: Extended pelvic lymph nodes dissection (EPLND) allows the removal of a higher number of lymph nodes than limited PLND. The aims of this study were to describe our robot-assisted EPLND (RAEPLND) technique with related complications, and to report the number of lymph nodes removed and the rate of lymph nodal metastasis.
Materials and Methods: 153 patients underwent RAEPLND prior to robot-assisted radical prostatectomy (RARP). Indications were defined according to Briganti nomogram, to predict risk of lymph-nodal metastasis. Lymphatic packages covering the distal tract of the common iliac artery, the medial portion of the external iliac artery, the external iliac vein and the internal iliac vessels, together with the obturator and the presacral lymphatic packages were removed on both sides.
Results: Median preoperative PSA was 7.5 ng/mL (IQR 5.5-11.5). Median operative time was 150 min (135-170). Median RAEPLND alone operative time was 38 min (32.75-41.25); for right and left side, 18 (15-29) and 20 min (15.75-30) (p=0.567). Median number of lymph nodes retrieved per patient was 25 (19.25-30); 13 (11-16) and 11 (8-15) for right and left side. In 19 patients (12.41%) metastasis was found at the level of pelvic lymph nodes. Median number of positive lymph nodes was 1 (1-4.6) per patient. Complications occurred in 11 patients (7.3%).
Conclusions: the number of lymph nodes removed was comparable to published data about open series, allowing the increase of detection rate of lymph nodal metastasis for minimally invasive approach without compromising complications' rate if performing the procedure following reported technique.

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INTRODUCTION

Pelvic lymph nodes dissection (PLND) is considered the surgical standard for staging of prostate cancer (PCa). The nomenclature and anatomic boundaries of PLND vary. Limited PLND is defined by many surgeons as the removal of lymphatic packages along the external iliac artery and vein, obturator fossa, and obturator nerve (1, 2). In case of extended PLND (EPLND), additionally, lymph nodes along the internal iliac artery together with presacral lymph nodes (the so called "superextended" PLND) are removed too (2-12). Indications of PLND vary: The European Association of Urology (EAU) guidelines recommend performing PLND in all men with a risk of lymph node invasion >5% based on the updated Briganti nomogram (13).

The American Urological Association (AUA) guidelines consider that PLND should be reserved for patients with higher risk of nodal involvement with no clear cut-off (14).

For these reasons some controversies have risen about the appropriateness of limited PLND as a staging tool and more recently increasing evidences support EPLND if PSA level is >10ng/mL or the Gleason Score is ≥7 (3, 5, 7, 10).

It is known that patients affected by high-risk PCa have a risk of lymph nodal metastasis of about 38% (10) but available literature data are derived from open and pure laparoscopic experiences.

Since the introduction of robotic Da-Vinci® system in urologic surgery, robot-assisted radical prostatectomy (RARP) has been becoming an increasingly popular procedure throughout Europe and the United States (10, 15-19).

In parallel with the beginning of RARP case-studies, experiences with robot-assisted PLND have started. The intraoperative magnification together with the higher degrees of freedom in movements allowed by robotic system have boosted performing of EPLND from the beginning in the majority of centers specialized in radical prostatectomy. To date, literature still lacks data about robot-assisted EPLND.

In our study, the primary aim was to describe our surgical technique for RAEPLND and to report surgery-related complications with discussion about how to prevent them; the secondary aim was to report the number of lymph nodes removed by this technique and the detection rate and location of lymph nodal metastasis.

MATERIALS AND METHODS

From January 2011 to December 2013, 153 patients consecutively underwent RAEPLND for PCa. Indications for RAEPLND were given according to the updated nomogram for prediction of lymph nodes invasion (LNI) by Briganti et al. (13). Patient's demographics were collected and reported in Table-1. All patients preoperatively underwent staging examinations by computed tomography (CT) scanning and/or magnetic resonance imaging (MRI) of the abdomen and pelvis. In patients with a preoperative serum PSA level above 20ng/mL, a total-body bone scanning was performed. Routine postoperative imaging assessment included ultrasonography at 1 month and three months to evaluate surgery-related complications.

Technique step-by-step

All procedures were performed by transperitoneal approach. Patient was placed in a 30° Trendelenburg position. Using a four-arm Si HD Da Vinci robotic system (Intuitive Surgical, Sunnyvale, CA, USA), six trocars were placed. A 10-mm port for the camera was placed just cranially the umbilicus through a midline incision; two 8 mm ports for the robotic working instruments were placed on the right and left pararectal lines at their intersection with umbilical line. The third 8 mm port (for the fourth robotic arm) was placed 8 cm laterally to the left robotic port. Two additional trocars were placed for the assistant: the first one (5-mm) placed between the camera and the right robotic port; the second one (10 mm)

Table 1 - Patient characteristics.

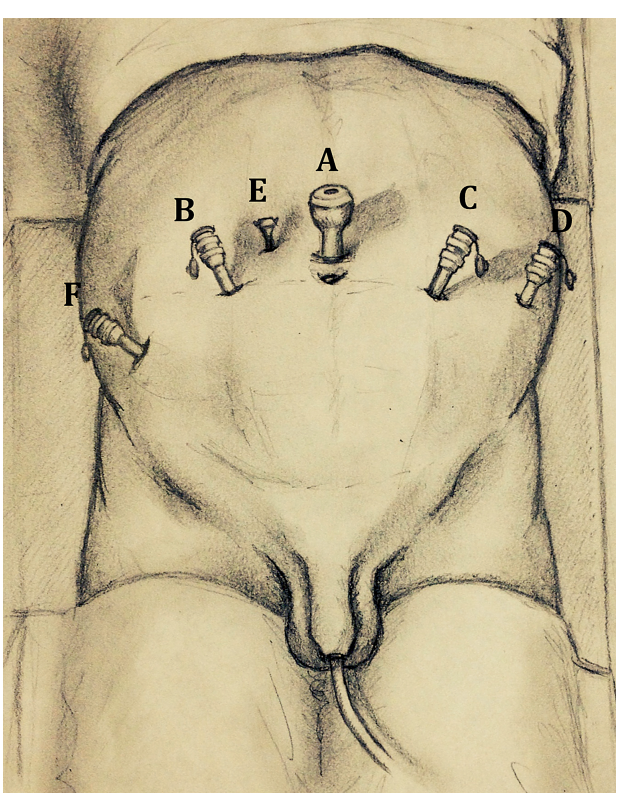
No. of patients	153
Median age, yr (IQR)	64 (59-68)
BMI, median (IQR)	26 (24-28.1)
Preoperative PSA, median, ng/mL (IQR)	7.5 (5.5-11.5)
Clinical T stage, No (%)	
T1	75 (49.0%)
T2a	31 (20.3%)
T2b	32 (20.9%)
T2c	9 (5.9%)
T3	6 (3.9%)
Preoperative Gleason Score, No (%)	
5	2 (1.3%)
6	22 (14.4%)
7a (3+4)	40 (26.2%)
7b (4+3)	39 (25.5%)
8	41 (26.7%)
9	9 (5.9%)
10	0

about 8 cm laterally to the right robotic trocar and about 4 cm cranially to the right anterior superior iliac spine (see Figure-1).

The anatomic landmarks of our technique of RAEPLND were the umbilical artery and the iliac vessels.

The anatomical limits were the bifurcation of the common iliac arteries, including the identification of the ureter cranially, the Cloquet's lymph node caudally, the external iliac artery laterally, and the bladder wall medially. The lymph nodes dissection included the lymphatic packages located at the angle between external and internal iliac artery and along the obturator nerve. The dissection was performed by bipolar forceps and monopolar scissors. We here report the specific steps of the procedure:

Figure 1 - A 10 mm trocar for the camera is placed 20mm above the umbilicus (A). Three 8 mm robotic trocars are placed pararectal on the right and on the left side (B, C), another 8 mm trocar is placed 80mm laterally on the left side (D). Two assistant trocars are placed: one 5 mm trocar between the camera and right working trocar (E) and one 12 mm trocar medial and cranial to the right anterior superior crest (F).



1-Conventionally, the RAEPLND is begun on the right side. After mobilizing the sigmoid colon, PLND starts with the incision of peritoneum, laterally to the umbilical ligament overlying the common iliac artery and parallel to the external iliac artery until the ureter.

Incision is performed at the level of the pubic bone until the crossing of the ureter with common iliac vessels. External iliac vessels are identified and exposed. Vas deferens is identified and sectioned.

A blunt dissection is performed (preserving pre-vesical fascia) in order to enlarge the operative field among lateral bladder wall, iliac vessels and lateral pelvic wall. The peritoneum covering hypogastric vessels and sacrum is medialized.

2-The ureter is identified at its crossing with common iliac artery, dissected, suspended (if necessary by using a vessel loop) and then displaced (we underline that vessel loop is generally used on the left side only because on the right side ureter adheres to peritoneum so that medialization of peritoneum itself is enough for ureter displacement). The operative field is now well prepared. We underline that presacral area is easy to get and dissect on the right side, while on the left side sacrum promontorium is usually covered by common iliac vein that limit the intraoperative vision and the dissection of presacral lymph nodes.

Once splitted the fibro-fatty tissue overlying the distal portion of the common and external iliac vessels, common iliac artery and its bifurcation are visible. The fibro-fatty tissue containing the lymphatics overlying the internal iliac artery, its medial vesical branches and the presacral lymph nodes are identified and dissected (Figure-2).

3-The external iliac lymph nodes are progressively dissected. The dissection of the external iliac packages starts with the division of the adventitia overlying the external iliac vein distally. Then dissection is carried out from the crossing of the ureter over the common iliac artery until the pubic bone at the level of circumflex vein

Figure 2 - Overview after suspension of the ureter (U) and removal of the fibrofatty tissue overlying the distal portion of the common (C.I.A) and external (E.I.A) iliac vessels (the bifurcation of the common iliac artery is now visible); the presacral (P.L.N) and hypogastric (H.L.N) lymph nodes are identified and dissected (left side). H.A hypogastric artery.

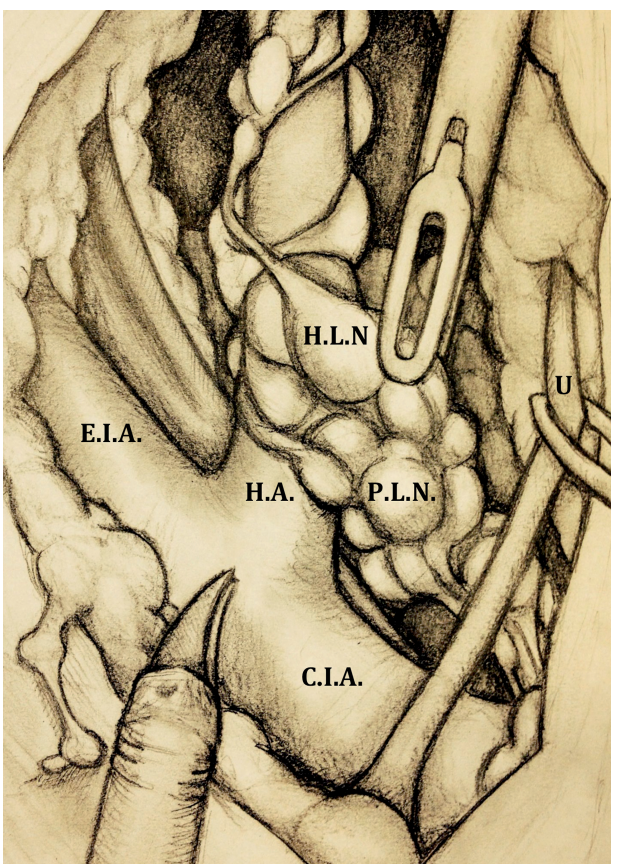
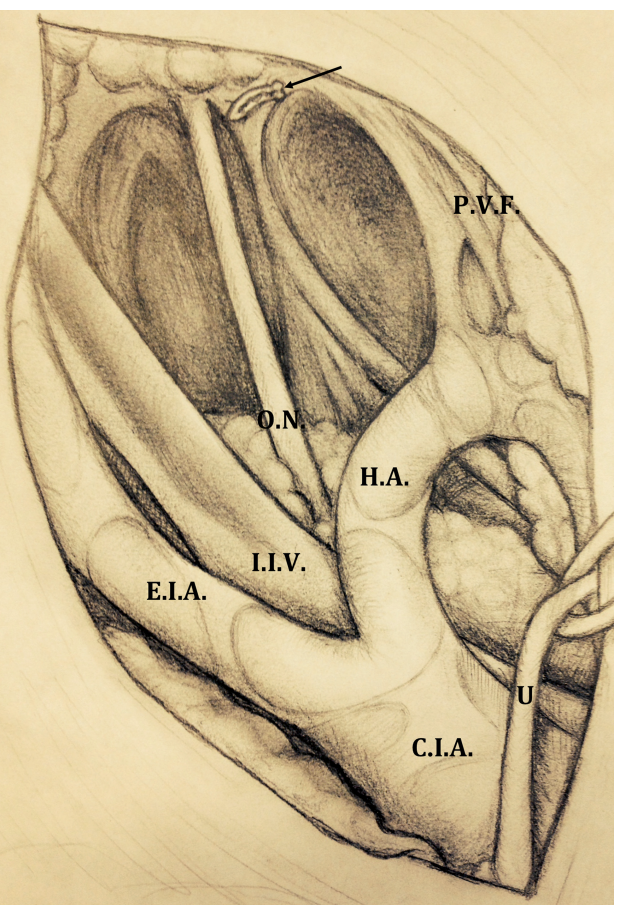


Figure 3 - The dissection is carried out from the pubic bone (a Hem o-lok clip is placed just cranially to the Cloquet lymph node, see the arrow) to the crossing of the ureter over the common iliac artery. Then, the obturator fossa is reached and the lymph nodes are progressively dissected until complete exposition of obturator nerve (O.N) is achieved. Prevesical fascia (P.V.F). Internal iliac vein (I.I.V).



that usually is preserved and dissected (one Hem-o-lok clip is placed just cranially to the Cloquet's lymph node, preserved in order to prevent lymphocele and lymphoedema) (Figure-3). Lateral limit of such a dissection is the medial portion of the external iliac artery: the tissue covering the lateral part of the artery is spared in order to prevent lymphoedema.

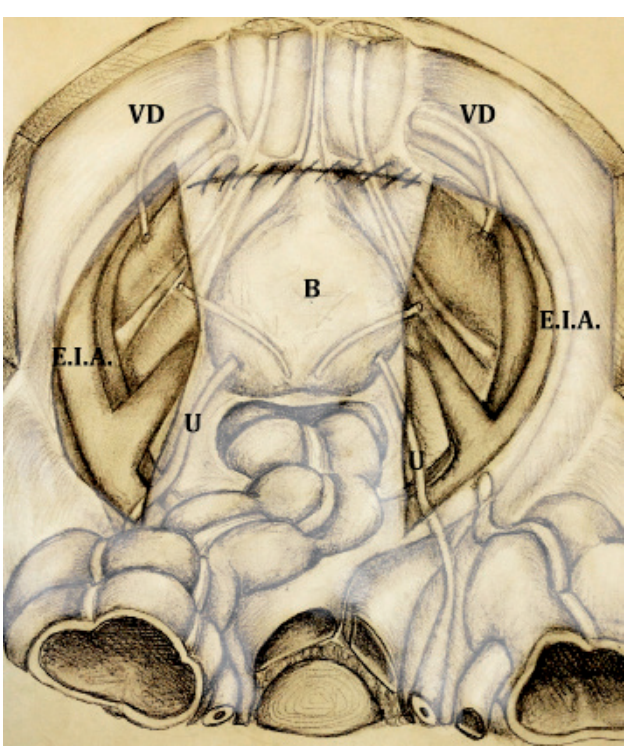
4-Then the obturator fossa is reached and the lymph nodes are progressively dissected until complete exposure of obturator nerve. Dissection is here performed with care in order to avoid any neural injury. The dissection is started at the angle between the external iliac vein and the pubic bone. The lymphatic package is dissected beneath the external iliac vein, proceeding until the pelvic side wall, which is the lateral limit of the dissection. The proximal attachments of the lymphatic packages are dissected by using a combination of either sharp or blunt dissection, paying attention in order to avoid any sharp, blunt, or thermal injury to the obturator nerve. The same technique is performed contra-laterally.

5-Once prostatectomy and its reconstructive phase is completed, the anterior peritoneum is sutured by using a running 3/0 "barbed" suture. At the end of the suture the peritoneal cavity and retropubic space are not in communication yet thanks to previously preserved pre-vesical fascia (Figure-4). Bilateral incisions of peritoneum done in order to perform RAEPLND are not sutured: at the end of the procedure, reconstruction is performed only at midline where parietal peritoneum covers the retropubic space.

Two drains are placed: one intraperitoneally and one, extraperitoneally, in the Retzius space: they are usually removed at the 1st and the 2nd postoperative day, respectively.

Specifically for the purpose of the study, in order to assess the location of lymph-nodal metastasis, lymph nodes were sent in two separated packages removed by two laparoscopic endo-catches from both sides (for convention, the right sided ones are secured by hem-o-lok clips to label them): one containing the lymphatics overlying the distal portion of the common, external (only medial portion) and obturator fossa's lymph nodes (they will be disposed on histopathological analysis Table from the caudalest to the cranialest in order to be recognized by pathologist); the other containing the internal iliac artery and the presacral lymphatic packages.

Figure 4 - At the end of the peritoneum suture the peritoneal cavity and retropubic space do not communicate thanks to prevesical fascia. Bladder (B). Vas deferens (V.D).



Histopathological analysis

A dedicated uro-pathologist performed all histopathological analysis. Tissue was submitted for permanent sectioning. Frozen section analysis was not routinely performed unless that in case of enlarged and/or clinically suspicious nodes.

Pathologic work-up to detect lymph nodes as well as lymph nodal metastases included direct visualization, palpation and standard hematoxylin-eosin staining.

Outcome measurements

Skin-to-skin time, RAEPLND (right, left and overall) operative time, estimated blood losses, intraoperative complications (as classified by modified Satava system (20)), postoperative complications (as classified according to the modified Clavien system (21)), duration of hospitalization, catheterization time and transfusion rate were collected and analyzed.

The number and the locations of dissected lymph nodes on each side and the rate of lymph nodal metastases were recorded. The daily amount of drainage secretion (mL) and duration of drainage (days) were registered. In case of patient with a drained volume over 200mL/24 hours, urinary leakage was excluded by creatinine measurement.

Statistical analysis

The descriptive statistics of patients characteristics are presented as median (IQR, inter quartile range) for continuous covariates, while as frequency (percentage) for categorical ones. No formal inferential test was performed, since the patients came from a single series. The data were analyzed by R 3.0.2 (R Foundation for Statistical Computing, Vienna-A, www.R-project.org).

RESULTS

Preoperative diagnostics were negative for metastasis in every case. All enrolled patients underwent RAEPLND+RARP. No patient received neo-adjuvant hormonal therapy. Median overall operative time was 150 (IQR 135-170) min. Median RAEPLND alone operative time was 38 (IQR 32.75-41.25) min; for right and left side, 18 (IQR 15-29) and 20 (IQR 15.75-30), respectively, p=0.567. No case was converted to open surgery. Patients were discharged after a median hospital stay of 5 days (IQR 4-7).

Median number of lymph nodes retrieved per patient was 25 (IQR: 19.25-30), specifically 13 (IQR: 11-16) and 11 (IQR: 8-15), right and left side, respectively. In 19 patients (12.41%) metastasis was found at the level of pelvic lymph nodes. Median number of positive lymph nodes was 1 (IQR: 1-4.6) per patient.

In lymph nodal metastatic patients, median PSA level was 8.2 (IQR 5.5-16.5) ng/mL versus 7.3 (IQR 5.3-11.4) ng/mL in negative lymph node-patients. Distribution of metastatic lymph nodes according to pathological stage and final Gleason Score is reported in Tables 2 and 3. Location and number of metastases per anatomic region are reported in Table-4.

In 11 patients (7.3%), RAEPLND-associated complications occurred: one patient (0.7%) had a temporary and reversible neuropraxia (involving ischiatic and obturator nerve). Ten (6.6%) patients were found to have lymphocele at ultrasonography performed at 1st month postoperatively with a

Table 2 - Metastatic lymph nodes according to pathologic staging.

Pathologic T stage	N° of patients	N° of patients with LNI (%)
Overall	**153**	**19 (12.4)**
pT2a	17	0
pT2b	5	0
pT2c	41	1 (2.4)
Total pT2	**63**	**1 (1.6)**
pT3a	56	6 (10.7)
pT3b	34	12 (35.3)
Total pT3	**90**	**18 (20.0)**
pT4	0	0

Table 3 - Metastatic lymph nodes according to pathologic grading.

Pathologic Gleason Score	Nº of patients	Nº of patients with LNI (%)
Overall	153	19 (12.41)
5	0	0
6	5	0
7a (3+4)	59	2 (3.38)
7b (4+3)	47	7 (14.89)
8	36	5 (13.88)
9	6	5 (83.33)
10	0	0

Table 4 - The location and number of metastases per anatomic region.

Anatomic region	Total lymph nodes, No	Metastatic lymph nodes, No (%)	Number of exclusively metastatic lymph nodes in this region
Iliac-obturator left	1611	31 (1.92)	**13**
Hypogastric-presacral left	236	8 (3.38)	**2* and 1****
Iliac-obturator right	1537	22 (1.43)	**7**
Hypogastric-presacral right	524	7 (1.33)	**2* and 1****
Total	**3908**	**68 (1.74)**	-

Evidenced by the pathologist only *in the hypogastric region or **in the presacral region

ranging size from 3.2 to 8.0cm. Among them only 5 (3.3%), with a lymphocele with ranging size from 4.5 to 8.0cm, were symptomatic complaining of lower abdominal pain and required percutaneous drainage. Non symptomatic lymphoceles (5 patients) measured lower than 4.5cm maximum diameter and were located in obturator fossa. At 3rd month control these lymphoceles were stable.

The overall median blood loss for RARP including the RAEPLND was 200 (IQR 150-350) mL; 1 patient (0.7%) who had preoperative serum haemoglobin concentration of 9.2g/dL received intraoperative blood transfusions (1 unit) during prostatectomy phase.

DISCUSSION

In men diagnosed with localized PCa who have opted for surgical treatment by radical prostatectomy, one of the key decision points of urologist is whether to include or not a staging PLND. Whether or not such a procedure has a therapeutic role in prostate cancer management still remains under investigation and even Guidelines do not agree on a uniform approach about it (22).

Up to date, standard imaging technologies (e.g. CT and MRI) are still able to detect enlarged lymph nodes over 1cm in diameter (23).

The rationale for regional lymph nodes dissection in prostate cancer would be the detection of occult micro-metastases for a proper staging of patients and identification of those who might benefit from adjuvant treatments.

Current indications for PLND vary. Increasing evidences support EPLND in patients with PCa if PSA level is over 10ng/mL or Gleason Score ≥7 (2, 9-12). Accordingly, recent data suggest avoiding lymph nodes dissection in low-risk patients (2, 8).

Briganti et al. reported that using a 5% nomogram cut-off for risk of LNI, about 70% of patients would be spared of EPLND, and LNI would be missed in only 1.5% (19). Indeed, we avoided EPLND in all patients with a nomogram-derived LNI risk <5%.

It is known that Briganti et al. predictive nomogram is based on easily available clinical parameters, such as pretreatment PSA, clinical stage, primary and secondary biopsy Gleason score, and percentage of positive cores (13). On the other side, the incidence of lymph nodal metastases is not exclusively dependent by such parameters: quality of surgical performance and extent of PLND have a crucial role. The lack of standardization in terminology and definitions of anatomic dissection landmarks has caused difficult comparisons among published data about this topic.

For this reason, some authors stressed the importance of evaluating the number of removed lymph nodes as a measure of the quality of PLND. The study by Weingärtner et al. on cadavers considered a total number of removed lymph nodes equal to twenty to be sufficient for accurate staging (24). However, given the fact that during the procedure nodes count is not available for surgeon, definition of extended rather than limited PLND is not based on the number of nodes removed but on the anatomical template.

Nowadays EPLND has been widely accepted as the standard of care when a regional surgical staging is required during surgery for PCa (25). It has been described as involving the lymphatic

packages along the external iliac artery and vein, the obturator fossa, along the obturator nerve, along the internal iliac artery and presacral lymph nodes. Its metastases-detection performance is two- to three-fold the limited PLND one, increasing the diagnostic value of lymph nodes dissection (11, 15, 19).

Extending PLND template may increase the risk of complications: this have to be counterbalanced by potential benefits. Overall and EPLND-related complications during RARP series are reported in Table-5. As expected the most frequently reported complication in literature was lymphocele formation, proportional with the number of lymph nodes removed (10.3% with >10 nodes versus 4.6% with <10 nodes) (8, 12, 14). Previous reports showed complications' rates for limited and standard PLND ranging from 2 to 9.8%, while EPLND complication's rates vary from 19.8% to 75% (15, 20). Seventy-five percent of complications in EPLND may be due to extensive dissection of lymphatic tissue along the external iliac artery that primarily supplies the lower extremities. Moreover, this area has never been shown to be affected by metastases in previous anatomical studies (7, 8).

To reduce or to prevent PLND-associated morbidities, advices regarding meticulous surgical technique have been provided by several authors (8, 9): first, all the lymphatic vessels coming from the lower extremities should be tied by using ligatures instead of clips. In our experience a Hem-o-lok clip only is placed just cranially to the Cloquet's lymph node, preserved in order to prevent lymphocele. Second, all lymphatics lateral to the external iliac artery should be spared in order to prevent lymphoedema. Third, two drainages should be placed, one per side of the pelvis and should not be removed until the total amount of fluid drained is below 50mL per 24 hours.

In the present prospective study we reported our experience with RAEPLND. Transperitoneal approach was chosen in all cases allowing an excellent working space. Our median number of

Table 5 - Overall and EPLND-related complications in RARP series.

Study	Overall, %	Potentially related to EPLND,%	Clavien grade 1-2, %	Clavien grade 3-4, %
Stone et al. (8)	14.6	10.6	Lymphocele, 6 Lymphoedema, 2	1
Feicke et al. (11)	-	7	Lymphatic fistula, 1 Lymphoedema, 2 Lymphocele, 4	0
Patel et al. (17)	12.3	8.2	Lymphocele, 2.1 Lower extremity oedema, 3	0
Zorn et al. (18)	13	5	Lymphocele, 2	Ureteral injury, 1 Bladder injury, 1 Vena cava compression, 1 Pulmonary embolus, 1
Katz et al. (44)	35.1	Limited: 8.1 Extended: 3.1	Neuropraxia, 3 Neuropraxia, 3.1	Deep vein thrombosis, 3 Pulmonary embolus, 1.6
Sagalovich et al. (45)	-	-	Lymphocele, 2.1	0
Davis et al. (46)	-	5	Lymphocele, 4 Neuropraxia, 3 Lower extremity oedema, 4	1
Present series	8.0	7.3	Neuropraxia 0.7 Lymphocele 3.3	Lymphocele 3.3

lymph nodes removed was 25, in line with published data on open series (1, 3, 17, 26-30) and comparable to the similar study published by Feicke et al. on robot-assisted approach who reported a median number of lymph nodes removed of 19 (11).

Among the 68 metastatic lymph nodes, 15 (22.05%) of them were localized either in the hypogastric or presacral regions (both not included in the limited PLND). In the vast majority, they were associated to the presence of lymph node metastases in the external/obturator iliac area. In our series, we observed just one patient (5.26%) with positive lymph nodes in bilateral presacral region only and two patients (10.52%) with hypogastric artery positive nodes only.

A dedicated comment about this finding is needed. It could seem this paper is actually an argument for not doing EPLND due to such a large number needed to treat (51:1) in order to record a benefit in undergoing EPLND (just 3 patients out of 153, 1.96%, exclusively found metastasis in hypogastric/presacral region). Moreover we underline that, in 5 patients having metastatic iliac-obturator lymph-nodes, 11 positive hypogastric/presacral region lymph-nodes were retrieved (3.26%). If we sponsor the therapeutic role of lymph-nodes dissection, this is an important finding. On the other side, we daily perform PLND in all patients, with a probability of lymph-nodal metastasis according to Briganti updated nomogram over 5%, which is not so different from this case-study percentages. If we add that our described technique was safe and that EPLND consumed just half an hour in the overall operative time, we believe that this is the right direction. Moreover, by performing hypogastric/presacral lymph-nodes removal, we are convinced about the fact that, in case of biochemical recurrence, further exams such as total body choline-PET will be more reliable.

After more than 100 procedures we suggest a possible solution to the most important surgical matter: the complete reconstruction of peritoneum at the end of RARP, in order to avoid communication between the peritoneal cavity and retropubic space is paramount. We underline that the bilateral peritoneal incisions above the iliac vessels are not deliberately sutured in order to facilitate lymphatic reabsorption by the peritoneum.

We believe that EPLND-related complications occur due to the fact that peritoneal end extraperitoneal space remains in communication at the end of the procedure, and this is particularly true for transperitoneal laparoscopic (pure or robot-assisted) approach.

The strength of our technique is the anatomical reconstruction of the two operative fields, that will be separated again thanks to the peritoneum reconstruction and the previous sparing of prevesical fascia.

The potential advantages are: first, the avoided risk of lymphatic leakage into the retropubic extraperitoneal space; second, the displacement of bowel loops into the retropubic space is avoided; third, eventual future surgeries are facilitated thanks to preserved and separated anatomical spaces. How were we able to remove a high number of lymph nodes in a relatively short operative time? Again thanks to our experience, we here underline some crucial technical steps: first, on the right side, ureter should be identified and then it should always remain inside intraoperative view; in case of any doubts, it should be suspended; second, on the left side, surgeon should know that ureter will not be sufficiently mobilized by medicalization of peritoneum only: indeed it is paramount to suspend it on this side; third, left iliac vessels anatomy is different: hypogastric vein partially covers sacrum: for this reason presacral lymp-nodes dissection is more challenging on this side.

Even if we were not able to perform long--term functional evaluation, our experience taught us that careful presacral lymph nodes dissection (thanks to robotic-system optical magnification) allows better functional outcomes.

The strengths of the paper are the prospective fashion which was designed by; all specimens were analysed by a dedicated expertise uro-pathologist; follow-up for complications occurrence was adequate. On the other side, the study is not devoid of limitations: case-study was a cohort of patients with high–risk prostate cancer selected according to Briganti nomogram for risk of lymph-nodal metastasis; no comparison with alternative technique was performed; the evaluations were confined about technical aspects, complications and how to prevent them. We disclose

for unreporting functional outcomes: they will be object of future researches.

CONFLICT OF INTEREST

None declared.

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Pregnancy and birth after intracytoplasmic sperm injection with normal testicular spermatozoa in a patient with azoospermia and tail stump epididymal sperm

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ABSTRACT

Main findings: An intriguing yet perplexing case report of a successful pregnancy and live birth with intracytoplasmic sperm injection using normal testicular sperm, after the finding of azoospermia in the semen analysis and discovering only tail stump abnormal sperm in the epididymis.
Case hypothesis: A tail stump sperm defect of genetic origin was suspected. However, after obtaining normal testicular sperm we concluded that obstructive azoospermia, either idiopathic or secondary to multiple minor genital trauma was the plausible scenario. This has rendered the search of previous reports on a similar condition, but none was found. However, it has raised scientific thoughts for future research.
Promising future implications: The importance of reporting this case is to alert urologists performing sperm retrieval that healthy and morphologically normal sperm may be found in the testis of azoospermic men with 100% tail stump epididymal sperm. Retrieval of normal testicular sperm obviates the need of a more complex investigation, including sperm electron microscopy. It also offers the possibility of utilizing such gametes for sperm injections rather than abnormal tail stump sperm that may be associated with a poor reproductive outcome.

ARTICLE INFO

Key words:
Sperm Injections, Intracytoplasmic; Sperm Retrieval; Sperm Tail

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INTRODUCTION

As many as 186 million people are estimated to be affected by infertility worldwide, of which the male factor accounts for more than 50% of all cases of childlessness (1). While the introduction of intracytoplasmic sperm injection (ICSI) has undoubtedly offered opportunities to treat the most severe cases of male infertility, the advancement and modernization of assisted reproductive technologies (ART), however, still fail to answer the etiology of some rare and enigmatic conditions, as described in this case report, gearing to future research.

Scenario

A 30-year-old male and his spouse were seeking treatment for their infertility condition at The Fertility Clinic Skive Regional Hospital. The couple presented with an infertility history of one year duration and no previous treatments. The male partner had undergone multiple semen analyses, all of which revealed azoospermia. The patient`s sexual, medical/surgical and family histories were essentially unremarkable. Notably, the patient was inquired about and confirmed that he had not had any history of sexually transmitted diseases or exposure to gonadoto-

xins. Childhood and pubertal development were also normal, but for the fact that he reported being a former goalkeeper playing handball with many hits on his genitals, which were mostly minor injuries with no need for medical assistance. He was a non-smoker with a normal body mass index, but reported alcohol consumption to a maximum of 2-4 units per day. His physical examination findings were unremarkable. The genitalia examination revealed a normal penis and both testes located in the scrotum, measuring approximately 20mL each in volume with no hydrocele. The epididymis were normal in size and consistency. Examination of the spermatic cord revealed a palpable normal vasa deferentia and absence of varicocele.

Semen analyses were repeated, confirming azoospermia after centrifugation of the liquefied specimens. Further investigations revealed a normal hormonal profile: FSH level of 1.5IE/L (normal range: 1.2-15.8IE/L), LH level of 4.4IE/L (normal range: 1.7-8.6IE/L), testosterone level of 17.87nmol/L (normal range: 11-34nmol/L) and prolactin of 274MIE/L (normal range: 90-580MIE/L). The chromosomal analysis showed a normal karyotyping with no Y chromosome deletion or cystic fibrosis mutations found.

A diagnostic percutaneous epididymal sperm aspiration (PESA) was carried out in the epididymis head, which found all spermatozoa to be morphologically abnormal, even after repeating the procedure at the contra-lateral side. Of note, all retrieved spermatozoa were immotile and showed the same tail defect, namely either 'tail stump' or no tail (Figures 1 a-c). Sperm viability testing was not performed.

With no normal spermatozoa yielded on PESA, a diagnostic testicular sperm aspiration (TESA) was performed at the same operative time. Surprisingly, normal spermatozoa were obtained (Figures 1d and 1e). A careful microscopic examination revealed no tail stump spermatozoa in the TESA sample, of which approximately 10% retrieved spermatozoa exhibited sluggish non-progressive motility. Both PESA and TESA specimens were frozen, using the liquid nitrogen vapor technique, and stored. Subsequently, the couple underwent an ICSI cycle. The mature retrieved oocytes were micro-inseminated with TESA-normal and motile spermatozoa from the male partner. The couple successfully conceived and delivered a healthy baby at term. Two years later, the couple came back for replacement of the remaining embryos that had been stored, however, the attempt was unsuccessful. Recently, they donated the straws of frozen tail stump spermatozoa obtained from PESA and the normal spermatozoa from TESA for research purpose.

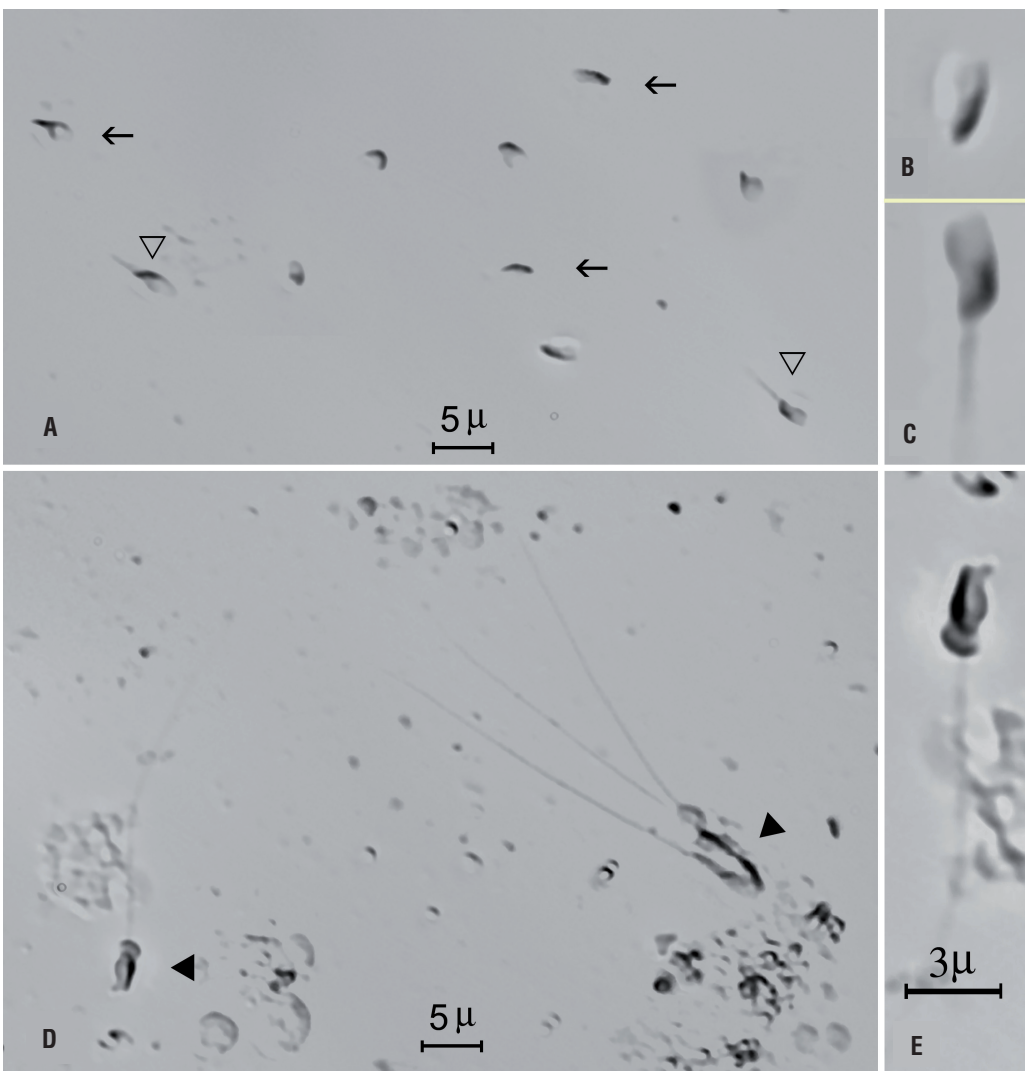
Case hypothesis and rational

Our initial hypothesis was "tail stump sperm defect of genetic origin" given the observation of 100% abnormal spermatozoa on PESA. However, after obtaining normal testicular sperm on TESA, of which about 10% were motile, we hypothesized that the patient had obstructive azoospermia either idiopathic or secondary to multiple minor genital trauma. Although viability studies would add to our report, we have not carried out viability testing in the PESA and TESA specimens. Given all spermatozoa retrieved from both epididymis were immotile and exhibited tail stump, our approach was to perform TESA in the same operative time. We retrieved sperm with normal tails by TESA, of which approximately 10% exhibited sluggish non-progressive motility, thus confirming viability in a proportion of the retrieved testicular spermatozoa. The importance of reporting this case is to alert urologists performing sperm retrieval that normal sperm may be found in the testis of men with tail stump epididymal sperm. The finding of normal testicular sperm by TESA will obviate the need of a more complex investigation including sperm electron microscopy. Also, it offers the possibility of utilizing such normal gametes for sperm injections rather than the abnormal tail stump sperm that may be associated with a poor reproductive outcome.

DISCUSSION AND FUTURE PERSPECTIVES

The excitement of obtaining a successful pregnancy and healthy offspring by ICSI after an

Figure 1 - Photomicrographs of wet preparations containing spermatozoa obtained by percutaneous epididymal sperm aspiration (PESA; a-c) and testicular sperm aspiration (TESA; d, e) from an infertile 30 year-old male with azoospermia. Open black arrows and triangles indicate tail stump and no tail epididymal spermatozoa (a). In 'b' and 'c', individual spermatozoa with no tail (b) and tail stump (c) are highlighted. Black triangles indicate normal spermatozoa retrieved from the testicle (d). A spermatozoon with a fully developed tail is highlighted (e). Photographs obtained using a Nikon Eclipse inverted microscope with Hoffman phase contrast optics (Nikon, Japan). The magnification bar shown in 'e' also applies for 'b' and 'c'.



unexpected diagnosis of azoospermia and 100% tail stump epididymal spermatozoa, and the subsequent finding of normal testicular sperm, was the driving force of reporting this intriguing case.

Although anecdotally pregnancies have been reported in humans with the utilization of tail stump spermatozoa and ICSI, the abnormally shaped sperm may negatively influence the reproductive outcome (2-4). Moreover, given that a genetic, but still unmapped defect has been postulated to be the cause of such tail defects, there is a concern of transmitting a genetic abnormality to the male offspring that will render them infertile (5-7). As a matter of fact, such a genetic linkage, either direct or indirect, has been associated with a number of sperm defects in different species (5, 8).

In humans, the finding of 100% tail stump spermatozoa is rare (9). Although the origin of this defect is unknown, testes sections revealed that damage might occur during spermiogenesis in the latest stages of flagellum elongation at the spermatid stage, which resulted in a generalized, blocked formation of the flagellum associated with an absence of axonemes and accessory fibers (9, 10). Electron microscopy studies revealed that the 'stump' category exhibits the sperm tail region organized as uniflagellate, and the extremely short axoneme can have a '9+2' or '9+0' arrangement generally with dynein arms, while the 'short tail' category has a biflagellate arrangement and a '9+0' or '9+1' axoneme almost devoid of dynein arms (5).

Although a genetic linkage has been discussed in previous reports involving tail stump sperm (2-7, 11), there may be other possible explanations. In an earlier study evaluating 247 men with severe asthenozoospermia, Chemes and colleagues reported that most men presented with nonspecific flagellar anomalies, which were random, secondary alterations that affected a variable number of spermatozoa in different samples (6). In such cases, there was no familial/genetic inheritance, and the flagellar anomalies were secondary to different andrological disorders. It has been thus suggested that this aforementioned type of flagellar anomaly be differentiated from the less common fibrous sheath dysplasia that is associated with genetic abnormalities or familial inheritance (6, 7).

In our reported case, azoospermia was found in multiple ejaculates after centrifugation. In such cases, a distinction should be made between obstructive and non-obstructive azoospermia. When normal genitalia (testes, epididymis and vasa deferentia) and endocrine profile are found in a normal virilized adult male, the finding of spermatozoa within the epididymis is highly indicative of obstructive azoospermia (12), as shown in our patient. Obstruction in the male reproductive system can be congenital or acquired. Acquired causes include vasectomy, infection and genital trauma (13, 14). The most common congenital form of obstructive azoospermia (OA) is congenital bilateral absence of vas deferens (CBAVD), which is linked to mutations in the cystic fibrosis transmembrane-conductance regulator (CFTR) gene (13). Our patient, however, neither had a clinical genitourinary infection or surgery, nor were the vasa deferentia absent on physical examination. Therefore, the possible explanation for his condition would be either genital trauma as noted in his medical history, or a congenital idiopathic obstruction.

The importance of investigating CFTR mutations in a case like ours relies on the fact that such mutations have been implicated in bilateral epididymal obstruction even in the presence of normal, bilateral palpable vasa (15), as many as 47% patients with idiopathic bilateral epididymal obstruction carry CFTR mutations (16, 17). The most common identified mutations were IVS8-5T, ΔF508, R117H and L206W, but none of them were found in our patient. Young syndrome is another rare disease primarily characterized by a bilateral epididymal obstruction with azoospermia. However, bronchiectasis and chronic sinusitis are common features in patients with this disorder, and epididymal sperm is normal and motile (15).

The reason why all spermatozoa retrieved from the epididymis of our patient had abnormal tails is unknown. However, given normal sperm were found within the testis it is presumptive that the morphological alterations occurred within the epididymis after sperm release from the seminiferous tubules. We therefore speculate that the presence of tail stump sperm in our case scenario might be due to prolonged epididymal stasis resulting in senescent sperm as sometimes seen in post-vasectomy vas and epididymal fluid aspirates (18-20). Such sperm tail defects may be associated with other concomitant epididymal pathologies, namely subclinical microbial infections and antisperm antibodies.

Lastly, the delivery of a healthy baby is the ultimate goal in assisted reproductive techniques. This has been achieved in our case with the micro-insemination of oocytes using normal sperm retrieved by TESA. The reproductive outcome of ICSI, using testicular sperm retrieved from men with OA has been reassuring, and TESA is associated with few complications (14, 21). The chances of achieving a live birth and the profile

of neonates born after sperm injection with a so--called normal epididymal or testicular sperm do not seem to be related to the cause of obstruction. Moreover, ICSI outcomes are comparable using frozen-thawed or fresh sperm retrieved from men with OA (22).

Despite it being an uncommon condition, the finding of azoospermia associated with 100% abnormal tail stump epididymal sperm may pose many challenges to the treating physicians. We hope the case presented here can help urologists to provide an even better management of patients, and encourage further research to enlighten the origin of this enigmatic condition.

CONCLUSIONS

Urologists performing sperm retrieval should be aware that healthy and morphologically normal sperm may be found in the testis of men with obstructive azoospermia and 100% tail stump epididymal sperm.

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CONFLICT OF INTEREST

None declared

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Adult granulosa cell tumor of the testis masquerading as hydrocele

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ABSTRACT

Adult testicular granulosa cell tumor is a rare, potentially malignant sex cord-stromal tumor, of which 30 cases have been described to date. We report the case of a 43-year--old male who complained of a left testicular swelling. Scrotal ultrasound showed a cystic lesion, suggestive of hydrocele. However, due to a clinical suspicion of a solid--cystic neoplasm, a high inguinal orchidectomy was performed, which, on pathological examination, was diagnosed as adult granulosa cell tumor.

Adult testicular granulosa cell tumors have aggressive behaviour as compared to their ovarian counterparts. They may rarely be predominantly cystic and present as hydrocele. Lymph node and distant metastases have been reported in few cases. Role of MIB-1 labelling index in prognostication is not well defined. Therefore, their recognition and documentation of their behaviour is important from a diagnostic, prognostic and therapeutic point of view.

ARTICLE INFO

Key words:
Granulosa Cell Tumor; Sex Cord-Gonadal Stromal Tumors; Testis; Immunohistochemistry; Neoplasms, Germ Cell and Embryonal

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INTRODUCTION

While granulosa cell tumor represents the most common sex cord-stromal tumor arising in the ovary (1), and juvenile testicular granulosa cell tumor (TGCT) is the commonest sex cord-stromal tumor seen in male children, adult TGCT remains an enigmatic entity. Due to its rarity, not much is known about its natural course; however, literature suggests that adult TGCTs are slow-growing neoplasms with potential for lymph node metastasis, even many years after initial diagnosis. We report a case of this rare tumor which was predominantly cystic, causing a diagnostic dilemma clinically, and ultimately diagnosed on histopathology.

Case Report

This 43 years old male, under follow-up in the Urology clinic for stone disease, complained of painless, progressively increasing left testicular swelling for two months. On physical examination, vitals were stable. No abdominal distension or mass was noted. Peripheral lymphadenopathy was absent. Left testicular enlargement was identified, caused by a cystic scrotal swelling. Scrotal ultrasound (Figure-1a) showed an anechoic cystic lesion measuring 5.5cm x 3.4cm, with nodular soft tissue shadows at the periphery and only a thin rim of testicular tissue. Based on ultrasonography, differential diagnoses included intra-testicular cystic neoplasm and a cystic lesion compressing

the testis. On investigation, routine haematological and biochemical parameters, as well as serum alpha-fetoprotein (AFP), lactate dehydrogenase and human chorionic gonadotropin levels were within normal limits. The patient was counselled for and submitted to a high-inguinal orchidectomy. Post-operative period was uneventful. The patient is doing well one year after surgery. CT abdomen revealed no retroperitoneal lymphadenopathy (Figure-1b).

Pathological examination

Left high inguinal orchidectomy specimen comprised of testis measuring 8cm x 5.5cm x 4.5cm, with attached spermatic cord measuring 6cm. A predominantly cystic tumor (Figure-2a) measuring 6cm x 3.5cm x 2.5cm was identified, almost completely replacing the testis, with a thin rim of compressed normal testicular parenchyma at the lower pole. Cysts varied from 0.5cm to 5cm in diameter, were smooth-walled, and contained clear fluid. Few solid nodules, 0.4cm to 1cm in maximum dimension, were seen within the cyst walls (Figure-2b). The tumor did not appear to infiltrate the tunica albuginea. No areas of hemorrhage or necrosis were identified.

On microscopic examination (Figure-3), the tumor was well-circumscribed, unencapsu-

Figure 1 - Scrotal ultrasound showing a cystic lesion with nodular soft tissue shadows at the periphery (a). CT abdomen one year post-surgery shows no lymphadenopathy (b).

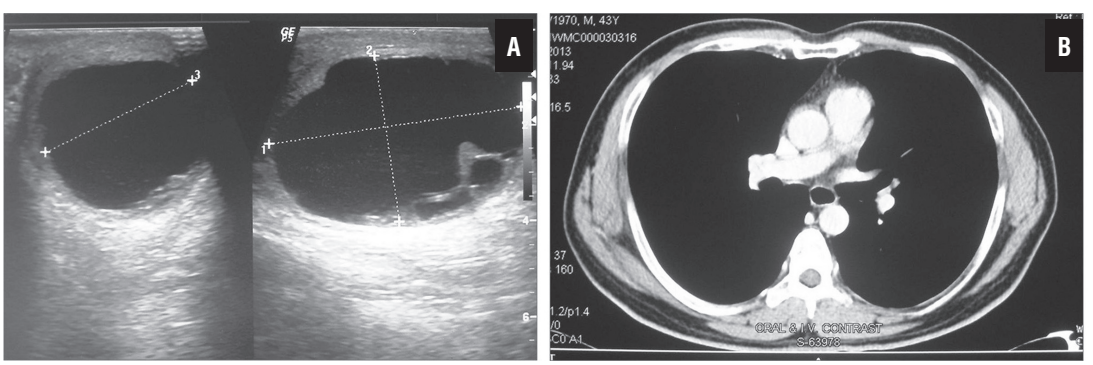


Figure 2 - Orchidectomy specimen showing a solid cystic tumor (a); multiple cysts with few small nodules (arrows) are seen, along with a rim of normal testicular parenchyma (N) at the periphery (b).

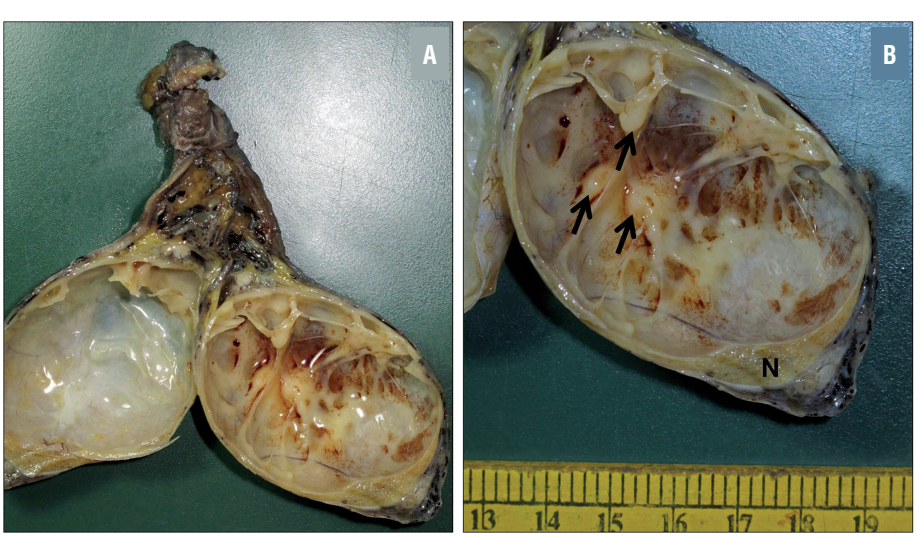
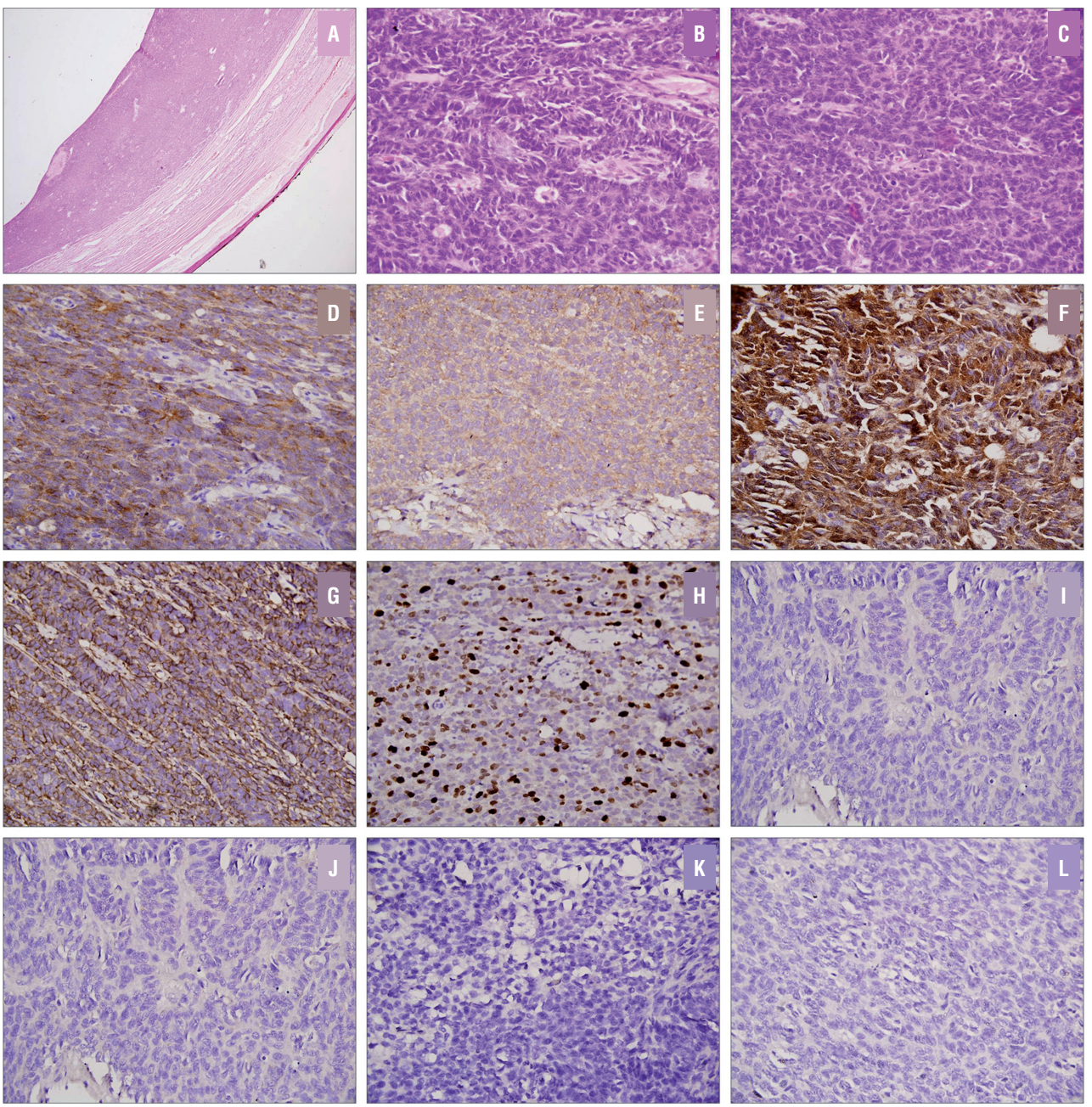


Figure 3 - Photomicrographs showing solid areas of tumor along with compressed seminiferous tubules at the periphery (a; HE, x40); tumor cells were arranged in trabeculae and microfollicles, had scant cytoplasm and ovoid nuclei with grooves (b; HE, x400); frequent mitotic figures seen (c; HE, x400). Tumor cells were positive for inhibin (d), MIC2 (e), calretinin (f) and vimentin (g), MIB1-LI was high (h); EMA (i), AFP (j), synaptophysin (k) and CD117 (l) were negative (IHC, x400)



lated, and was composed of monomorphic cells arranged in sheets and trabeculae. Focally, microfollicular structures (Call-Exner bodies) were seen. Tumor cells had scant cytoplasm, ill-defined cytoplasmic borders, and ovoid medium-sized nuclei with fine chromatin and inconspicuous nucleoli. Longitudinal nuclear grooves were seen at places. Frequent mitotic figures (8-10/10 high power fields) were identified. No necrosis, lymphovascular invasion or pseudosarcomatous areas were present. On immunohistochemistry, tumor cells were diffusely immunopositive for vimentin, inhibin, MIC2, and calretinin. They were negative for pancytokeratin (CK), epithelial membrane antigen (EMA), leukocyte common antigen (LCA), AFP, placental alkaline phosphatise (PLAP), CD117 and synaptophysin. MIB-1 labelling index (LI) was high (18% in highest proliferating areas). Based on histomorphological and immunohistochemical features, a diagnosis of adult granulosa cell tumor of the testis was made. Section from resected end of spermatic cord was free of tumor.

DISCUSSION

Adult testicular granulosa cell tumor (TGCT) is a rare sex cord-stromal neoplasm arising in the testes, of which thirty cases have been reported until now (2-15). Majority of patients present with testicular enlargement which is painless and of variable duration (3). Some may present with features of hormonal dysregulation, like gynaecomastia (4, 5). In some instances, however, this tumor may be detected incidentally, as in our case (6). The age range at presentation is wide, varying from 16 to 77 years (2).

Adult TGCTs occur as solid, circumscribed tumors, in contrast to the multicystic juvenile TGCTs. While it is not unusual to identify small cystic foci within adult TGCTs (1), predominantly cystic tumors are rarely encountered, contributing to lack of suspicion of this diagnosis preoperatively, as in the case under discussion. Only two cases have been previously documented as presenting with hydrocele on the same side of tumor (7, 8).

A variety of microscopic patterns may occur in adult TGCTs, making differential diagnosis from Sertoli-Leydig cell tumor, germ cell tumors, non-Hodgkin lymphoma and neuroendocrine tumors difficult. Microfollicular, macrofollicular, trabecular, and insular growth patterns are commonly observed (3). Typical Call-Exner bodies may or may not be present. These tumors are immunopositive for vimentin, inhibin, MIC2, calretinin, and smooth muscle actin (3), and are usually negative for CK, EMA, LCA, synaptophysin, chromogranin, PLAP, and AFP (9). Morphological features along with immunohistochemistry help in clinching the correct diagnosis (Table-1). A recent review by Rabban et al. succinctly summarizes the immunohistochemical differential diagnosis between sex cord stromal tumors and germ cell tumors using newer IHC markers (16). While PLAP is a fairly reliable marker for germ cell tumors like dysgerminoma, yolk sac tumor, choriocarcinoma, and embryonal carcinoma, SALL4 is a novel marker that has higher sensitivity for the above-mentioned tumors with the exception of choriocarcinoma. OCT4 is a transcription factor which is expressed in dysgerminoma and gonadoblastoma. Sex cord-stromal tumours including adult granulosa cell tumours are, however, negative for PLAP, OCT4 and SALL4. Glypican-3 is another new marker with high specificity for yolk sac tumors, and is not expressed in sex cord stromal tumors. Newer markers for the sex cord stromal family of tumors include SF-1 and FOXL2, which are highly specific and are not expressed by germ cell tumors.

In contrast to their more common juvenile counterparts that have a benign course and are cured by simple orchidectomy, adult TGCTs are believed to be potentially malignant tumors, which behave more aggressively than their namesakes in the ovary (2, 3). Reports of metastases to lymph nodes as well as distant sites, even as late as ten years after initial surgery, have served to consolidate this opinion (10). Definitive criteria for malignancy are lacking, and identification of aggressive cases remains difficult. Few features have been proposed by Jimenez-Quintero et al. (10), including size greater than 7cm, lymphovascular invasion, necrosis, and hemorrhage, while Hanson et al. have suggested that tumors greater than 5cm may behave aggressively (3). However, it is not clear whether size of the entire tumor should be taken into consideration in tumors that are solid-cystic, or only the solid

Table 1 - Immunohistochemical panel for differential diagnosis of adult testicular granulosa cell tumor from morphologically similar tumors.

Differential diagnosis	Vimentin	CK	EMA	Inhibin	Calretinin	MIC2	PLAP	CD117	SALL4/ OCT4	Glypican-3	SF-1/ FOXL2	AFP	LCA	Chromogranin/ Synaptophysin
Adult TGCT	+	-/focal	-	+	+	+	-	-	-	-	+	-	-	-
Seminoma	+/-	-/+	-	-	-	-	+	+	+	-	-	-	-	-
Yolk sac tumor	-	+	-	-	-	-	+	-	+	+	-	+	-	-
Non-Hodgkin lymphoma	+	-	-	-	-	-	-	-	-	-	-	-	+	-
Sertoli-Leydig cell tumor	+	+	-/+	+	+	+	-	-	-	-	+	-	-	-
Neuroendocrine tumors	-	-	-	-	-	-	-	-	-	-	_	-	-	+

CK = cytokeratin; **EMA =** Epithelial membrane antigen; **PLAP =** Placental alkaline phosphatase; **AFP =** alpha feto protein; **LCA =** leukocyte common antigen

areas. The role of MIB-1 LI (proliferation marker) in predicting malignant behavior is not clear and needs to be ascertained. As our case showed high MIB-1 LI, the patient was followed-up closely, but has remained free of disease one year post-surgery.

In conclusion, adult TGCTs are potentially malignant tumors that may have unusual clinical presentation occasionally. Recognition of these rare neoplasms and documentation of their behaviour is important from diagnostic, prognostic and therapeutic points of view. As delayed lymph nodal and distant metastases have been reported, patients need to be followed-up over a prolonged period after orchidectomy. Utility of MIB-1 LI in identification of tumors that may behave aggressively needs further evaluation.

CONFLICT OF INTEREST

None declared.

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Pancake kidney with bladder herniation

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CASE PRESENTATION

A 61-year-old man presented to the Emergency Department with vomiting and progressively worsening abdominal pain. A computed tomography (CT) was performed. The diagnosis of patient was acute cholecystitis and the patient was referred to general surgery clinic. In addition CT scan showed bilateral ectopic kidneys with urinary bladder herniation (Figures 1 and 2). Both kidneys were fused at the medial borders of each pole. To our knowledge, the case of pancake kidney with bladder herniation was not published yet in the literature.

Pancake kidney is very rare type of congenital fusion anomaly of the kidney. It is described as a renal mass located in the pelvis which is formed by complete medial fusion of renal parenchyma without intervening septum (1). Each kidney has its own collecting system and anteriorly placed short ureters entering the bladder orthotopically (1). The presence of a pancake kidney may predispose the formation of stones due to the probable rotation anomaly of the collecting system and short ureters which are prone to stasis and obstruction. Patients with pancake kidney are usually asymptomatic, but may present with features of urinary tract infection, fever and vague lower abdominal pain (1). If a pancake kidney has to undergo surgery, division of the parenchyma presents potential problems such as renal vascular damage, postoperative renal failure and eventual renal failure (2). Asymptomatic cases can be managed conservatively with long-term follow-up of renal function (1). If there are symptoms of renal failure, surgery is warranted. Ultrasonography, excretory urography and CT were efficient in detection and evaluation of pancake kidney anomaly (1).

Figure 1 - Axial CT images show pancake kidney and bladder herniation (dashed arrows).

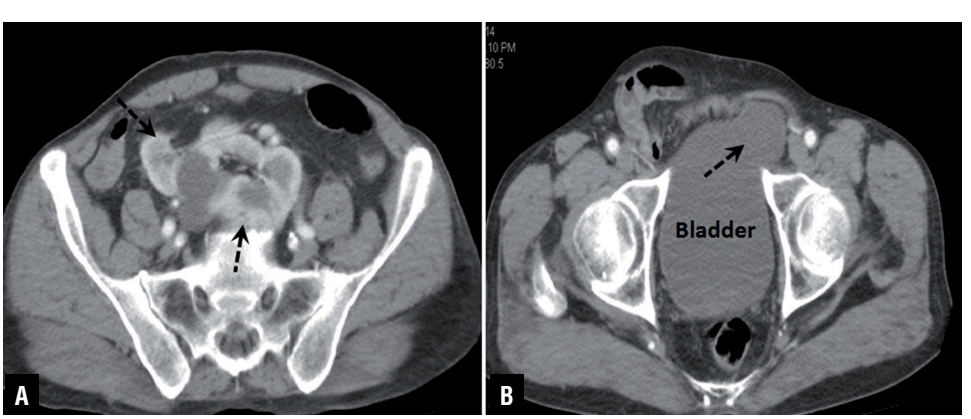
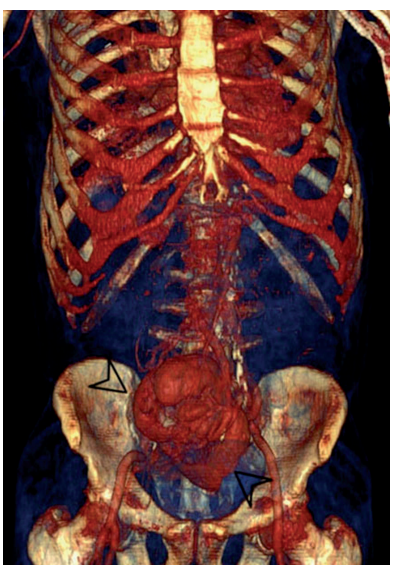


Figure 2 - Three dimensional volume rendering image shows pancake kidney (arrowheads).



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VIDEO SECTION

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Resection of the Urethral Plate and Augmented Ventral Buccal Graft in Patients with Long Obliterative Urethral Strictures

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ABSTRACT

The treatment of long urethral strictures is based on the use of buccal mucosa graft (BMG). Postoperative failures commonly occur in patients with the obliterative strictures, and the long augmented part of the urethra which is prone to fibrotic changes.

Combined approach with the resection of the obliterative part of the urethral plate located in the bulbar urethra, together with the ventral placement of BMG was performed in 36 patients. Etiology of the stricture was: idiopathic in 19/36 (52.7%), iatrogenic in 14/36 (38.8%), and other causes in 3/36 (8.3%). Mean length of the stricture was 7.2±1.6 cm, and the length of the augmented graft 4.5±1.2 cm (due to resected urethral plate) so, the single BMG was enough in 25/36 (69.4%) patients. The medium postoperative follow up was 24 months (20-28 months) months. Success of the surgery was defined as no need for additional surgery neither dilatation. Cystoscopy was performed 4-6 months after the surgery and additional follow up with IPSS and uroflowmetry.

Overall success was achieved in 31/36 (86.1%) patients. Mean postoperative IPSS was 9.5±2.1 in these patients. Complications were according to Clavien Dindo scale: grade II in 11/36 (30.5%-infection, orchialgia, scrotal pain), grade III in 4/36 (11.1%- fistula) and grade IV in 5/36 (14.5% - restenosis). Postoperative Q_{max}= 13.2±1.2 ml/s. Bell shaped curve was present in 14/36(38.8%).

Our results suggest that overall success rate is similar to the expected values for BMG surgery, and the number of the grafts used is lower due to reduced stricture length.

ARTICLE INFO

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EDITORIAL COMMENT

The surgical treatment of urethral stricture diseases is continually evolving. In recent years there has been continuous discussion with regard to the etiology, location, length, and management of extensive urethral stricture disease. Various tissues such as genital and extragenital skin, buccal mucosa, lingual mucosa, small intestinal submucosa, and bladder mucosa have been proposed for urethral reconstruction (1). Although various surgical techniques are available for the treatment of long anterior urethral stricture, no one technique has been identified as the method of choice. Basically, in patients with a wide, soft urethral plate and no fibrous spongiosum tissue, use of a graft is preferred. Contrary, in patients with a narrow, rigid urethral plate and fibrous spongiosum tissue, use of a flap is preferred. Although a buccal mucosa seems to be better than a skin graft, the difference in success rate is so slight (82% vs. 78%) that it does not justify the use of a buccal mucosa as a first choice (2). In this Video, the authors presented one of the procedures for long urethral stricture. Scientific and technical demonstration of their procedure looks excellent. The authors are to be congratulated on this complex and precize work.

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LETTER TO THE EDITOR

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Re: Mini incision open pyeloplasty – Improvement in patient outcome

Vishwajeet Singh [1], Manish Garg [1], Pradeep Sharma [1], Rahul Janak Sinha [1], Manoj Kumar [1]

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Int Braz J Urol. 2015; 41: 927-34

To the editor,

The authors of "Mini incision open pyeloplasty - Improvement in patient outcome" are to be congratulated for publishing their work in the era of minimally invasive surgery (MIS) (1). Refinement of open surgery should still be ongoing and published because not every surgeon can do MIS, and not every patient can afford MIS which is more expensive than that of open surgery.

However, two issues need to be clarified. The first issue is how to select the right patient. Most of the patients presented as lumbar pain and hydronephrosis. Is the pain related to hydronephrosis/ UPJO? The mean T1/2 of diuretic renal scan was 26.7±6.4 minutes. It means that some of the patients actually had a T1/2 shorter than 20 minutes. What were the surgical indications for this group of patients?

The second issue is how to define success? The authors reported an overall success rate of 98.6% without clear definition. By grade of ultrasound, there were at 5 patients (7.0%) with postoperative grade 3-4 hydronephrosis; by grade of IVP, there were 7 patients (10%) with moderate to severe hydronephrosis (table 2). Could we define these as success? T1/2 was greatly improved postoperatively. However, how many of them still had obstructive curve or T1/2 >20 minutes?

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