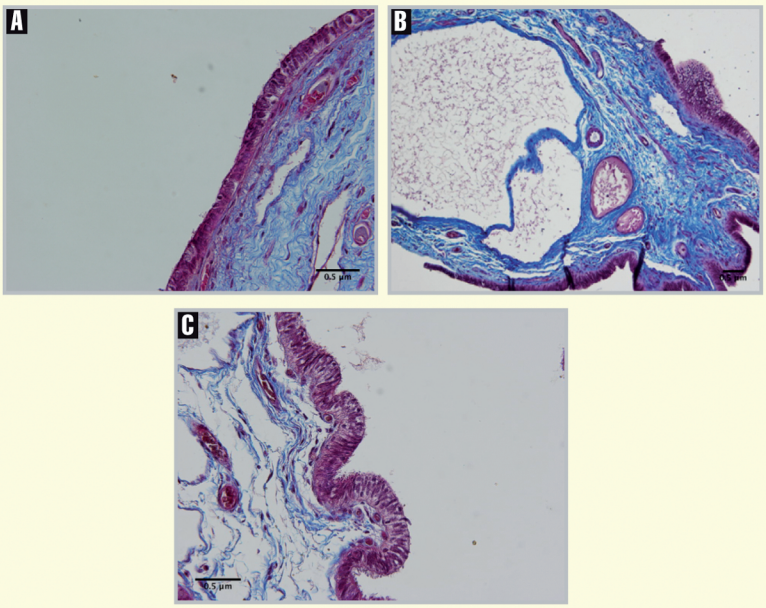
INTERNATIONAL BRAZ J UROL

ISSN 1677-5538

INTERNATIONAL BRAZ J UROL

OFFICIAL JOURNAL OF THE BRAZILIAN SOCIETY OF UROLOGY

VOLUME 39, NUMBER 2, MARCH - APRIL, 2013



Appendix epithelium. A) Patient with cryptorchidism with 3-years-old, showing the vascular stroma lined with pseudostratified epithelium of the testicular appendix.Masson's trichrome x200. B) Patient of the control group with 13-year-old, showing epididymal appendix with cavitation covered by pseudostratified epithelium. Masson's trichrome x 200. C) Patient of the control group with 13-years-old, showing pseudostratified epithelium of the entire testicular appendix. Masson's trichrome x200. (Page 243)

XXXIV Brazilian Congress of Urology
November 16-20, 2013 - Natal - RN - Brazil

SOCIEDADE BRASILEIRA DE UROLOGIA

Full Text Online Access Available
www.brazjurol.com.br

VOLUME 39, NUMBER 2, MARCH - APRIL, 2013 (Pages 152 - 301)

INTERNATIONAL

BRAZ J UROL

OFFICIAL JOURNAL OF THE BRAZILIAN SOCIETY OF UROLOGY - SBU

INTERNATIONAL

BRAZ J UROL

INTERNATIONAL

BRAZ J UROL

INTERNATIONAL

BRAZ J UROL

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

http://www.brazjurol.com.br

Correspondence and Editorial Address:

Rua Bambina, 153 – 22251-050 – Rio de Janeiro – RJ – Brazil
Tel.: + 55 21 2539-6787; Fax: + 55 21 2246-4088; E-mail: brazjurol@brazjurol.com.br

Editorial and Graphic Composition
J Sholna Reproduções Gráficas Ltd.

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

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

CNPq
Conselho Nacional de Desenvolvimento Científico e Tecnológico

The International Braz J Urol, ISSN: 1677-5538 (printed version) and ISSN: 1677-6119 (electronic version) is the Official Journal of the Brazilian Society of Urology– SBU, has a circulation of 6,000 copies per issue and is published 6 times a year (bimonthly, starting in January - February).
The issue date is up to 2 weeks after the month of issuefor the hard copy and up to 1 week after the month of issue for the electronic version.

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

Manuscript submission: **submission@brazjurol.com.br**

DISCLAIMER

CONTENTS

Volume 39 | number 2 | March . April, 2013 | INT BRAZ J UROL

EDITOR'S COMMENT

Volume 39 | number 2 | March . April, 2013 | INT BRAZ J UROL
doi: 10.1590/S1677-5538.IBJU.2013.02.01

Basic Research in Prostate Cancer, Kidney Cancer, Urinary Incontinence, Pediatric Urology and Erectile Dysfunction

The March-April 2013 issue of the International Braz J Urol presents original contributions with a lot of basic research papers in different fields. The papers come from many different countries such as Brazil, USA, China, Turkey, Jordan, Iran and Italy, Israel, Germany, Canada and Korea and as usual the editor´s comment highlights some papers.

Doctor da Silva and collegues, Department of Uro-oncology and Center of Oncologic Evidences, Universidade Estadual de Campinas, UNICAMP, Campinas, Brazil performed on page 155 an interesting study Systematic review of literature and meta-analysis to evaluate the results of magnetic resonance image 1.5T with endorectal coil in the diagnosis and evaluation of extra-prostatic extension and involvement of seminal vesicles of prostate cancer, compared to the histopathological results of the radical prostatectomy specimen.

Doctor Cabral and collegues from the Ipiranga Hospital and Brazilian Institute of Cancer Control, Sao Paulo, Brazil performed on page 173 an interesting study about testosterone as a predictor of aggressive disease in subjects with clinically localized PCa in 164 patients submitted to radical prostatectomy. This study indicates that testosterone may be a useful predictive tool once pathological extraprostatic extension was somewhat signaled by lower TT levels preoperatively. However, it does not consolidate a clear association between aggressive tumor biology and hypogonadism.

Doctor Wang and collegues from Changhai Hospital in China performed on page 189 a interesting basic research about the pahologic sizes of renal tumors. They concluded that the renal tumor size was overestimated by radiography as compared with pa- thology. The difference was just 0.22 cm with little clinical significance, suggesting that CT provides an accurate method to estimate renal tumor size preoperatively.

Doctor Faddegon and collegues Department of Urology, University of Texas Southwestern Medical Center, Dallas, TX, USA, performed on page 195 an interesting study about Horseshoe kidney is an uncommon renal anomaly often associated with ureteropelvic junction (UPJ) obstruction. Advanced minimally invasive surgical (MIS) reconstructive techniques including laparoscopic and robotic surgery are now

Volume 39 | number 2 | March . April, 2013 | INT BRAZ J UROL

being utilized in this population. However, fewer than 30 cases of MIS UPJ reconstruction in horseshoe kidneys have been reported. We herein report our experience with these techniques in the largest series to date.

Doctor Camara-Lopes and collegues from University of Sao Paulo Medical School, Sao Paulo, Brazil, performed on page 222 a interesting study about the Prostatic artery embolization (PAE) for the treatment of patients with symptomatic benign prostatic hyperplasia (BPH). This is the first description in BPH patients treated by PAE, a new procedure that is being used increasingly as a therapeutic intervention. The recognition of the changes caused by this new modality of treatment has become a very important differential in a chronic granulomatous reaction of the prostate tissue.

Doctor Tostes and collegues from Urogenital Research Unit - State Universtiy from Rio de Janeiro, Brazil, performed on page 240 a interesting basic research about the incidence and structure of testicular appendices (TAs) in patients with cryptorchidism, comparing their incidence with epididymal anomalies (EA) and patency of the vaginal process (PVP) and analyzes the structure of TAs in 55 patients with cryptorchidism. The authors shows that there was no difference in the incidence of testicular appendices in relation to the testicular position in the patients with cryptorchidism. There also was no increased incidence of anatomical anomalies associated with the testes containing appendices. The testicular appendices showed a significant structural alteration in the patients with cryptorchidism: although the epithelium was not changed, the testicular appendices of the patients with cryptorchidism had a larger quantity of elastic fibers and smaller quantity of smooth muscle cells and predominance of type III collagen, remodeling in patients with cryptorchidism.

Doctor Shynlova and Collegues from the University of Toronto, Canada, performed on page 257 a interesting basic research analyze the expression of genes involved in extracellular matrix (ECM) biogenesis and remodeling in vaginal tissue of women clinically normal pelvic floor support according to the phase of menstrual cycle and postmenopausal women with and without pelvic organ prolapse (POP) and concluded that ovarian cycle and age-related changes influence the expression of genes encoding proteins responsible for ECM metabolism in human vagina.

Moreover, POP is associated with alteration in vaginal ECM components after menopause.

Doctor Reges and collegues from Unicamp and UFC, Brazil, performed on page 268 a study about the potential of acute administration of the PDE5i sildenafil to improve detrusor overactivity (DO) induced by Nω-nitro-L-arginine methyl ester

EDITOR'S COMMENT

hydrochloride (L-NAME), an nitric oxide sinthase (NOS) inhibitor, in rats. They observed a systemic reduction of nitric oxide causes detrusor overactivity and acute in- fusion of sildenafil reduces the number of micturition cycles in chronic NO-deficient rats.

Luciano A. Favorito, MD, PhD

Associated Editor
Internacional Braz J Urol

REVIEW ARTICLE

Vol. 39 (2): 155-166, March - April, 2013
doi: 10.1590/S1677-5538.IBJU.2013.02.02

Magnetic Resonance Image in the diagnosis and evaluation of extra-prostatic extension and involvement of seminal vesicles of prostate cancer: a systematic review of literature and meta-analysis

Rogério Cardoso da Silva, André Deeke Sasse, Wagner Eduardo Matheus, Ubirajara Ferreira

Department of Uro-oncology (RCS, WEM, UF) and Center of Oncologic Evidences (ADS), Universidade Estadual de Campinas, UNICAMP, Campinas, Brazil

ABSTRACT

Objective: Systematic review of literature and meta-analysis to evaluate the results of magnetic resonance image 1.5T with endorectal coil in the diagnosis and evaluation of extra-prostatic extension and involvement of seminal vesicles of prostate cancer, compared to the histopathological results of the radical prostatectomy specimen.
Materials and Methods: It was conducted a systematic review of literature and meta-analyses of all studies data published after 2008. In those studies, the patients with prostate cancer with indication to radical prostatectomy were submitted to magnetic resonance image (MRI) at pre-operatory period and the results were compared to those of histopathological studies after the surgery. The selected terms for research included prostate cancer, magnetic resonance, radical prostatectomy, and prostate cancer diagnosis, in the databases EMBASE, LILACS, PUBMED/MEDLINE and Cochrane Library. The data were collected using a specific qualitative instrument and the meta-analysis data were presented in the forest plot graphics, homogeneity test and sROC curves and funnel plot.
Results: A total of seven studies were included, with a total of 603 patients. Among these studies, six evaluated the value of MRI for the detection of prostate cancer, and the median sensitivity of meta-analysis was 0.6 and specificity 0.58, but with heterogeneity among the studies. Three studies evaluated extra-prostatic extension with a median sensitivity of 0.49, specificity 0.82 and heterogeneity only for sensitivity. Three studies evaluated invasion of seminal vesicles, with median sensitivity of 0.45 and specificity 0.96, with heterogeneity in both analysis.
Conclusion: Magnetic resonance of 1.5T with endocoil showed low values of sensitivity and specificity for the diagnosis and staging of prostate cancer. The reviewed studies showed a significant heterogeneity among them. The best observed result was MRI specificity for invasion of seminal vesicles. More studies are necessary to evaluate new techniques and parameters before recommending the routine use of MRI in clinical practice.

ARTICLE INFO

Key words:
Prostatic Neoplasms; Magnetic Resonance Spectroscopy; Diagnosis

Int Braz J Urol. 2013; 39: 155-66

Submitted for publication:
December 10, 2012

Accepted after revision:
April 02, 2013

INTRODUCTION

Prostate cancer (PC) is the most frequent malign tumor among men older than 50 years and presents a tendency of increase in the next decades due to the rise of life expectancy (1).

Although the natural history of the disease is not completed understood, several prognostic

factors influence the evolution of PC. Among these, some are very important: histological grade, PSA level, tumor volume and extension of the disease (2,3).

There is a lack of consensus in clinical practice regarding diagnostic, staging and treatment techniques that may lead to excessive and unnecessary image exams with increasing costs, waste of time and excessive exposure to ionizing radiation (depending upon the used method) (4,5).

Magnetic resonance image (MRI) with endorectal coil is the most accepted diagnostic method for the evaluation of the prostate tumor, with the advantage of not exposure to ionizing radiation. But there are still several doubts regarding its usefulness and real applicability.

The objective of the present study was the systematic review (SR) of studies that used MRI with conventional 1.5T images in the diagnosis and staging of PC compared to the results of histopathological studies of the radical prostatectomies (RP).

It was performed a systematic meta-analyses, including sensitivity and specificity, comparing the results regarding diagnosis, evaluation of extra-prostatic extension (EPE) and invasion of seminal vesicles (ISV).

MATERIALS AND METHODS

We selected studies in which MRI was used for PC diagnosis, extension of extra-prostatic disease evaluation and involvement of seminal vesicles compared to histopathological studies of RP.

We included clinical trials with the following inclusion criteria: transversal studies published after 2008 and all patients presenting PC at biopsy with indication of RP without previous treatment. All patients should have been submitted to MRI previous to surgery with conventional 1.5T images.

The following databases were reviewed using the terms prostate cancer, magnetic resonance, radical prostatectomy and diagnosis: PUBMED, MEDLINE, EMBASE, LILACS and Cochrane Library. The following terms were used for PUBMED: ((“prostaticneoplasms ”[MeSHTerms] OR (“prostatic”[AllFields] AND “neoplasms”[AllFields]) OR “prostaticneoplasms ”[AllFields]) AND (“prostatectomy”[MeSHTerms] OR “prostatectomy”[AllFields]) AND (“magn eticresonancespectroscopy”[MeSHTerms] OR (“magnetic”[AllFields] AND “resonance”[AllFields] AND “spectroscopy”[AllFields]) OR “magn eticresonancespectroscopy”[AllFields] OR (“magnetic”[AllFields] AND “resonance”[AllFields]) OR “magneticresonance”[AllFields]))AND Diagnosis/Narrow[filter]. The same search strategy was adapted for the remaining databases. There was no restriction of the published language. Two independent researchers selected the papers and collected the relevant data. For this purpose, it was used a data collection protocol, depicting the studies characteristics and the collected results. In order to solve any possible disagreements between the researchers, a third one was convened.

To evaluate the individual quality of the studies it was used the question form QUADAS (Quality Assessment of Diagnostic Accuracy Studies). The questions included the main sources of bias and each question should have been answered with “yes”, “no”, “not clear”. The greatest the number of negative or not clear answers, the worst was the quality of the study (6).

Meta-analysis and presentation of results

We used the following software for the meta-analysis: RevMan version 6 (Cochrane) and Meta DiSc 1.4. The presentation of the results used four statistical tools: forest plot, homogeneity test, sROC curve and funnel plot.

1. Forest plot: it was used a statistical combination of sensitivity and specificity of each study, obtaining a median integrated value (pooling) (7).
2. Homogeneity tests: it was used the chi-square test (Q), and the inconsistency index (I-squared, I^2) to estimate the heterogeneity of the individual studies. $P < 0,05$ suggested heterogeneity. I-squared (I^2) describes the percentage of total variation among the studies and $I^2 > 50\%$ suggested significant heterogeneity among data (8).
3. sROC curve: summarizes and combines rates of true positive and false positive diagnosis

of the different studies. AUC (area under the curve) summarizes the quality of the curve, representing an index of accuracy of the test. A test with low performance has AUC < 0.5 and a satisfactory one AUC > 0.70. Q* evaluates the point at the sROC curve where sensitivity and specificity are equal, being equivalent to the point of symmetry of the sROC curve. If Q* is ≤ 0.5 the test is not worth for the studied evaluation and as much Q* approaches 1.0 the best performance has the test (9).

4. Funnel plot: the published bias can be detected by the use of the funnel plot chart, in which the size of the sample is the strongest correlate of the bias of the publication. In the absence of bias, the data have a more funneled symmetric distribution. The presence of a not symmetric funneled distribution indicates the presence of bias of publication (10,11). Cochran-Q with $P < 0.05$ suggests the presence of heterogeneity beyond what expected singly. I-squared (I^2) describes the percentage of variability effect due to heterogeneity and not due to random distribution. $I^2 > 50\%$ disclose heterogeneity. T^2 estimates variability among studies, and $T^2 > 1$ suggests heterogeneity (10,11).

RESULTS

General Aspects

Using the search terms, 234 summaries were selected from the online databases. After careful evaluation of the summaries, 27 articles were selected for careful review, and after that, 20 were excluded. The main causes of exclusion of the 20 articles included: 6 with any previous treatment prior do RP, 4 without correct identification of the use of the endocoil, 5 without sufficient data for the calculus of sensitivity and specificity. Seven studies were selected, 6 in English and 1 in German (Figure-1).

All seven selected studies were transversal (without calculus of the size sample), unicentric (one only institution involved) and summed 603 patients (Table-1).

Most studies were considered with good consistency after the use of QUADAS question form, since there were mainly "yes" answers after methodological evaluation (greater than 50% of the answers). The question "if the results of the gold-standard test (histopathological study of RP) were interpreted without the knowledge of the test being evaluated (pre-operatory MRI)" had not a satisfactory answer.

Figure 1 - Organogram of the selected, examined and included studies of SR.

Analysis of the diagnosis of PC

Six of the seven selected articles studied the capacity of MRI with endocoil and 1.5T conventional images to diagnose PC. The mean sensitivity of this meta-analysis was 0.6 and specificity 0.58 (the data were obtained from the florest plot).

The homogeneity test for sensibility and specificity showed Q = 36.49 (P = 0.000), I^2 = 86.3% and Q = 24.89 (P = 0.0001), I^2 = 79.9%, respectively (Figures 2a and 2b). sROC curve showed AUC = 0.7090 and Q* = 0.6601(Figure-3).

Funnel plot showed Cochran-Q = 26.80 (P = 0.0001), I^2 = 81.3% and T^2 = 1.1861 (Figure-4). There was a tendency of displacement of the graphic symmetry to the right demonstrating heterogeneity in the statistical parameters of the Q test of Cochran, I^2 and T^2.

Table 1 - Summarized data of the studies used in the SR.

Author	Year	Country	TR	Nº Patients	Age (Years)	PSA(ng/dl)	RP
Chabanova et al (15)	2010	Denmark	P	43	64.1 (51-74)	10.2 (1,3-28)	Open
Kim et al (18)	2010	Canada	R	32	59.1(52.4-65.8)	#	Open
Brajtbord et al (19)	2011	USA	R	179	59.3	6.6	RALP
Janane et al (14)	2011	Morocco	NC	190	62.9 (50-73)	10.8 (2-18,5)	Open
Jeong et al (12)	2010	South Korea	R	88	66 (41-76)	5.3 (1.5-9.8)	Open
Yoshizako et al (13)	2008	Japan	R	23	65 (52-76)	NM	Open
Beyersdorff et al (16)	2011	Germany	P	48	63.43 (49–71)	8.17 (2.7-31.4)	Open

< 10 ⇒ 2 patients; 10-20 ⇒ 5 patients; > 20 ⇒ 5 patients; RALP; Robotic assisted

TR: Temporal relation; P: prospective, R: retrospective, NC: not clear; RP: Radical prostatectomy; NM: Not mentioned

Figure 2 - Forest plot of sensitivity and specificity regarding the diagnosis of PC.

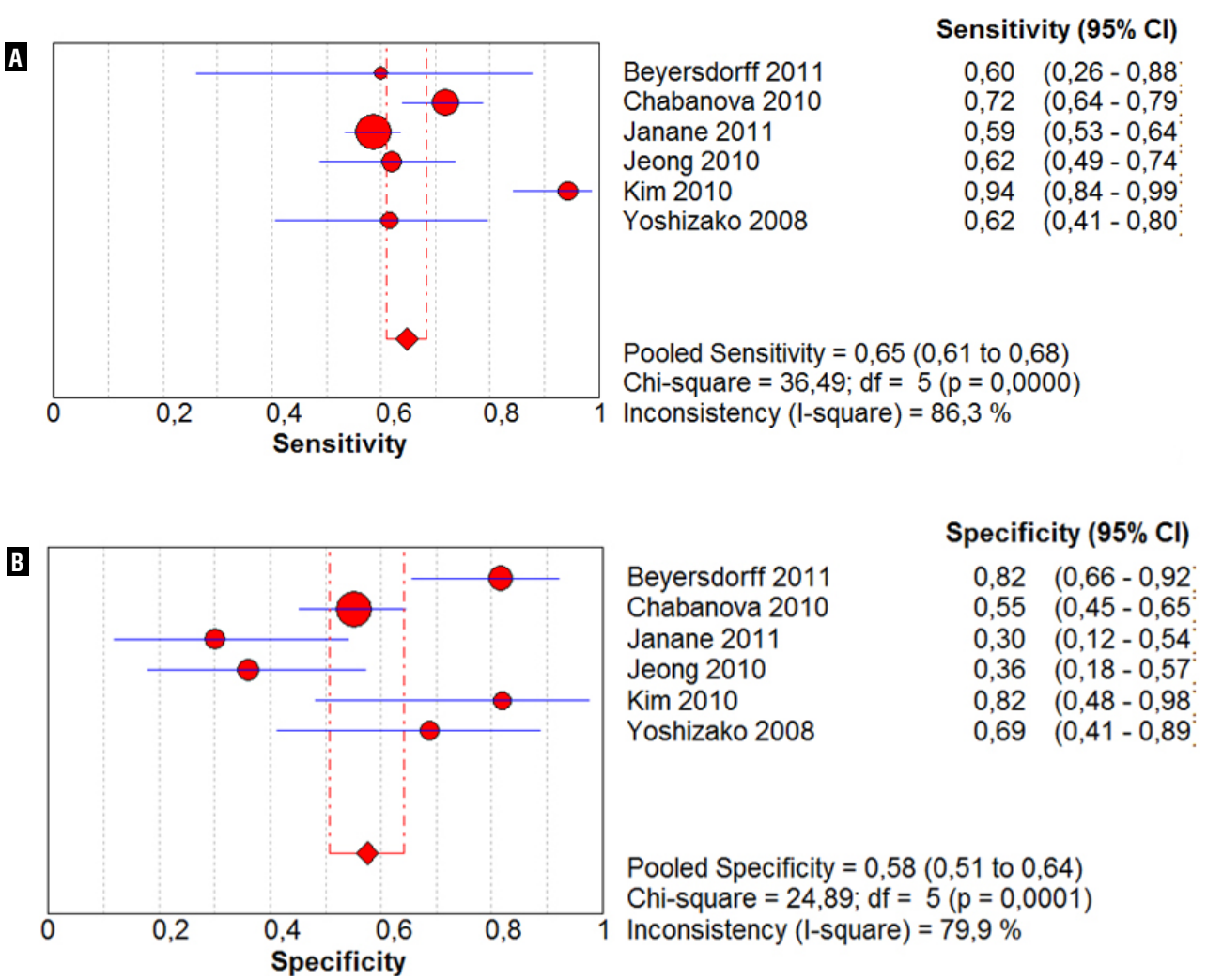


Figure 3 - sROC curve depicting the representation of sensitivity versus 1-specificity of the diagnosis of PC.

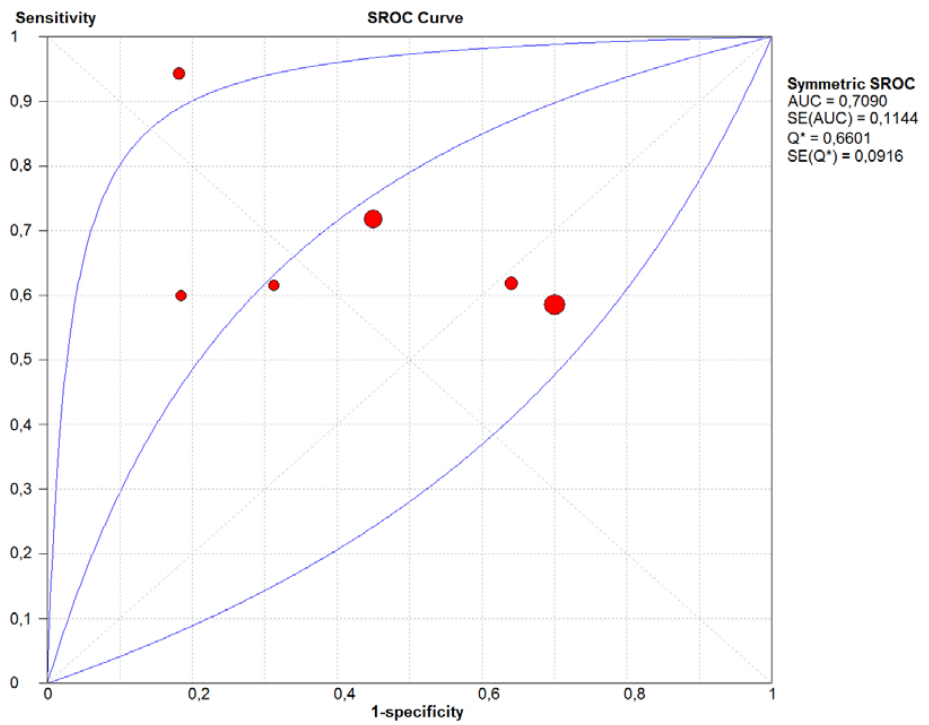
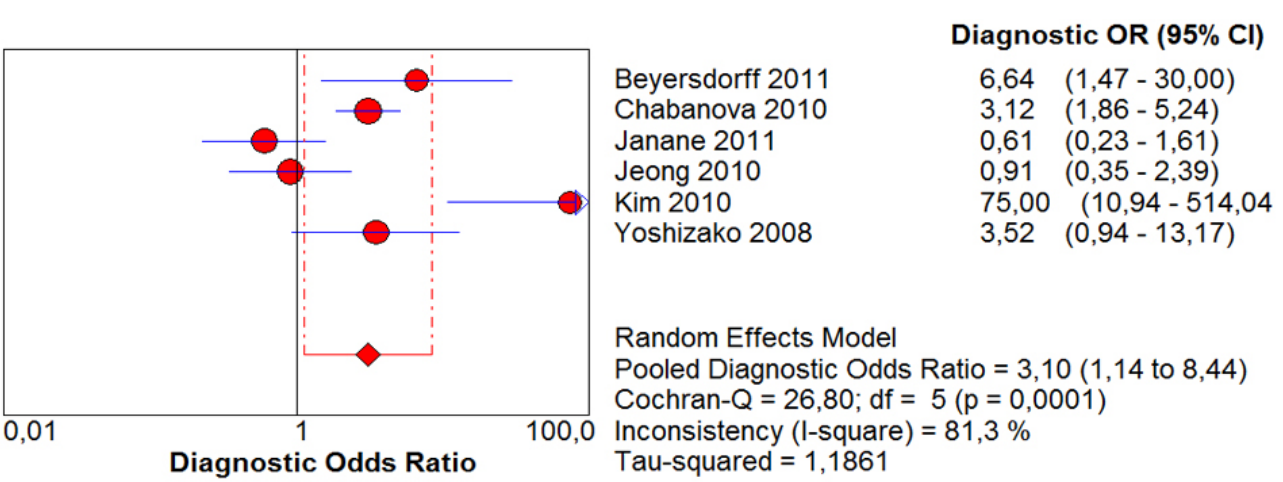


Figure 4 - Funnel plot of the studies regarding the diagnosis of PC.



Analysis of EPE

Three of the seven studies evaluated the capacity of MRI with conventional 1.5T images to detect EPE, with a median sensibility of 0.49 and specificity of 0.82.

The homogeneity test for sensitivity and specificity showed Q = 16.28 (P = 0.0006), I^2 = 87.7% and Q = 1.09 (P = 0.5799), I^2 = 0.0%, respectively (Figures 5a and 5b). sROC curve showed AUC = 0.9298 and Q* = 0.8649 (Figure-6).

The statistical calculi in the funnel plot were: Cochran-Q = 12.03 (P < 0.05), I^2 = 83.4% and T^2 = 0.9707. P < 0.05 suggested the presence of heterogeneity in the individual studies far from what would be expected singly. I^2 > 50% gave a dimension of the percentage of total variation of the studies due to heterogeneity (Figure-7).

Analysis of SVI

The same three articles that evaluated EPE also evaluated SVI with 1.5T conventional images and showed a median sensitivity of 0.45 and specificity 0.96.

The result of the homogeneity test for sensitivity was Q = 9.98 (P = 0.0068) and I^2 = 80.0%, while for specificity Q = 8.91 (P = 0.0116) and I^2 = 77.6% (Figures 8a and 8b). sROC curve: AUC = 0.9241 and Q* = 0.8581 (Figure-9).

Funnel plot obtained Cochran-Q = 5.73 (P = 0.0570), I^2 = 65.1% and T^2 = 1.1657. P = 0.05 and I^2 lightly over 50% suggested the presence of discrete heterogeneity of the individual studies far from what expected singly. T^2 > 1 suggested heterogeneity among the studies (Figure-10).

Funnel plot showed asymmetric format, suggesting the presence of bias publication (chance reasons away from the baseline vertical 1). Graphic with asymmetric tendency P = 0.0570 (limit of the significance statistical level), I^2 > 50% and T^2 > 1 suggesting heterogeneity of the single studies, due to the variability of the size sample of each paper and due to the threshold effect for the diagnosis of SVI (9,10).

Figure 5 - Florest plot of sensitivity and specificity for EPE.

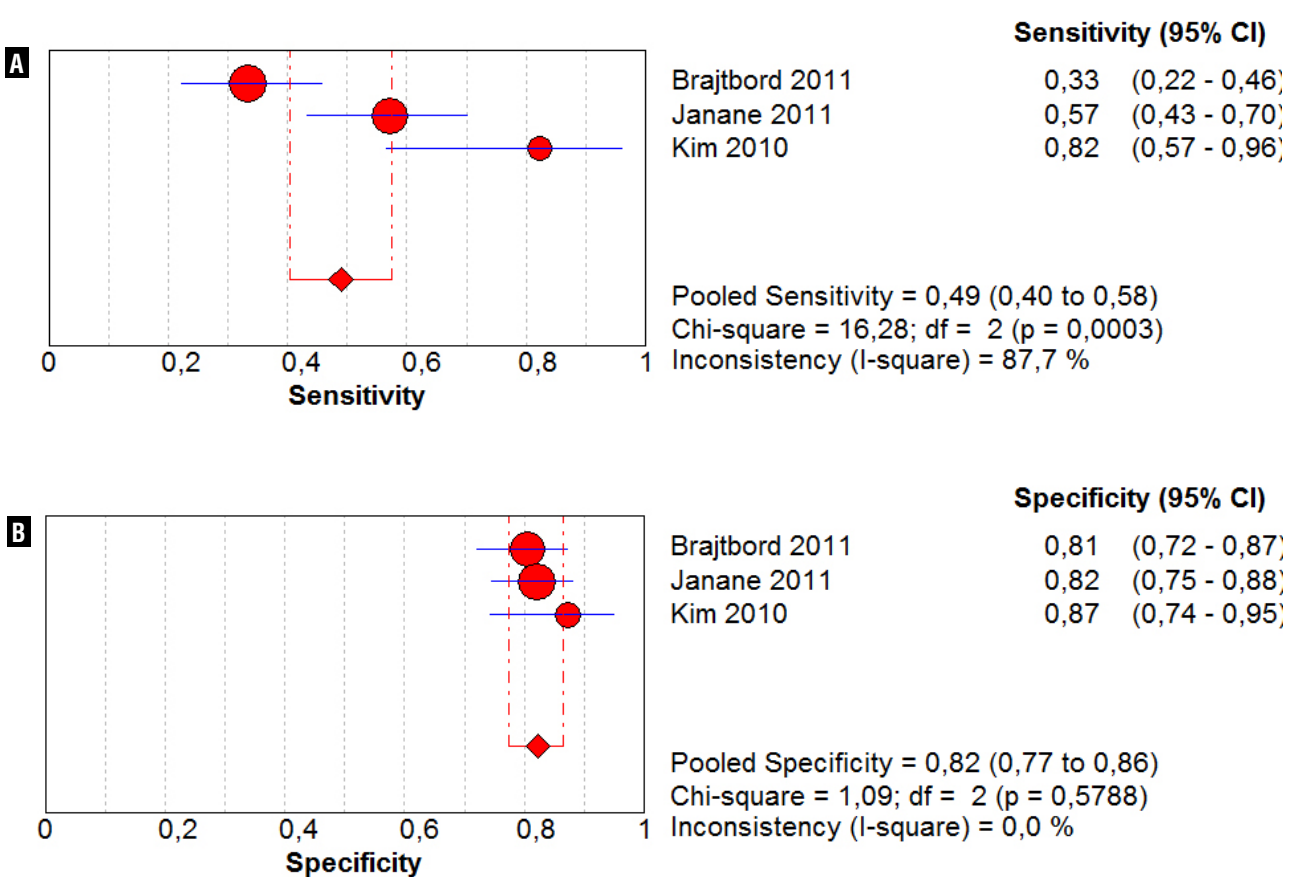


Figure 6 - sROC curve depicting the representation of the sensitivity versus 1-specificity of EPE.

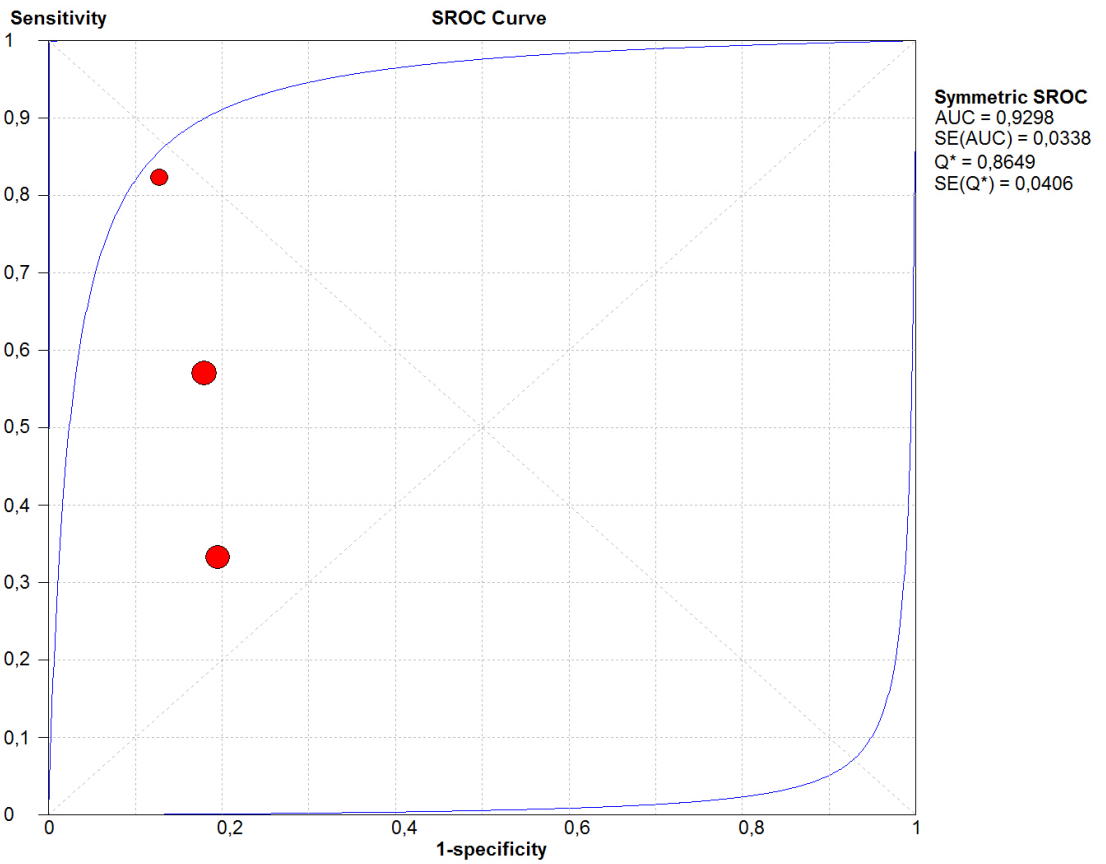


Figure 7 - Funnel plot of the studies regarding EPE.

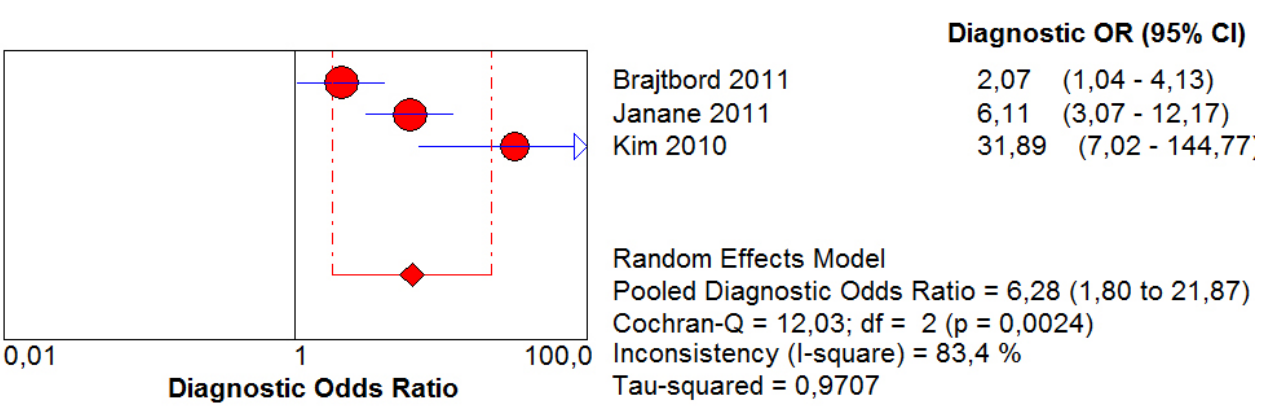
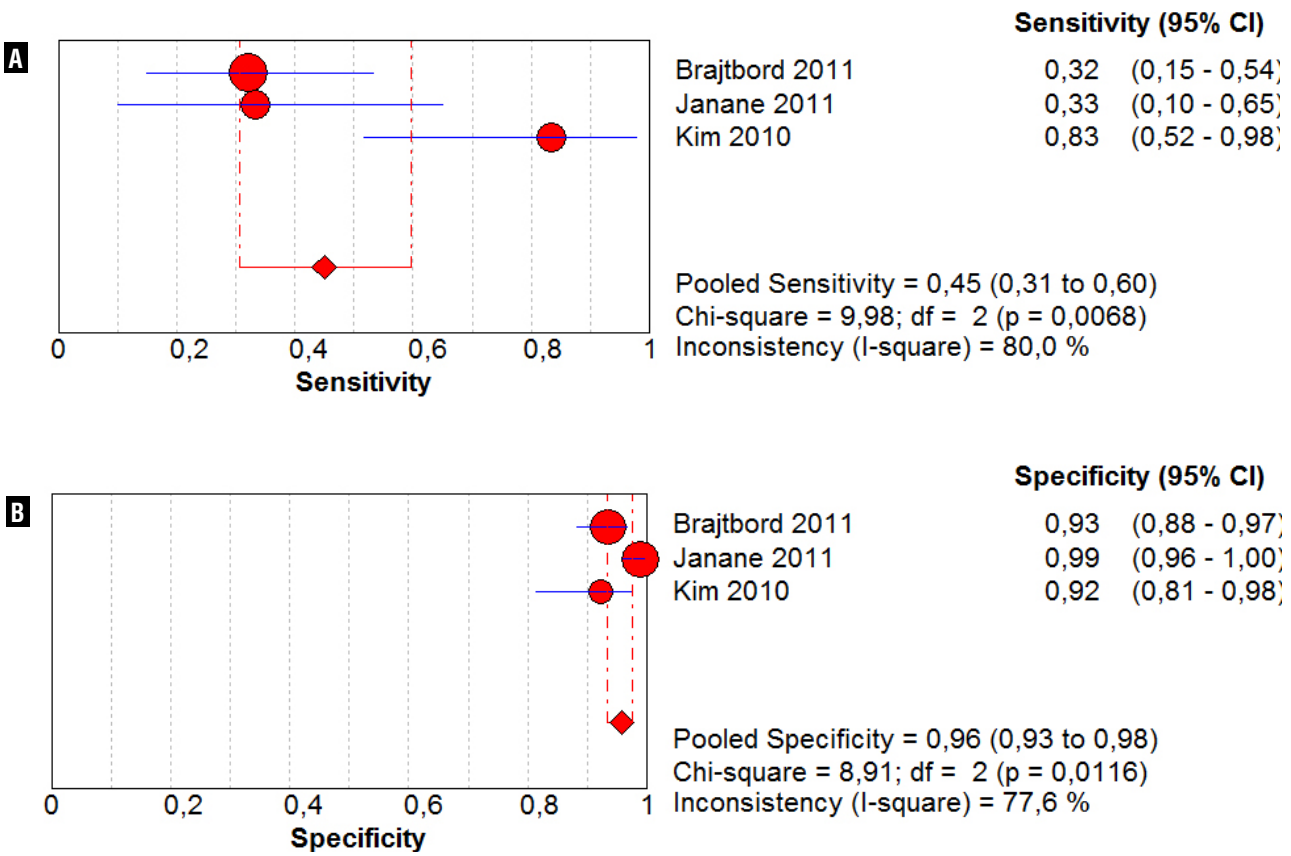


Figure 8 - Florest plot of sensitivity and specificity regarding VSI.



DISCUSSION

In this SR all studies compared the histopathological results with pre-operatory MRI. Although there were more than 200 studies about MRI in the diagnosis and staging of PC, only seven were selected to evaluate pre-operatory 1.5T MRI in patients submitted to RP compared to histopathologic results.

This limited number of studies must be analyzed carefully and will always evoke serious thoughts during systematic reviews, due to the small number of well elaborated articles, with a clear methodology and scientific relevance.

In relation to the selected articles, there were no calculi of the size of the sample in none of them. The studies were conducted in the respective institutions, without any common protocol, ignoring the differences and particularities of each study center. Only two of the seven studies (28.57%) were prospective and none of them informed financial disclosures. In spite of that, the analysis of quality of the studies using the QUADAS question form demonstrated that most questions regarding the study (more than 50%) were answered "yes", suggesting that the quality of the studies was adequate.

Although independent, a few characteristics of the studies were the same in all, such as lack of previous treatment before PR, the use of 1.5T conventional images of MRI and a period of more than a month between the prostatic biopsy and RP surgery.

The difference of the values of sensitivity and specificity for the diagnosis of PC, as well as of the respective low medians, may be explained by the threshold limits or the cutoff of the particular exam.

Jeong et al. and Yoshizako et al. described the standardization of the diagnosis of PC in five

Figure 9 - sROC curve depicting the representation of the sensitivity versus 1-specificitity of VSI.

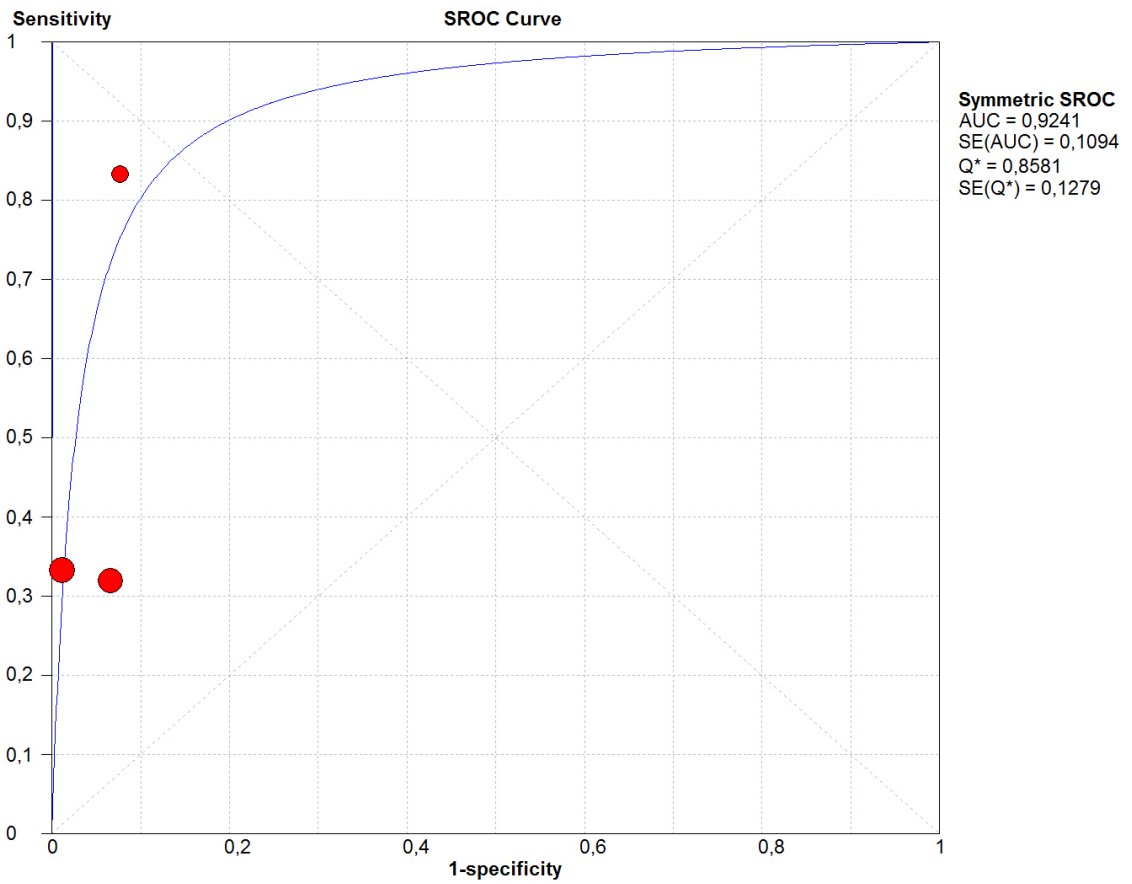
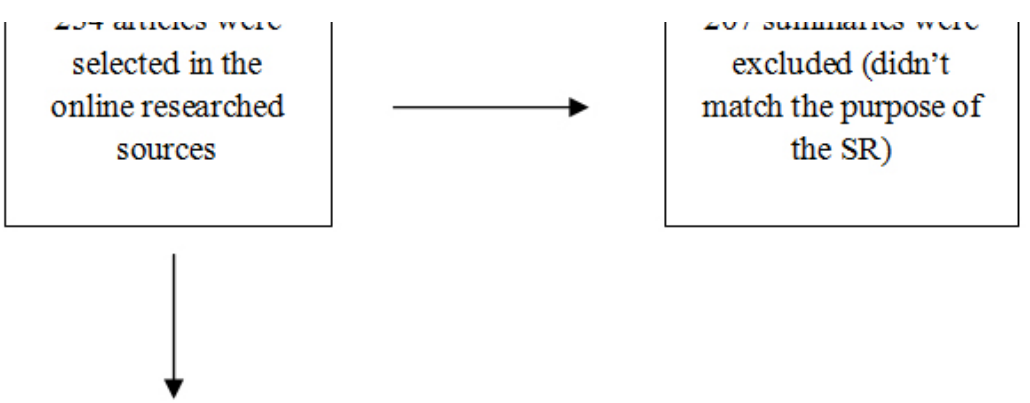


Figure 10 - Funnel plot of the studies regarding VSI.



2

categories: cancer certainly absent, cancer probably absent, cancer possibly present, cancer probably present and cancer certainly present (12,13). Other authors categorized in two options: presence or absence of cancer. Also, in only those two studies it was clear that the radiological evaluation was made by two well trained radiologists. Another aspect is the lack of standardization of the radiological parameters for the diagnosis of PC used by most studies, and some actually don't mention them. Finally, the size of the tumor was not considered for the diagnosis of PC, except on the study of Janane et al. (14).

Chabanova et al. studied conventional images of MRI and spectroscopy and perfusion methods in the diagnosis of PC. In relation to spectroscopy, they found sensitivity of 0.46 and specificity of 0.78. In relation to perfusion, sensitivity and specificity were respectively 0.48 and 0.68. They demonstrated that the combination of conventional MRI, spectroscopy and perfusion could diagnose all patients with PC, indicating a positive combination of the three methods. However, this association is expensive and troublesome, and can derail the routine use of MRI (15).

Yoshizako et al. also studdied the combination of conventional MRI images with difusion and perfusion. The combination of conventional MRI and diffusion showed sensitivity of 0.80 and specificity of 0.87, and when combined with perfusion showed sensitivity of 0.69 and specificity of 0.68. The combination of the three methods showed sensitivity of 0.69 and specificity of 0.93. The combination of the three methods was more accurate for the diagnosis of PC than conventional MRI alone, but this data referred only to PC of the prostatic transition zone (13).

Jeong et al. studied the conventional images and the combination of them with the diffusion method and found sensitivity and specificity of 0.87 and 0.72 respectively. The authors did not report the data of the diffusion method alone (12).

Beyersdorff et al. compared conventional MRI with perfusion images alone. In relation to perfusion, the sensitivity and specificity were 0.78 and 0.79 respectively. The sensitivity was superior to that of conventional MRI (0.60) and the specificity a little lower than that of conventional MRI (0.82) (16). The heterogeneity of the studies can be explained by the variable size of the sample of each study, by the different criteria to diagnose PC using MRI and by the different studied populations of each study (8).

The suspicion of EPE at MRI is made by specific signs, as the presence of solid tissue in the periprostatic fat, irregular bulging of the prostatic capsule and obliteration of the retoprostatic angle, as well as non-specific signs as capsular thickening, capsular retraction and regular bulging of the capsule (17).

The values of sensitivity of the three studies that evaluated EPE are different, leading to a considerable heterogeneity of the individual studies ($P < 0.05$ and I^2 greater than 50%) and medium of 0.49 (CI 95%: 0.40 - 0.58).

Kim et al. considered EPE the presence of obliteration of the retoprostatic angle, blurring of the periprostatic fat, invasion of the neurovascular bundle or transcapsular tumor continuation (18). Janane et al also included discontinuity of the prostatic capsule, obliteration of the prostatic veins and a distance between the tumor and the prostatic capsule equal or superior to 1 cm (14). Brajtbord et al didn't disclosure the used criteria to consider the presence of EPE as a positive MRI (19).

Another important aspect was that the analyzed populations were different among the revised studies. Janane et al included a particular north-african population and didn't mention the categorization of the population according to extra-prostatic risk of the disease (14). Kim et al excluded low risk patients and included only high or intermediate risk patients (18). Brajtbord et al didn't state the categorization risk, although presented the histopathological results: Gleason < 7: 13%, Gleason = 7: 69%, Gleason > 7: 16% (19).

The reduced sensitivity rates for EPE are due to the incapacity of the used images techniques, including MRI, to detect microscopic extra-prostatic tumors. The low specificity is due to the difficulty of the image techniques to distinguish prostatic tumors and benign inflammatory diseases (20).

Regarding the study of SVI sensitivity, there was a discrepancy between Kim study and

the two others, with heterogeneity ($P < 0.05$ and $I^2 > 50\%$). Kim et al worked exclusively with intermediate and high risk patients, increasing the MRI sensitivity in relation to the two other authors (18).

When evaluating the specificity, each individual study presented results greater than 0.9, with a median of 0.96 (IC 95%: 0.93 - 0.98). Kim et al suggested the presence of VSI when there was a low intensity sign causing dilation and an asymmetric gland (18). Janane et al identified the VSI as the focal shortening of the wall or the presence of low intensity sign inside the vesicles (14). Brajtford et al only informed presence or absence of VSI (17).

In this SR, it is concluded that MRI with 1.5T conventional images using endorectal coil should not be routinely used in all patients with PC. It should only be used as a complementary method for the diagnosis and staging of the disease and indicated for only specific patients.

More studies evaluating new technologies (for example, 3T MRI) and multiple parameters (diffusion, perfusion and spectroscopy) are necessary before recommending the routine use of MIR in clinical practice.

CONCLUSIONS

There are very few good studies comparing pre-operatory pelvic MRI using 1.5T conventional images and histopathological results of patients with PC submitted to RP. Also, the analysed studies showed a significant heterogeneity. The best results of SR are related to specificity for VSI. This SR suggests that 1.5T MRI using endorectal coil is not indicated routinely for the diagnosis and staging of patients with PC.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Hsing AW, Chokkalingam AP: Prostate cancer epidemiology. Front Biosci. 2006; 11: 1388-413.
2. Chodak GW: The role of watchful waiting in the management of localized prostate cancer. J Urol. 1994; 152: 1766-8.
3. Dodds PR, Caride VJ, Lytton B: The role of vertebral veins in the dissemination of prostatic carcinoma. J Urol. 1981; 126: 753-5.
4. Lorente JA, Morote J, Raventos C, Encabo G, Valenzuela H: Clinical efficacy of bone alkaline phosphatase and prostate specific antigen in the diagnosis of bone metastasis in prostate cancer. J Urol. 1996; 155: 1348-51.
5. Abuzallouf S, Dayes I, Lukka H: Baseline staging of newly diagnosed prostate cancer: a summary of the literature. J Urol. 2004; 171: 2122-7.
6. Iared W, Valente O: Revisões sistemáticas de estudos de acurácia. Diagn Tratamento. 2009; 14: 85-8.
7. Moher D, Cook DJ, Eastwood S, Olkin I, Rennie D, Stroup DF: Improving the quality of reports of meta-analyses of randomised controlled trials: the QUOROM statement. Quality of Reporting of Meta-analyses. Lancet. 1999; 354: 1896-900.
8. Higgins JP, Thompson SG, Deeks JJ, Altman DG: Measuring inconsistency in meta-analyses. BMJ. 2003; 327: 557-60.
9. Walter SD: Properties of the summary receiver operating characteristic (SROC) curve for diagnostic test data. Stat Med. 2002; 21: 1237-56.
10. Egger M, Smith GD: Bias in location and selection of studies. BMJ. 1998; 316: 61-6.
11. Moayyedi P: Meta-analysis: Can we mix apples and oranges? Am J Gastroenterol. 2004; 99: 2297-301.
12. Jeong IG, Kim JK, Cho KS, You D, Song C, Hong JH, et al.: Diffusion-weighted magnetic resonance imaging in patients with unilateral prostate cancer on extended prostate biopsy: predictive accuracy of laterality and implications for hemi-ablative therapy. J Urol. 2010, 184: 1963-9.
13. Yoshizako T, Wada A, Hayashi T, Uchida K, Sumura M, Uchida N, et al.: Usefulness of diffusion-weighted imaging and dynamic contrast-enhanced magnetic resonance imaging in the diagnosis of prostate transition-zone cancer. Acta Radiol. 2008; 49: 1207-13.
14. Janane A, Hajji F, Ismail TO, Elondo JC, Ghadouane M, Ameur A, et al.: WITHDRAWN: Endorectal MRI accuracy in auguring tumour location, tumour extent, capsular perforation and seminal vesicle invasion of prostate cancer in north-African men. Eur J Radiol. 2011; 26. [Epub ahead of print]
15. Chabanova E, Balslev I, Logager V, Hansen A, Jakobsen H, Kromann-Andersen B, et al.: Prostate cancer: 1.5T endocoil dynamic contrast-enhanced MRI and MR spectroscopy--correlation with prostate biopsy and prostatectomy histopathological data. Eur J Radiol. 2011; 80: 292-6.
16. Beyersdorff D, Lüdemann L, Dietz E, Galler D, Marchot P, Franiel T. Dynamic contrast-enhanced MRI of the prostate: comparison of two different post-processing algorithms. Rofo. 2011; 183: 456-61.

17. Yu KK, Hricak H, Alagappan R, Chernoff DM, Bacchetti P, Zaloudek CJ: Detection of extracapsular extension of prostate carcinoma with endorectal and phased-array coil MR imaging: multivariate feature analysis. Radiology. 1997; 202: 697-702.
18. Kim B, Breau RH, Papadatos D, Fergusson D, Doucette S, Cagiannos I, et al.: Diagnostic accuracy of surface coil magnetic resonance imaging at 1.5 T for local staging of elevated risk prostate cancer. Can Urol Assoc J. 2010; 4: 257-62.
19. Brajtbord JS, Lavery HJ, Nabizada-Pace F, Senaratne P, Samadi DB: Endorectal magnetic resonance imaging has limited clinical ability to preoperatively predict pT3 prostate cancer. BJU Int. 2011; 107: 1419-24.
20. Epstein JI, Chan DW, Sokoll LJ, Walsh PC, Cox JL, Rittenhouse H, et al.: Nonpalpable stage T1c prostate cancer: prediction of insignificant disease using free/total prostate specific antigen levels and needle biopsy findings. J Urol. 1998; 160: 2407-11.

Correspondence address:
Dr. Rogério Cardoso da Silva
Department of Uro-oncology, Universidade Estadual de Campinas, UNICAMP
Campus Universitario Zeferino Vaz SN
Cidade Universitaria - Campinas, 13083-970
E-mail: rogeriocardosodasilva@hotmail.com

ORIGINAL ARTICLE

Vol. 39 (2): 167-172, March - April, 2013
doi: 10.1590/S1677-5538.IBJU.2013.02.03

Radical cystectomy with W-shaped orthotopic ileal neobladder constructed with non-absorbable titanium staples-long term follow-up

Sergey Kravchick, Leonid Lobik, Adrian Paz, Eugeny Stepnov, David Ben-Dor, Shmuel Cytron

Department of Urology (SK, LL, AP, ES, SC) and Department of Pathology (DBD), Barzilai Med. Center, Ashkelon, Israel

ABSTRACT

Purposes: We retrospectively assessed our experience with the W-shaped orthotopic ileal pouch, which was constructed with non –absorbable titanium staples. For these purpose, we discuss the results of bladder capacity, urinary continence and early and long-term postoperative complications.
Materials and Methods: We included in the study 17 patients who underwent radical cystoprostatectomy followed by construction of an orthotopic W-shaped ileal pouch between October 2000 and November 2009. A 65-70 cm segment of ileum was isolated and prearranged into a W- configuration, leaving two 10 cm intact segments on both sides of the ileal fragment. In our technique we entirely anatomized all adjacent limbs in order to create a sphere-shaped pouch. The ureters were directly anastomized to both intact segments of the ileal division. All our patients underwent pouchscopy 6 months after operation and annually.
Results: Mean operative time for neobladder reconstruction and ureteral anastomoses was 87 ± 7.67 minutes. In one patient a leak from the ileo-ileal anastomosis was confirmed on the 3rd day after operation. In 2 cases unilateral stricture of the ureteral-neobladder anastomosis was documented. Staple lines were mostly covered with ileal mucosa after 6 months. The mean functional bladder capacity was 340 ± 27.6 mL and 375 ± 43.4 mL at 6 and 12 months, respectively. First-year daytime and nighttime continence was good and acceptable in 90% and 78% of patients, while it increased to 95% during the 2nd year.
Conclusions: The long term follow-up shows that non-absorbable titanium staples can be safely used for creation of an orthotopic ileal neobladder. However, these data should be further validated in a larger series of patients.

ARTICLE INFO

Key words:
blabber neoplasm, cystectomy, laparoscopy, urinary diversion

Int Braz J Urol. 2013; 39: 167-72

Submitted for publication:
August 21, 2012

Accepted after revision:
January 18, 2013

INTRODUCTION

Since the first report of successful laboratory and clinical data of stapled bladder closure with titanium staples (1), several studies have adopted this technique with encouraging results (2-6). This technique possesses several advantages, such as decreased operative time, excellent tissue adaptation and presumably watertight closure. However, the possibility of urinary stone formation on the staples requires long term follow-up (1). In this study we describe our technique for W-shaped orthotopic ileal pouch construction with non-absorbable titanium staples and report the results of long term follow-up.

MATERIALS AND METHODS

Between October 2000 and November 2009 seventeen males with invasive high grade carcinoma of urinary bladder underwent radical cystoprostatectomy with orthotopic W-shaped ileal pouch, which was constructed with non-absorbable staples. Six patients underwent this operation with a laparoscopic approach, in which case the pouch was created after surgical specimen removal through an additional 7 cm incision. The criteria for inclusion in the study were: non-metastatic disease, negative biopsies from prostatic urethra, adequate renal function (serum creatinine < 1.5mg/dL), normal liver function, no active inflammatory bowel disease or previous extensive bowel resection (for laparoscopic procedure), physical and mental ability to live with a bladder substitute and the ability to perform self catheterization if needed, and compliance with routine follow-up.

Technique

Open (n-11) radical cystoprostatectomy was performed in the usual manner. In all laparoscopic cases (n-6) we used a 5-port transperitoneal approach (7-9) and a 2-arm spring-loaded articulating instrument holder (Endoholder). In laparoscopic cases the surgical specimen was removed intact through an additional 7 cm incision. Afterwards we isolated a 65-70 cm segment of ileum 20 cm proximally from the ileocecal valve. The most dependent part of the segment, that could easily reach the top of the symphysis pubis, was pointed and marked with a suture. The ileum was then divided between bowel clamps and a standard bowel anastomosis was performed with staples. The mesenteric trap was closed. A 40-45 cm segment of the isolated ileum was prearranged into W configuration, leaving two intact segments on both sides of the ileal fragment in order to decrease the tension on the ureteral anastomoses. In our version of the W-shaped technique all adjacent limbs were entirely anastomosed "side by side". For this purpose, an opening was created on the pointed distal loop of the segment and a 80X3.5 mm mechanical stapler (Multifire GIA - US Surgical) was inserted through the opening and fired (Figures 1 A and B). Later, additional openings were made on the proximal part of the segment and 60/80X3.5 mm mechanical staplers were inserted and fired in order to create a sphere-shaped pouch (Figures 1 C and D).

Figure 1A - A 40-45 cm segment of the isolated ileum is prearranged into W configuration, leaving two intact segments on both sides of the ileal fragment. An opening was created on the pointed distal loop.

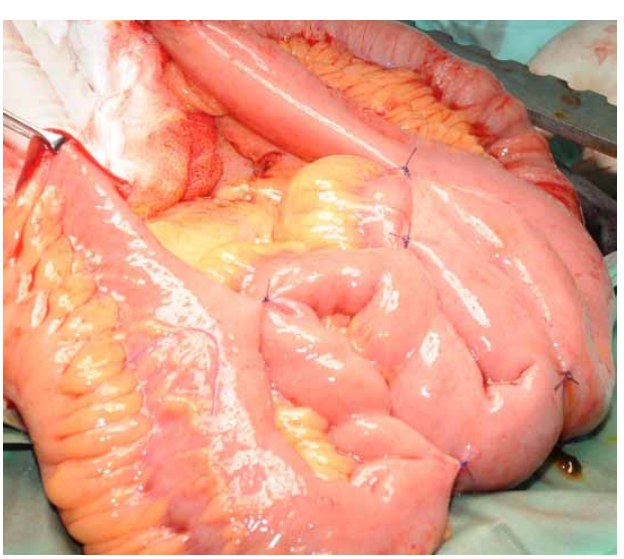


Figure 1B - 80X3.5 mm mechanical stapler (Multifire GIA - US Surgical) is inserted through the distal opening and fired.

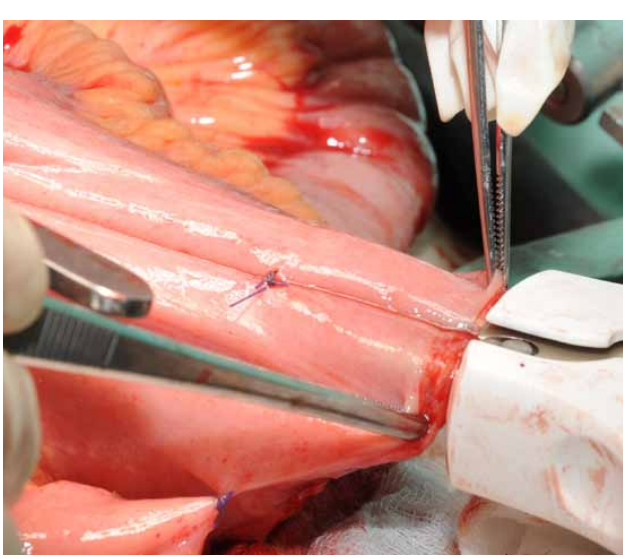


Figure 1C - The additional opening is made on the proximal part of the segment and 60/80X3.5 mm mechanical staplers is inserted and fired.

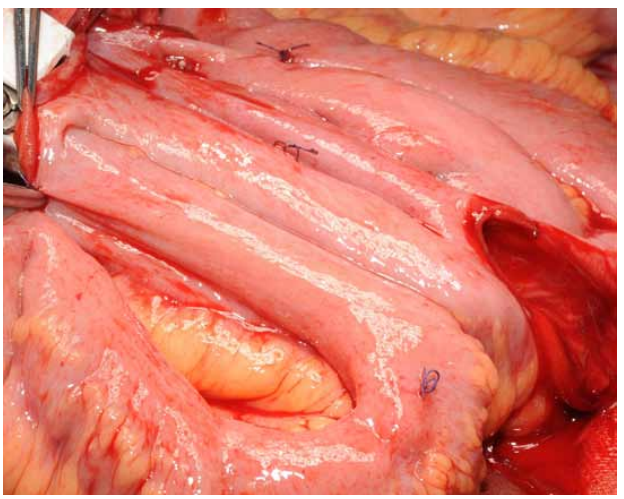
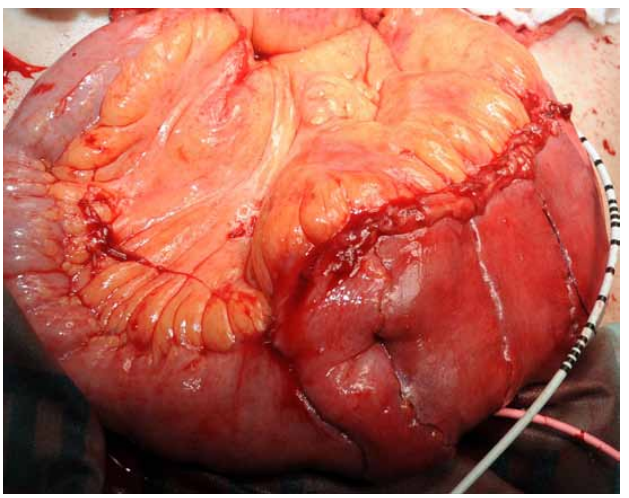


Figure 1D - A sphere-shaped pouch is created.



Afterwards, 2 single J stents were inserted up to the renal pelvis and the ureters were directly anastomosed to the intact segments of the ileal division, according to the Wallace technique. The distal ends of the stents were taken out through the separate small openings on the lateral walls of the pouch. Finally, the proximal openings in the pouch, which were made for the staplers insertion, were closed with running absorbable sutures, leaving the distal outlet open. The neobladder was placed inside the abdominal cavity and a 22 Fr silicone Foley catheter was inserted in the urethra. The pouch was anastomosed to the urethra with six interrupted 3-0 monocryl sutures over a 22F Foley. All knots were tied in the outside manner.

Postoperative and follow-up protocols

The urethral Foley catheter was irrigated with 50 mL of saline every 4 to 6 hours for the first 2 to 3 postoperative days. Starting from the 4th day it was irrigated with 100 mL every 8 hours. The Jackson-Pratt drains were removed as drainage decreased to less than 100 mL. If the cystogram on postoperative day 14 excluded any urinary extravasation, the left ureteral stent was removed first, followed 24 hours later by the right stent. The urethral catheter was removed between days 18-21.

During the first months all patients were instructed to void in a sitting position by relaxation of the pelvic floor, followed by slight abdominal straining. Effectiveness of emptying was maintained by hand pressure on the lower abdomen and bending forward. We asked our patients to perform daily self catheterization and pouch irrigation during the first month in order to decrease the chance of mucous blockage. Through the first 2 months patients were instructed to void every 3-4 hours during the day and every 4 hours at night. All our patients underwent pouchscopy 6 months after operation and annually.

We carried out cystometry 6/12 months postoperatively in all patients. Maximal neobladder capacity was identified based on the maximal discomfort in the lower abdomen or urethral leakage. We asked patients to fill out voiding charts to assess functional neobladder capacity and postvoiding residual volume (measured by catheter insertion). We also inquired that the patients grade their day and night time continence as good (no need for pad), acceptable (1-2 pads) or poor (> 2 pads). To exclude the detrimental effect (obstruction) of the anti- peristaltic segment of ileum which was used for uretero-ileal anastomosis, all patients underwent renal scan.

RESULTS

Patients' ages ranged from 47 to 72 years with a mean age of 68 ± 6.23. Mean time for

orthotopic ileal-neobladder reconstruction and ureteral anastomoses was 87 ± 7.67 minutes. No tumor was found in 2 postoperative specimens. In the other patients pathology reported T1 low grade (n-2), T1 high grade (n-4), T2 high grade (n-5), T2 high grade with CIS (n-1) and T3 a-b high grade TCC (n-3). Positive lymph nodes were found in 4 specimens. No sign of leakage was found on postoperative cystograms. Three patients suffered from upper tract UTI in the early postoperative period (Clavien Grade 2). In one patient a leak was confirmed from the intestinal anastomosis (ileo-ileal) on the 3rd day after operation (Clavien Grade 3b).

Five patients died from metastatic disease in the first 3 years after operation and two patients died from concomitant diseases (2 and 6 years, respectively). Mean follow-up was 64.76 ± 23.6 months. Seven patients remained in follow-up > 5 years (6-8 years) and six patients ≥ 3 years (3-5 years), while 4 patients were followed for ≤ 2 years.

During the first six months after operation daytime continence was good and acceptable in 90% (no or 1-2 pads), while nighttime continence was good and acceptable in 78% of patients. However, starting from the second half of the year daytime continence increased to 95%. One half year after surgery the mean maximal neobladder capacity was 395 ± 65.3 mL, while one year after operation it was 463 ± 59.4 mL. The mean functional bladder capacity was 340 ± 27.6 mL and 375 ± 43.4 mL at 6 and 12 months, respectively. Only three patients still used self catheterization 3 years after operation.

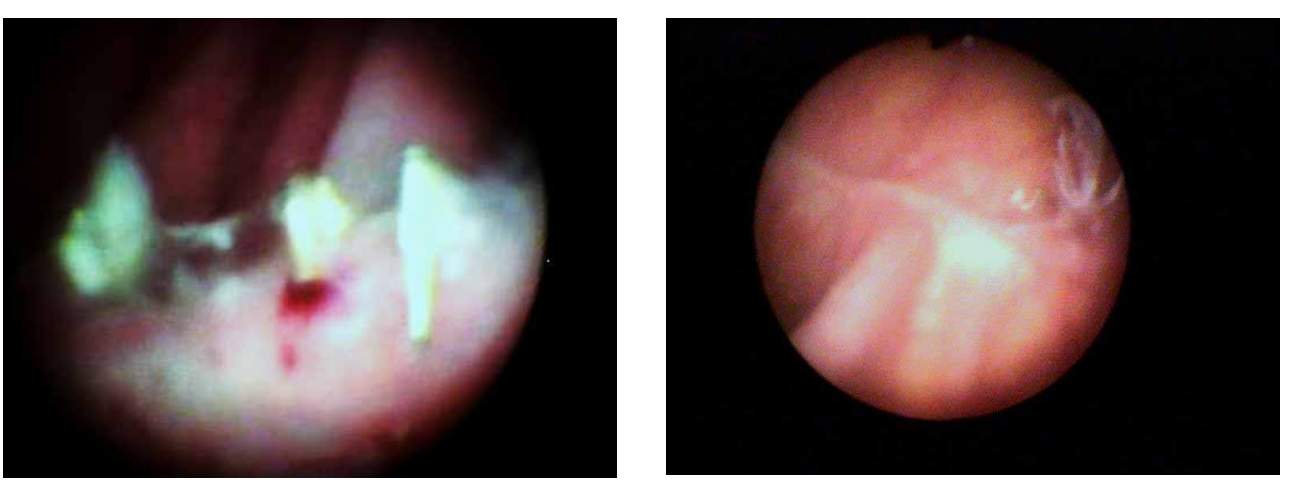
In 2 patients UTI developed 6 to 37 months after operation. Creatinine rose > 0.5 mg/dL from the "baseline" in 5 patients, while renal scan confirmed deterioration of the renal function in three of them (Clavien Grade 4a): two in the right and one in the left unit. In 8 patients treatment with vitamin B12 was started due to anemia. In two cases mild unilateral stricture of the right ureteral-neobladder anastomosis was suspected on control CT urography. In both cases renal scan (99mTc-MAG3) was followed by furosemide administration with a separate 20-min assessment and was interpreted as an equivocal result.

Cystoscopic control of the pouch revealed that the staple lines were mostly covered with ileal mucosa 6 months after operation (Figures 2 A and B). Small calculi 0.5 - 1 cm were found in 4 patients 2-6 years after operation and in all these cases the calculi passed spontaneously or were removed with a basket during follow-up flexible cystoscopy. We failed to find staple material in the nucleus of the stones.

DISCUSSION

The experience of general surgeons, who pioneered staplers for anastomosis construction during bowel resection and reconstruction, has shown that this device may decrease operative time, simplify tissue adaptation and guarantee leak proof closure (10-12). Consequently, the results of operation can become less dependent on

Figures 2 - A and B - Staple lines are mostly covered with ileal mucosa.



the operators' experience. Since 1993 urologists also began using stapling devices for laparoscopic bladder closure (1,2). Kerbl et al. performed laboratory and clinical studies and reported no intra- and postoperative complications, with no episodes of stone formation 3-5 months after operation (1). However, at that time, these authors warned that previous experience with stainless steel staples in ileal loop diversion and Kock pouch had shown stone occurrence in 1.8-10% and in 16.7% of the patients, respectively. Later on, Shalhav et al. discussed the results of long-term follow-up (greater than 7 years) after bladder closure with titanium staples and reported no stone formation on the closure line or within the bladder (3). In addition, Grubb et al. reported that Endo-GIA stapler used for renal pelvis closure possessed no risk for stone formation in the short term period (4).

Endoscopic control of neobladder in our study showed that urothelium had covered the staple line six months after operation. These results are in accordance with the data reported by Abreu et al. (5,6). However, small stones were found in 4(26%) of 17 patients. In all of these cases, the stones passed spontaneously or were removed with a basket. Subsequent analysis failed to find staple material in the nucleus of the calculi. Therefore, we agree that calculi formation in neobladder might be explained by another cause, including metabolic changes and other processes that take place in the pouch (13,14). Although the rates of stone formation in our study were a little bit higher than those previously reported (5,6,13,15,16), we believe that long-term follow--up and relative frequent endoscopic control might explain this discrepancy.

Ureteral stricture is one of the most controversial issues in ileal pouch construction, because it can cause renal damage. In an attempt to decrease their incidence, one should avoid excessive ureteral mobilization, devascularization and anastomotic tension. Based on the previous study of Montie et al., we used chimneys on the both sides of ileal loop in order to advance them towards the end of ureters (17). Anastomotic strictures occurred in two cases and were successfully treated with minimal endoscopic procedures. Although the stricture rate in our study was higher than that reported by Fontana et al., it is still comparable with the results of other studies (18). We also failed to find any sign of obstruction of the uretero-ileal anastomosis on the antiperistaltic site of neobladder.

Our version of the W-neobladder construction provided comparable maximal and functional bladder capacity due to the long detubularized segment and entirely anastomosed limbs. The storage capacity of our reservoir was much better than that reported by Montie et al. (17). As a result, good/acceptable daytime and nighttime continence was reported in 90% and 78% of the patients six months after operation. By the end of the first year daytime continence improved and reached 95%. These results are very similar to those previously reported by other authors, who constructed different types of neobladder (19,20). However, it must be emphasized that other studies had reported superior functional bladder capacity at the end of the first year (21). In these studies absorbable sutures were used, and this fact makes us to suggest that staplers line may restrain bladder functional capacity during the first years.

We presume that our study comes from a relatively "low volume" hospital and this fact can inversely affect the results of long-term follow-up (22). However, the success rates and the percentage of complications are very comparable with the data reported by "high volume hospitals/surgeons". In this context we would like to agree that mechanical staplers simplify the operation and makes its results less dependent on operator experience.

CONCLUSIONS

Long term follow-up shows that non-absorbable titanium staples can be safely used for the creation of an orthotopic ileal neobladder. Staple lines are typically covered with urothelium 6 months after operation. With strict annual follow--up the rates of small stone formation in this type of neobladder were higher than in the other series. Storage characteristics of ileal reservoir constructed by our version are comparable with other types of orthotopic pouches. This technique is fast, reliable and may help to decrease the difference between the low and high volume centers.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Kerbl K, Chandhoke P, McDougall E, Figenshau RS, Stone AM, Clayman RV: Laparoscopic stapled bladder closure: laboratory and clinical experience. J Urol. 1993; 149: 1437-9; discussion 1439-40.
2. Chandhoke PS, Clayman RV, Kerbl K, Figenshau RS, McDougall EM, Kavoussi LR, et al.: Laparoscopic ureterectomy: initial clinical experience. J Urol. 1993; 149: 992-7.
3. Shalhav AL, Dunn MD, Portis AJ, Elbahnasy AM, McDougall EM, Clayman RV: Laparoscopic nephroureterectomy for upper tract transitional cell cancer: the Washington University experience. J Urol. 2000; 163: 1100-4.
4. Grubb RL 3rd, Sundaram CP, Yan Y, Chen C, McDougall EM, Clayman RV: Use of titanium staples during upper tract laparoscopic reconstructive surgery: initial experience. J Urol. 2002; 168: 1366-9.
5. Abreu SC, Messias FI, Argollo RS, Guedes GA, Araujo MB, Fonseca GN: Laparoscopic assisted radical cystoprostatectomy with Y-shaped orthotopic ileal neobladder constructed with non-absorbable titanium staples through a 5 cm Pfannensteil incision. Int Braz J Urol. 2005; 31: 362-7; discussion 368-9.
6. Abreu SC, Araújo MB, Silveira RA, Regadas RP, Pinheiro DG, Messias FI, et al.: Laparoscopic-assisted radical cystectomy with U-shaped orthotopic ileal neobladder constructed using nonabsorbable titanium staples. Urology. 2006; 68: 193-7.
7. Gill IS, Fergany A, Klein EA, Kaouk JH, Sung GT, Meraney AM, et al.: Laparoscopic radical cystoprostatectomy with ileal conduit performed completely intracorporeally: the initial 2 cases. Urology. 2000; 56: 26-9; discussion 29-30.
8. Moinzadeh A, Gill IS: Laparoscopic radical cystectomy with urinary diversion. Curr Opin Urol. 2004; 14: 83-7.
9. Basillote JB, Abdelshehid C, Ahlering TE, Shanberg AM: Laparoscopic assisted radical cystectomy with ileal neobladder: a comparison with the open approach. J Urol. 2004; 172: 489-93.
10. Kracht M, Hay JM, Fagniez PL, Fingerhut A: Ileocolonic anastomosis after right hemicolectomy for carcinoma: stapled or hand-sewn? A prospective, multicenter, randomized trial. Int J Colorectal Dis. 1993; 8: 29-33.
11. Gustavsson K, Gunnarsson U, Jestin P: Postoperative complications after closure of a diverting ileostoma--differences according to closure technique. Int J Colorectal Dis. 2012; 27: 55-8.
12. Leung TT, MacLean AR, Buie WD, Dixon E: Comparison of stapled versus handsewn loop ileostomy closure: a meta-analysis. J Gastrointest Surg. 2008; 12: 939-44.
13. Terai A, Ueda T, Kakehi Y, Terachi T, Arai Y, Okada Y, et al.: Urinary calculi as a late complication of the Indiana continent urinary diversion: comparison with the Kock pouch procedure. J Urol. 1996; 155: 66-8.
14. Osther PJ, Poulsen AL, Steven K: Stone risk after bladder substitution with the ileal-urethral Kock reservoir. Scand J Urol Nephrol. 2000; 34: 257-61.
15. Fontana D, Bellina M, Fasolis G, Frea B, Scarpa RM, Mari M, et al.: Y-neobladder: an easy, fast, and reliable procedure. Urology. 2004; 63: 699-703.
16. Turk TM, Koleski FC, Albala DM: Incidence of urolithiasis in cystectomy patients after intestinal conduit or continent urinary diversion. World J Urol. 1999; 17: 305-7.
17. Montie JE, Pontes JE, Parulkar BG, Selby T: W-stapled ileal neobladder formed entirely with absorbable staples. J Urol. 1994; 151: 1188-92.
18. Shaaban AA, Abdel-Latif M, Mosbah A, Gad H, Eraky I, Ali-El-Dein B, et al.: A randomized study comparing an antireflux system with a direct ureteric anastomosis in patients with orthotopic ileal neobladders. BJU Int. 2006; 97: 1057-62.
19. Madersbacher S, Möhrle K, Burkhard F, Studer EU: Long-term voiding pattern of patients with ileal orthotopic bladder substitutes. J Urol. 2002; 167: 2052-7.
20. Rogers E, Scardino PT: A simple ileal substitute bladder after radical cystectomy: experience with a modification of the Studer pouch. J Urol. 1995; 153: 1432-8.
21. Marim G, Bal K, Balci U, Girgin C, Dinçel C: Long-term urodynamic and functional analysis of orthotopic "W" ileal neobladder following radical cystectomy. Int Urol Nephrol. 2008; 40: 629-3.
22. Goossens-Laan CA, Gooiker GA, van Gijn W, Post PN, Bosch JL, Kil PJ, et al.: A systematic review and meta-analysis of the relationship between hospital/surgeon volume and outcome for radical cystectomy: an update for the ongoing debate. Eur Urol. 2011; 59: 775-83.

Correspondence address:
Dr. Sergey Kravchick
HaAzmaut 87 / 35
Ashdod, 77452, Israel
Fax.: + 9 728 674-5704
E-mail: cambell@netvision.net.il

ORIGINAL ARTICLE

Vol. 39 (2): 173-181, March - April, 2013
doi: 10.1590/S1677-5538.IBJU.2013.02.04

Study of testosterone as a predictor of tumor aggressiveness in patients with prostate cancer

Pedro Henrique Oliveira Cabral, Marcelo Wassano Iwamoto, Victor Silvestre Soares Fanni, Luciano da Rocha Barros, Sandro Nassar Cardoso, Luiz Figueiredo Mello, Sidney Glina

Ipiranga Hospital (PHCO, LRB, SNC, LFM, SG), Department of Urology and Brazilian Institute of Cancer Control - IBCC (MWI, VSSF), São Paulo, Brazil

ABSTRACT

Purpose: A growing body of evidence suggests that low testosterone can be an independent predictor of adverse clinicopathological features and worse prognosis in prostate cancer (PCa) patients. However, this association is still incompletely understood and the results are divisive. The aim of this study was to analyze testosterone as a predictor of aggressive disease in subjects with clinically localized PCa.

Materials and Methods: A cohort was conducted including the patients submitted to radical prostatectomy in our institution during a period of four years. The patients had clinically localized disease and their total testosterone (TT) was routinely measured preoperatively in the morning before surgery. They were stratified in groups with low (< 300 ng/dL) and normal TT (≥ 300 ng/dL). Tumor aggressiveness was inferred based on preoperative PSA levels, pathological Gleason score (lower, equal or greater than 7), TNM stage and surgical margins status.

Results: After analyzing 164 patients we found a significant association between mean preoperative TT and extraprostatic disease (379 for pT3 vs. 421 ng/for pT2 - $p < 0.001$, AUC > 0.99). Conversely, men with high Gleason score had similar mean TT compared to those with lower scores. Preoperative low TT (defined as TT < 300 ng/dL) could not be statistically correlated with either preoperative PSA levels, pathological Gleason score, extraprostatic extension, positive surgical margins or seminal vesicles involvement.

Conclusions: This study indicates that testosterone may be a useful predictive tool once pathological extraprostatic extension was somewhat signaled by lower TT levels preoperatively. However, it does not consolidate a clear association between aggressive tumor biology and hypogonadism.

ARTICLE INFO

Key words:
Prostate cancer; Testosterone; Hypogonadism; Prognosis

Int Braz J Urol. 2013; 39: 173-81

Submitted for publication:
August 08, 2012

Accepted after revision:
November 30, 2012

INTRODUCTION

Prostate cancer is a biologically heterogeneous disease and both indolent and aggressive tumors are found in clinical practice (1). Defining in which group a patient fits is critical for selecting the adequate treatment. In fact, there has been extensive research in this area and three major prognostic factors were universally established, namely the clinical TNM stage of the disease, preoperative levels of PSA, and degree of tumor differentiation as expressed by the Gleason score (1).

Testosterone is a hormone necessary for the development of the prostate and has been considered for more than 70 years an inductor of proliferation of normal and cancerous cells (2). This concept was introduced by Huggins' landmark study demonstrating that androgen deprivation caused

tumoral regression in men with metastatic (but not localized) prostate cancer (3). Interestingly, when analyzing the failure cases, the author found that those with small testes at the time of castration had a poor prognosis, the first description of a more ominous cancer arising in men with low testosterone. Surprisingly, it was not until recently that preoperative testosterone has been investigated as a new marker to identify aggressive disease among men with non-metastatic cancers (4).

While many controversies and uncertainties regarding the correlation between testosterone and the aggressiveness of non-metastatic PCa persist (5), an increasing body of evidence demonstrates not only an association between low total testosterone (TT) and pathologically advanced disease (6-8), but also with more undifferentiated tumors (9-11) and worse prognosis (12).

In addition, the usefulness of testosterone as a prognostic factor for clinically localized PCa in the Brazilian population has yet to be determined. To our best knowledge, there´s only one previous retrospective Brazilian survey of 64 patients that failed to validate TT as a predictor of either pathological stage or Gleason score (13).

The aim of this study was to evaluate prospectively the association between serum TT and clinicopathological features (preoperative PSA, Gleason score, pathological stage and surgical margins status) in patients submitted to radical retropubic prostatectomy (RRP) for the treatment of clinically localized PCa.

MATERIALS AND METHODS

We analyzed a prospective cohort of 164 patients submitted to open RRP and bilateral obturatory lymphadenectomy for the treatment of clinically organ-confined PCa. None of the patients received any type of neoadjuvant therapy or had previous testosterone replacement therapy. We excluded those on medications that could induce testosterone levels decrease, such as glucocorticoids, loop diuretics, cimetidine, digoxin, neuroleptic drugs, opiates, cannabinoids and others. The surgeries were performed by the team of urologists according to the technique previously described by Walsh (14), at the Department of Urology of the Ipiranga Hospital (Brazil), from April 2005 to May 2009. Nerve-sparing was pursued in all the procedures, except when it was judged to compromise oncological principles, in those cases in which there was an induration palpable in the lateral pelvic fascia after the endopelvic fascia was opened or when the neurovascular bundle seemed to be fixed to the prostate at the time it was being released.

The diagnosis of PCa was done by transrectal ultrasound-directed biopsy of a minimum of 12 fragments. The indications for biopsy were PSA > 4 ng/dL or suspect digital rectal examination.

Total testosterone was determined by a single sample of venous blood using a commercially available radioimmunoassay collected in the morning of the day before surgery. Two groups were devised: one with normal TT (≥ 300 ng/dL) and other with low TT (< 300 ng/dL). This threshold to delineate the low TT group was adopted because it is recommended by the American Society of Clinical Endocrinologists to indicate hypogonadism depending on symptoms and widely used in previous studies on testosterone and PCa (15).

The pathological staging of the surgical specimens was based on the 1997 TNM classification (AJCC/UICC). The surgical specimens were assessed for Gleason score, tumor volume, extracapsular extension, seminal vesicle invasion and lymph node involvement. Organ-confined tumors (pT2) included those tumors without extracapsular extension or seminal vesicles invasion. Locally advanced tumors (pT3-T4) included those with extracapsular extension (pT3a) or seminal vesicle invasion (pT3b). According to the Gleason score, patients were divided into low (Gleason < 7), intermediate (Gleason = 7) and high-grade disease (Gleason ≥ 8).

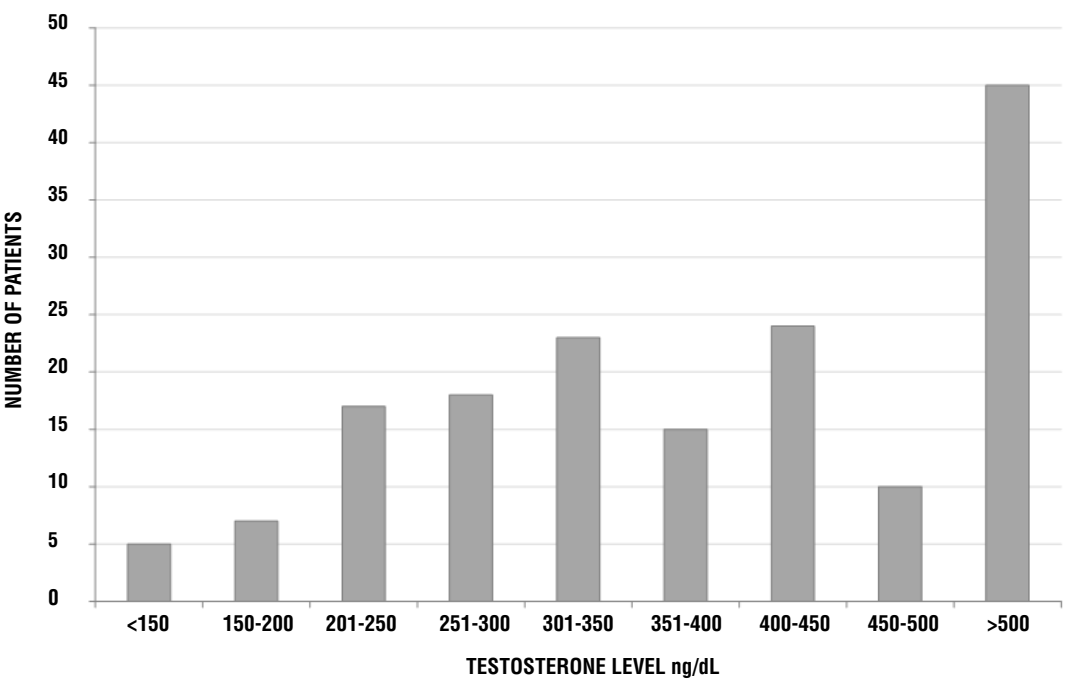
Collected data was allocated in an electronic spreadsheet and statistical analysis was accomplished by a statistician using the Mann-Whitney and Kruskall-Wallis tests for comparing the means of continuous numeric variables, and the likelihood ratio test to analyze proportions of categorized variables (groups with low and normal testosterone). Results were considered significant when $p < 0.05$.

The study was approved by the Ethics Committee of the Institution and informed consent was obtained from all patients before enrollment.

RESULTS

Of the 164 patients included, the mean age, PSA and TT levels were 63.6 years (range: 44-76 years), 9.35 ng/mL and 400.4 ng/dL (range: 92-1050 ng/dL), respectively. Forty-seven patients (28.6%) had low TT. Figure-1 shows the distribution of TT levels in the population.

Figure 1 - Total testosterone levels in the study population.



PSA levels, age or suspicious digital rectal examination did not differ significantly between the groups, but hypogonadal men had higher BMIs (Table-1).

One hundred twenty patients (73.2%) had stage organ-confined disease and 44 (26.8%) were pT3. Mean TT was 421 ng/dL for pT2 and 379 ng/dL for pT3 tumors (Table-2). This difference was statistically significant, with an area under the curve (AUC) > 0.99. In the categorized analysis, the rate of extraprostatic disease was higher in the hypogonadic group: 34% vs. 23.9%, but without statistical significance (Table-3).

Involvement of seminal vesicles was noted in 14 (8.5%) and positive surgical margins in 44 patients (26.8%). The occurrence of these events was comparable in both groups (Table-3). There wasn´t any case of lymph node involvement or T4 tumors.

In regard to tumor differentiation, 70 (42.7%) patients had Gleason < 7, 73 (44.5%) Gleason = 7 and 21 (12.8%) Gleason ≥ 8. The mean levels of TT were statistically equivalent in each one of these groups: 400.6, 432.2 and 365.8 ng/dL, respectively (Table-2). In the categorized analysis,

Table 1 - Baseline clinical characteristics stratified by total serum testosterone.

	Total Testosterone - ng/dL		p value
	< 300	≥ 300	
No. of pts (%)	47 (28.6)	117 (71.4)	Not applicable
Mean age (range)	63.6 (44-76)	62.6 (46-76)	0.50*
BMI - kg/m2 (range)	27.3 (21-34)	25.5 (17-32)	0.006*
Suspicious DRE (%)	17 (36.2)	41 (36)	0.9**
Mean PSA (± SD)	9.25 (7.64)	9.46 (5.57)	0.45*

* Mann-Whitney test. ** Likelihood ratio test
DRE: digital rectal examination.
BMI: body mass index.

Table 2 - Mean total testosterone levels according to the pathological outcomes.

Pathological feature	No. Pts (%)	Mean testosterone level - ng/dL (± SD)	p value
Organ-confined disease (pT2) p(pT2)(p(pT2)	120 (73.2)	421.6 (± 173)	
Extraprostatic disease (pT3)	44 (26.8)	379.1 (± 178)	< 0.001*
Gleason < 7	70 (42.7)	400.6 (± 172)	0.4 **
Gleason = 7	73 (44.5)	432.2 (± 183)	
Gleason ≥ 8	21 (12.8)	365.8 (± 153)	
Total	164 (100)	410.2 (± 175)	

* Mann-Whitney test
** Kruskall-Wallis test.

Gleason scores were also similar in groups with low and normal testosterone (Table-3).

DISCUSSION

The selection of the adequate method of treatment in oncology relies greatly on the balance between the aggressiveness of the disease and the benefits and morbidity of the therapy. This is particularly valid for PCa, a malignancy that is frequently indolent and which treatment (regardless of the method chosen) may be both deleterious and unnecessary. For better patient selection, D´Amico and others have stratified risk

Table 3 - Gleason score and pathological features stratified by total serum testosterone level.

	Total Testosterone - n (%)		p value
	< 300 ng/dL	≥ 300 ng/dL	
No. of pts	47	117	Not applicable
Gleason score			
< 7	23 (48.9)	47 (40.2)	
7	18 (38.3)	55 (47)	0.55
≥ 8	6 (12.8)	15 (12.8)	
Organ-confined disease (pT2)	31 (66)	89 (76.1)	0.19
Extraprostatic disease (pT3)	16 (34)	28 (23.9)	
Seminal vesicles compromised			
Yes	6 (12.8)	12 (10.3)	0.6
No	41 (87.2)	105 (89.7)	
Urethral margin positive			
Yes	10 (24.4)	20 (17.4)	0.3
No	31 (75.6)	95 (82.6)	
Vesical margin positive			
Yes	5 (12.5)	9 (7.9)	0.39
No	35 (87.5)	105 (92.1)	

Likelihood ratio test

groups considering only three major prognostic markers: clinical stage, Gleason score and PSA levels (1). Despite universally accepted these criteria are not flawless and urologists are still limited in their ability to predict pathological tumor stage in a reliable manner (4,5). Understanding other determinants of disease aggressiveness may be extremely helpful in selecting appropriate therapy for individual patients and advances in the comprehension of other prognostic factors such as cancer density in biopsy, third Gleason grade, genetic mutations, tumor characteristics on MRI and, more recently, testosterone have been made (12,16).

Although the data on the association between low testosterone and prognosis of metastatic prostatic cancer is solid (17), the link between serum testosterone and clinically localized PCa

is still incompletely understood and divisive, as we depict in Table-4. While there is evidence that cancers in a low testosterone environment tend to be more aggressive (6-10,12,18,19), many groups failed to demonstrate this association (1,20-22).

Approximately one third of our patients had TT deficiency, accordingly to surveys that also noted an increased incidence of biochemical hypogonadism in PCa patients compared to the general population (15). Again, this scenario is not unequivocal and a recent trial noted a rate of 15% of hypogonadism, which is comparable to the populational prevalence (7).

We adopted as primary endpoints the pathological features (Gleason score, stage, surgical margins status) as determined by the analysis of the surgical specimen because it´s the most reliable manner to determine the actual status of disease and biopsy frequently understages the tumor (16). In our view, this avoids confusing and conflicting results of others who relied exclusively on clinical staging and non-standardized biopsies.

The major finding of this survey was the significant difference in the mean preoperative TT levels when there was non-organ confined disease (421 vs. 379 ng/dL). This association was

Table 4 - Synthesis of the principal studies on the relationship between clinically localized prostate cancer and tumor aggressiveness.

			Clinicopathological features associated with low testosterone				
	No. of cases	Design	Gleason	TNM stage	PSA	Surgical margins	Recurrence
Hoffman (11) (2000)	57	Retrospective	Yes***	No	No	NA	NA
Schatz (13) (2001)	156	Retrospective	Yes	NA	Yes	NA	NA
Massengill (10) (2003) # *	879	Retrospective	No	Yes	No	No	No
Teloken (16) (2005)	64	Retrospective	No	No	No	Yes	NA
Isom-Batz (12) (2005) #*	326	Retrospective	No	Yes	No	NA	No
Imamoto (30) (2005) *	82	Retrospective	No	Yes	No	NA	Yes
Yamamoto (14) (2007)*	272	Retrospective	No	No	No	No	No
Lane (17) (2008)	455	Prospective	Yes	No	No	No	No
Pierorazio (19) (2010)	781	Retrospective	No	No	NA	NA	Yes
Xylinas (8) (2010)**	107	Retrospective	Yes	Yes	No	No	No
Botto (7) (2011)	431	Prospective	Yes	No	Yes	Yes	NA
Salonia (21) (2011)	673	Prospective	No##	No###	No	No	NA
Isbarn (9) (2009)	---	Review	Uncertain	Uncertain	Uncertain	NA	No

NA - not analyzed; # included patients previously to PSA adoption; ## On multivariate analysis, but higher proportion of gleason 8 in the hypogonadic group; ### Association with seminal vesicles invasion when TT < 100 ng/dL, but not with extracapsular extension; * Testosterone not collected in a systematic manner; ** included patients submitted to laparoscopic procedure; *** Relied on biopsy results.

very strong, with an AUC > 0.99. Curiously, when patients were divided in groups of low and normal TT, the rate of pT3 disease was 11% higher in the hypogonadic group, but still not statistically significant. The reasons for this are unknown to the authors. Possibly, this difference may become significant with an inclusion of a higher number of patients. Another pertinent explanation addresses the TT cut-off level of 300 ng/dL adopted by us and other authors. Clearly, while a threshold of 300 ng/dL may be adequate to hypogonadism diagnosis according to consensus definition of endocrinology and urology societies (15), it may be inappropriate to predict tumor aggressiveness. The relatively high mean TT values we found in the groups (421 and 379 ng/dL) support this idea by themselves. Hoffman also reported a mean TT of 490 and 390 ng/dL when Gleason was < 8 or ≥ 8 respectively (9), levels similar to ours and to Imamoto et al., who also correlated lower mean TT with locally advanced PCa (18).

This ability to predict extraprostatic extension in prostatectomy specimens is important because it´s a proven indicator of aggressive disease, determining greater likelihood of clinical progression, greater risk of a positive surgical margin and poorer long-term cancer control (5). Massengill et al. were the first to demonstrate, in a retrospective cohort, results similar to ours less than ten years ago (6). In that study, there was a higher likelihood of non-organ confined disease (pT3–T4) as TT decreased, but testosterone was collected "at the discretion of the treating physician", potentially imparting a selection bias. The only previous study in a Brazilian population is retrospective and analyzed retrospectively 64 patients after RRP, with the only statistically significant association found between low TT and positive surgical margins, which in our experience was not more frequent in the men with TT < 300 ng/dL (13).

We failed to demonstrate that Gleason score or preoperative PSA levels are influenced by preoperative TT levels, like some groups (12,13,18,23) and in contrast to others (9,10,20). In our opinion, this seems somewhat logical because dihydrotestosterone (the most biologically active prostatic androgen) concentration in prostate cells does not reflect the concentration of total testosterone (24). Notably, when DHT was inhibited by finasteride or dutasteride in PCPT (25) and REDUCE (26) trials, a higher proportion of high grade tumors was detected.

Some of the most important outcomes in oncologic treatment are disease recurrence and actual clinical progression. Their relationship with testosterone lacks confirmation (20). Interestingly, there are studies demonstrating a correlation with Gleason score (20) and pathological staging (6,10) but not with PSA recurrence or clinical progression (18,20). In 2007, Yamamoto et al. demonstrated that preoperative TT was an independent predictor of biochemical recurrence, but paradoxically it did not correlate with any pathologic features (Gleason score, pathologic stage, surgical margins). The authors state that the reason of these discrepancies is unclear (12). In a well-conducted prospective study, Lane et al. concluded that low pretreatment TT was associated with Gleason pattern 4-5 cancer at prostatectomy, but not with pathological stage or disease progression thereafter (20). They affirm that "at present, routine measurement of TT in men treated by prostatectomy does not appear to be of any clinical value". An argument can be done, however, because this study used TT, which is not the most biologically active form. In this regard, Hoffman et al. showed that free testosterone correlated with mean percent of biopsies demonstrated cancer (47% vs. 28%, p = 0.018) and also with pathological stage while TT did not (9).

To further confound the scenario, Miller, Zangh and others have demonstrated a normalization of serum testosterone following RRP, raising the question if low TT may be a consequence and not the cause of more aggressive prostate cancer (27-29). They propose the lower TT in patients with more advanced pathological stage may be due to inhibition of the hypothalamic-pituitary by the neoplasia itself (13-15). Another theory is that there is a disruption in the normal growth and maintainance of the prostatic caused by a low testosterone hormonal milieu, leading to compensatory hyperplasia that might result in cell mutations and consequent selection of androgen independent, aggressive prostate cells (10). Actually, the exacts mechanisms of interaction between testosterone and PCa remain unknown.

The greater strength of our study was its prospective design, allowing routine morning testosterone measurement before surgery during a 4 years period and the formation of a cohort of men representative of the reality in which PCa is treated in Brazil, including both high and low-risk disease. To our knowledge, this is also the first prospective study to address testosterone as a predictor of aggressive disease in Brazilian men with clinically localized PCa. Validation of a prognostic factor in a different population is important because prostate cancer may be genetically and clinically diverse in different populations (30).

The limitations of our study include the absence of central pathological review and unavailability of data on long-term post-operative follow-up and survival. Body mass index was lower in the hypogonadic group (a finding shared by others (7)) and we did not control the groups for ethnicity because it´s particularly complex to discriminate race in the Brazilian population, that´s multiracial and heterogeneous. Free and bioavailable testosterone (considered more biologically active forms) were not determined. Furthermore, a single dosage of TT in the day before surgery could imply on an incorrect value, once the stress of preoperative period could modify testosterone levels on an individual fashion (15).

CONCLUSIONS

Preoperative TT was associated with extraprostatic disease and may become a useful tool to improve our ability to recognize more advanced carcinomas. This correlation was not validated for other variables indicative of tumor aggressiveness and is not unequivocally consolidated in the literature. Nonetheless, the concept that testosterone and other androgens have a permissive role and promote the development of PCa seems to be incorrect and an oversimplification in view of the current evidences in the field.

ABBREVIATIONS

BMI: Body mass index
DRE: Digital rectal examination
NA: Not analyzed
PCa: Prostate cancer
PSA: Prostate Specific Antigen
TT: Total testosterone

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Partin AW, Kattan MW, Subong EN, Walsh PC, Wojno KJ, Oesterling JE, et al.: Combination of prostate-specific antigen, clinical stage, and Gleason score to predict pathological stage of localized prostate cancer. A multi-institutional update. JAMA. 1997; 277: 1445-51. Erratum in: JAMA 1997; 278: 118.
2. Morgentaler A: Testosterone deficiency and prostate cancer: emerging recognition of an important and troubling relationship. Eur Urol. 2007; 52: 623-5.
3. Huggins C, Hodges CV: Studies on prostatic cancer. I. The effect of castration, of estrogen and androgen injection on serum phosphatases in metastatic carcinoma of the prostate. Cancer Res. 1941; 22: 293-7.
4. Morgentaler A: Turning conventional wisdom upside-down: low serum testosterone and high-risk prostate cancer. Cancer. 2011; 117: 3885-8.
5. Isbarn H, Pinthus JH, Marks LS, Montorsi F, Morales A, Morgentaler A, et al.: Testosterone and prostate cancer: revisiting old paradigms. Eur Urol. 2009; 56: 48-56.
6. Massengill JC, Sun L, Moul JW, Wu H, McLeod DG, Amling C, et al.: Pretreatment total testosterone level predicts pathological stage in patients with localized prostate cancer treated with radical prostatectomy. J Urol. 2003; 169: 1670-5.
7. Botto H, Neuzillet Y, Lebret T, Camparo P, Molinie V, Raynaud JP: High incidence of predominant Gleason pattern 4 localized prostate cancer is associated with low serum testosterone. J Urol. 2011; 186: 1400-5.
8. Xylinas E, Ploussard G, Durand X, Fabre A, Salomon L, Allory Y, et al.: Low pretreatment total testosterone (< 3 ng/mL) predicts extraprostatic disease in prostatectomy specimens from patients with preoperative localized prostate cancer. BJU Int. 2011; 107: 1400-3.
9. Hoffman MA, DeWolf WC, Morgentaler A: Is low serum free testosterone a marker for high grade prostate cancer? J Urol. 2000; 163: 824-7.
10. Isom-Batz G, Bianco FJ Jr, Kattan MW, Mulhall JP, Lilja H, Eastham JÁ: Testosterone as a predictor of pathological stage in clinically localized prostate cancer. J Urol. 2005; 173: 1935-7.

11. Schatzl G, Madersbacher S, Thurridl T, Waldmüller J, Kramer G, Haitel A, et al.: High-grade prostate cancer is associated with low serum testosterone levels. Prostate. 2001; 47: 52-8.
12. Yamamoto S, Yonese J, Kawakami S, Ohkubo Y, Tatokoro M, Komai Y, et al.: Preoperative serum testosterone level as an independent predictor of treatment failure following radical prostatectomy. Eur Urol. 2007; 52: 696-701.
13. Teloken C, Da Ros CT, Caraver F, Weber FA, Cavalheiro AP, Graziottin TM: Low serum testosterone levels are associated with positive surgical margins in radical retropubic prostatectomy: hypogonadism represents bad prognosis in prostate cancer. J Urol. 2005; 174: 2178-80.
14. Walsh PC: Radical prostatectomy in locally confined prostatic carcinoma. Prog Clin Biol Res. 1990; 359: 199-207; discussion 223-9.
15. Wang C, Nieschlag E, Swerdloff R, Behre HM, Hellstrom WJ, Gooren LJ, et al.: ISA, ISSAM, EAU, EAA and ASA recommendations: investigation, treatment and monitoring of late-onset hypogonadism in males. Int J Impot Res. 2009; 21: 1-8.
16. Freedland SJ, Presti JC Jr, Terris MK, Kane CJ, Aronson WJ, Dorey F, et al.: Improved clinical staging system combining biopsy laterality and TNM stage for men with T1c and T2 prostate cancer: results from the SEARCH database. J Urol. 2003; 169: 2129-35.
17. Chodak GW, Vogelzang NJ, Caplan RJ, Soloway M, Smith JÁ: Independent prognostic factors in patients with metastatic (stage D2) prostate cancer. The Zoladex Study Group. JAMA. 1991; 265: 618-21.
18. Imamoto T, Suzuki H, Fukasawa S, Shimbo M, Inahara M, Komiya A, et al.: Pretreatment serum testosterone level as a predictive factor of pathological stage in localized prostate cancer patients treated with radical prostatectomy. Eur Urol. 2005; 47: 308-12.
19. Pierorazio PM, Ferrucci L, Kettermann A, Longo DL, Metter EJ, Carter HB: Serum testosterone is associated with aggressive prostate cancer in older men: results from the Baltimore Longitudinal Study of Aging. BJU Int. 2010; 105: 824-9.
20. Lane BR, Stephenson AJ, Magi-Galluzzi C, Lakin MM, Klein EA: Low testosterone and risk of biochemical recurrence and poorly differentiated prostate cancer at radical prostatectomy. Urology. 2008; 72: 1240-5.
21. Salonia A, Gallina A, Briganti A, Abdollah F, Suardi N, Capitanio U, et a.: Preoperative hypogonadism is not an independent predictor of high-risk disease in patients undergoing radical prostatectomy. Cancer. 2011; 117: 3953-62.
22. Salonia A, Gallina A, Briganti A, Suardi N, Capitanio U, Abdollah F, et al.: Circulating estradiol, but not testosterone, is a significant predictor of high-grade prostate cancer in patients undergoing radical prostatectomy. Cancer. 2011; 117: 5029-38.
23. Monda JM, Myers RP, Bostwick DG, Oesterling JE: The correlation between serum prostate-specific antigen and prostate cancer is not influenced by the serum testosterone concentration. Urology. 1995; 46: 62-4.
24. Marks LS: Words of wisdom. Re: Endogenous sex hormones and prostate cancer: a collaborative analysis of 18 prospective studies. Eur Urol. 2009; 55: 750-1.
25. Thompson IM, Goodman PJ, Tangen CM, Lucia MS, Miller GJ, Ford LG, et al.: The influence of finasteride on the development of prostate cancer. N Engl J Med. 2003; 349: 215-24.
26. Andriole G, Bostwick D, Brawley O, Gomella L, Marberger M, Tindall D, et al.: Chemoprevention of prostate cancer in men at high risk: rationale and design of the reduction by dutasteride of prostate cancer events (REDUCE) trial. J Urol. 2004; 172: 1314-7
27. Miller LR, Partin AW, Chan DW, Bruzek DJ, Dobs AS, Epstein JI, et al.: Influence of radical prostatectomy on serum hormone levels. J Urol. 1998; 160: 449-53.
28. Zhang PL, Rosen S, Veeramachaneni R, Kao J, DeWolf WC, Bubley G: Association between prostate cancer and serum testosterone levels. Prostate. 2002; 53: 179-82.
29. Imamoto T, Suzuki H, Yano M, Kawamura K, Kamiya N, Araki K, et al.: Does presence of prostate cancer affect serum testosterone levels in clinically localized prostate cancer patients? Prostate Cancer Prostatic Dis. 2009; 12: 78-82.
30. Lima MM Jr, Oliveira MN, Granja F, Trindade AC, De Castro Santos LE, Ward LS: Lack of association of GSTT1, GSTM1, GSTO1, GSTP1 and CYP1A1 polymorphisms for susceptibility and outcome in Brazilian prostate cancer patients. Folia Biol (Praha). 2008; 54: 102-8.

Correspondence address:
Dr. Pedro Henrique Oliveira Cabral
Rua Barão de Indaiá, 639
Condomínio Laranjeiras Premium, casa 121
Flores, Manaus, Amazonas, 69058-448, Brazil
Telephone: + 55 11 8263-1559
E-mail: pedro.he@uol.com.br

ORIGINAL ARTICLE

Vol. 39 (2): 182-188, March - April, 2013
doi: 10.1590/S1677-5538.IBJU.2013.02.05

Results of preoperative electrical stimulation of pelvic floor muscles in the continence status following radical retropubic prostatectomy

Carla Elaine Laurienzo, Carlos Alberto Ricetto Sacomani, Telma Ribeiro Rodrigues, Stênio de Cássio Zequi, Gustavo Cardoso Guimarães, Ademar Lopes

Department of Physiotherapy (CEL, TRR), Department of Pelvic Surgery (CARS, SCZ, AL) and Department of Urology (GCG), A.C. Camargo hospital, Antonio Prudente foundation, Sao Paulo, Brazil

ABSTRACT

Purpose: To evaluate preoperative rectal electrical stimulation in the recovery of urinary continence in patients who undergo radical retropubic prostatectomy.
Materials and Methods: Patients were divided into 3 randomized groups: control, pelvic exercises, and electrical stimulation. A 1 hour pad-test, the ICIQ-SF, and the SF-36 were performed 1, 3, and 6 months after the surgical procedure.
Results: Of the 58 patients who were initially included in the study, 9 were excluded due to radiotherapy after surgical intervention, an indwelling urethral catheter for more than 30 days, high surgical risk, loss of follow-up, or incomplete participation in the study routines and spontaneous interruption. Forty-nine patients concluded the study (15 in the control group, 17 in the exercise group, and 17 in the electrical stimulation group). We did not observe any significant difference in the pad test ($p > 0.05$), the 8 domains of the SF-36, or ICIQ-SF score compared with control groups (control, exercise, and electrical stimulation).
Conclusion: Preoperative rectal electrical stimulation has no impact on continence status in patients who undergo radical retropubic prostatectomy. There is no difference in the three above mentioned groups with regard to urinary leakage and quality of life.

ARTICLE INFO

Key words:
Neoplasms; Prostatectomy; Urinary Incontinence; Pelvic Floor; Quality of Life

Int Braz J Urol. 2013; 39: 182-8

Submitted for publication:
April 24, 2012

Accepted after revision:
January 10, 2013

INTRODUCTION

Radical retropubic prostatectomy (RPP) is the chief option in the treatment of prostate cancer. Although it is a routine procedure that has improved with regard to technique, urinary incontinence (UI) remains a significant condition (1,2). Despite total urinary control, the majority of patients experiences some period of UI following surgery (2). Because UI negatively affects quality of life and can delay a return to social and professional activities (3), patients desire a rapid recovery of continence.

Several studies have described various modalities of pelvic floor muscle rehabilitation (PFMR), including Kegel exercises with or without biofeedback and electrical stimulation post-RPP (2,4-6). PFMR can enhance the return of urinary control after RPP. Some groups have conducted PFMR preoperatively and postoperatively (7), but few have analyzed the impact of PFMR that is performed exclusively before the procedure (8,9). PFMR has been hypothesized to prepare the urethral sphincter mechanism and pelvic floor muscles preoperatively, which remains to be demonstrated.

The aim of our study was to evaluate electrical stimulation of the pelvic floor muscles prior to radical retropubic prostatectomy to accelerate the recovery of continence.

MATERIALS AND METHODS

Patients with prostate cancer (stage T2) and candidates for RPP who were referred for treatment at the Pelvic Surgery Department of A.C. Camargo Hospital were eligible for the study. Exclusion criteria included: radiotherapy (previous or after RPP); previous transurethral resection; pre-existing neurological disease; urinary fistula after RPP; prolonged indwelling urethral catheterization (more than 15 days); clinical situations that rendered the patient unsuitable for surgical procedure; failure to attend all PFMR or electrical stimulation sessions; loss of follow-up and desistance. The surgical intervention (RPP) was performed by four highly skilled and experienced surgeons and included nerve-sparring technique, according to Walsh.

Outcome evaluation

After approval by the ethical committee and internal review board, 58 consecutive males were included in this analysis. All subjects received and signed an informed consent form. The patients were randomized (computer-generated list using Research Randomizer, v4) and divided into 3 groups: control (only verbal instructions to contract the perineum); Kegel exercises alone; and electrical stimulation plus Kegel exercises.

The electrical stimulation (ES) group underwent ten preoperative physiotherapy sessions, with variable frequency (respecting scheduled surgery), using electrical stimulation and rectal pelvic exercises.

Electrical stimulation of this group was conducted with the Phenix equipment (VIVALTIS®) via rectal probe length of approximately 12 cm, width 2.5 cm and approximate weight 04 g.

The parameters used included parameters for muscle strengthening, to tonic fibers and phasic fibers:

To tonic fibers: Frequency: 20 Hz (Hertz); Pulse Width: 700μs (microseconds), Rise Time: 02 seconds Descent time: 02 seconds Working Time: 06 seconds Rest Time: 06 seconds. Intensity was used to determine the visible contraction of the pelvic floor. The duration of the stimulation was 10 minutes.

To phasic fibers: Frequency: 65Hz; Pulse Width: 150μs, Rise Time: 02 seconds; Descent time: 02 seconds Working Time: 06 seconds Rest Time: 18 seconds. It was also used to determine the intensity visible contraction of the pelvic floor. The duration of the stimulation was 05 minutes.

The electrical stimulation group, in the same preoperative sessions, also performed five (5) types of exercises to contract the pelvic floor muscles: consecutive contractions of pelvic floor muscles for 5 seconds in dorsal decubitus (10 times), in the same position with the waist elevated (10 times), lying down with legs adduction against a plastic ball (10 times), and tenfold exercises standing and flexing the hips to approximately 60º.

The exercise group performed 10 (ten) preoperative physiotherapy sessions, with variable frequency (respecting scheduled surgery), using only the pelvic exercises. The exercises were exactly the same exercises of the electrical stimulation group, already described above.

The control group did not perform any type of therapeutic intervention in the preoperative period. The patients in this group were examined only once, before the surgery, when the evaluation was performed and when they received information about the anatomy of the prostate region (as in the other groups).

PFMR was performed in the preoperative period by the same physiotherapist (C.E.L.). All patients were then evaluated after RPP at 1, 3, and 6 months by 1 hour pad test, International Consultation on Incontinence Questionnaire- Short Form (ICIQ-SF), and the Medical Outcomes Study 36-Item Short Form Health Survey (SF-36). No patient received PFMR postoperatively.

The 1 hour pad test was the primary outcome measure and was administered according to the International Continence Society (ICS). During the test, the patient was instructed to drink 500 mL of water, wait in the seated position for 30 minutes, walk, and do some exercises for another 30 minutes.

The ICIQ-SF and SF-36 were considered secondary outcome measures and were self-administered. Possible doubts were solved with a different physiotherapist (T.R.R.).

Statistical analysis

The groups were compared by chi-square association test (for qualitative variables) and Kruskal-Wallis test (for quantitative variables). Comparisons of the SF-36, ICIQ-SF, and pad test scores between the groups during the follow-up period were made by two-way analysis of variance (ANOVA), in which 1 factor was a repeated measurement (month) and the other was an independent factor (group).

When there was a significant difference, post hoc Tukey HSD test was used to detect the difference. Differences between groups were considered, whereas within-group differences were disregarded. Odds ratios of the groups at each assessment (1, 3, and 6 months after the operation) were also calculated to determine the likelihood of developing urinary incontinence. In the pad test, the cutoff point for continence was ≤ 2 grams and > 2 grams for incontinence.

In all analyses, differences were significant at $p < 0.050$. The statistical analyses were performed using Statistica, version 7 (for ANOVA) and SPSS, version 16.0 (for all other analyses).

RESULTS

Nine men were excluded during the evaluation (2 for failing to attend all sessions, 2 due to desistance, 1 adjuvant radiotherapy, 1 postoperative urethral stenosis, 1 urinary fistula, 1 unsuitable for surgery due to cardiovascular risk, and 1 inadequate follow-up). Thus, 49 remained eligible for the study (15 in Group 1; 17 in Group 2; 17 in Group 3). The mean age of the patients was similar ($p = 0.556$) in all groups (Group 1: 64 ± 8 years; Group 2: 62 ± 7 years; Group 3: 60 ± 8 years).

There were no significant differences between groups with respect to clinical and demographic data. Table-1 shows baseline characteristics of the groups, regarding the qualitative variables.

The pad test (Table-2 and Figure-1) showed no significant difference between the 3 groups at first, third, and sixth months of follow-up ($p > 0.05$). Based on the odds ratios (ORs) between groups, there was no significant difference ($p > 0.05$), with a 95% confidence interval. Using a cutoff of 2 grams, the ORs between Groups 1 and 2 and between Groups 1 and 3 were 1.2 and 0.71 at 1 month, 1.14 and 1.14 at 3 months, and 1.39 and 2.71 at the end of the study (Month 6), respectively.

There was no significant difference in ICIQ-SF score between the 3 groups at 1, 3, and 6 months of follow-up ($p > 0.05$) (Table-3).

There were no differences between groups on the various domains of the SF-36 ($p > 0.05$).

DISCUSSION

Urinary control usually recovers gradually during the first year after RPP (10). However, even temporary UI can negatively affect one's quality of life and delay his return to social and professional activities.

Previous studies have focused on PFMR as a method to anticipate adequate urinary control after RPP, most of which reported better results when PFMR was used after RPP. Unfortunately, the optimal start and stop points, modality of PFMR, and duration and frequency of the treatment remain unknown.

Filocamo et al. (4) analyzed 300 consecutive patients who were randomized into 2 groups. One hundred fifty men were referred to a physiotherapist for pelvic exercises, and another 150 individuals were not given formal instructions for PFMR. Nineteen percent of the treated group achieved adequate continence during the first month following RPP, rising to 95% after 6 months. In the control group, 8% reported good urinary control at the first month versus 65% after 6 months.

Ribeiro et al. (6) also recently described good results in 26 patients who were treated with biofeedback-pelvic muscle training once per week for 3 months compared with a control group (28 men) who received only verbal instructions. At 12 months, 96% of patients in the biofeedback-trained

Table 1 - Comparison between groups according to qualitative variables control (N = 49).

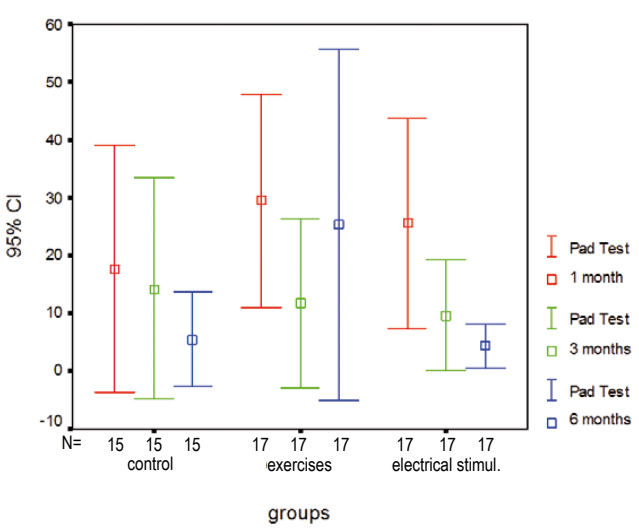
Variable	Category	Group			P
		Control N (%)	Exercises N (%)	Electrical stim. N (%)	(ϰ2)
Gleason	6	10 (66,7)	13 (76,5)	11 (64,7)	0,730
	7-9	5 (33,3)	4 (23,5)	6 (35,3)	
Systemic Arterial Hipertension	No	5 (33,3)	12 (70,6)	11 (64,7)	0,077
	Yes	10 (66,7)	5 (29,4)	6 (35,3)	
Use of diuretics	No	8 (53,3)	14 (82,4)	13 (76,5)	0,164
	Yes	7 (46,7)	3 (17,6)	4 (23,5)	
Heart disease	No	13 (86,7)	17 (100)	16 (94,1)	0,291
	Yes	2 (13,3)	0	1 (5,9)	
Diabetes	No	13 (86,7)	13 (76,5)	17 (100)	0,111
	Yes	2 (13,3)	4 (23,5)	0	
Respiratory disease	No	14 (93,3)	16 (94,1)	17 (100)	0,571
	Yes	1 (6,7)	1 (5,9)	0	
Smoking	No	14 (93,3)	16 (94,1)	13 (76,5)	0,213
	Yes	1 (6,7)	1 (5,9)	4 (23,5)	
Performs Physical Exercises	No	8 (53,3)	11 (64,7)	8 (47,1)	0,578
	Yes	7 (46,7)	6 (35,3)	9 (52,9)	
Urinary loss preoperatively	No	12 (80)	14 (82,4)	16 (94,1)	0,464
	Yes	3 (20)	3 (17,6)	1 (5,9)	
Other Cancer	No	15	17	17	@
Total		15 (100)	17 (100)	17 (100)	

@: statistical test was not done because not all responded

Table 2 - Comparison of pad test scores between groups during follow-up.

	Groups			
Period	Control (N=15)	Exercises N=(17)	Electrical Stimulation (N=17)	p*
	Mean (sd)	Mean (sd)	Mean (sd)	
1 month	17.6 (38.5)	29.5 (35.8)	25.5 (35.4)	> 0.05
3 months	14.3 (34.4)	11.8 (28.4)	9.6 (18.8)	> 0.05
6 months	5.5 (14.16)	25.3 (59.0)	4.35 (7.3)	> 0.05

Figure 1 - Comparison of pad test results between groups during follow-up.



group were continent versus 75% in the verbal instruction group. They also described a short period of incontinence immediately after RPP.

Few studies have examined PFMR before RPP. Although Burgio et al. (11) noted an improvement in continence and a decreased time to achieve urinary control in patients who attended one session of assisted biofeedback pelvic floor training and performed exercises at home, other groups failed to describe any benefits of PFMR exclusively in the preoperative period.

Considering the possibility that chronic electrical stimulation of tonic and phasic myofibers increases muscle strength, we also evaluated preoperative electrical stimulation of pelvic floor muscles using a rectal probe, comparing this method with Kegel exercises without biofeedback and with a control group that received only verbal instructions. The primary outcome was the 1 hour pad test score; quality of life questionnaires (ICIQ-SF score and SF-36) were used as secondary measures. Neither variable differed, and the social and physical aspects of the SF-36 were unaffected by PFMR. The ICIQ-SF revealed no changes in the impact of incontinence on quality of life between the 3 groups, reinforcing the poor results of PFMR regarding this area.

These data confirm the findings of previous groups with regard to PFMR in the preoperative period and support that PFMR is effective only when used postoperatively. Although PFMR might be unnecessary before the striated sphincter fibers have been manipulated, Parekh et al. (12) demonstrated good results with PFMR before and after the surgical procedure. Further studies are needed to determine whether preoperative and postoperative PFMR is more effective than PFMR only after RRP.

Table 3 - Comparison of ICIQ-SF scores between groups during follow-up.

	Groups			
Period	Control (N=15)	Exercises (N=17)	Electrical stimulation (N=17)	p
	Mean (sd)	Mean (sd)	Mean (sd)	
1 month	7.5 (5.0)	14 (3.6)	9.6 (6.3)	> 0.05
3 months	5.4 (5.2)	6.9 (5.8)	7.2 (6.4)	> 0.05
6 months	3.7 (5.3)	4.8 (5.3)	5.3 (5.5)	> 0.05

CONCLUSIONS

Preoperative rectal electrical stimulation has no impact on continence status in patients who undergo radical retropubic prostatectomy regarding urinary leakage and quality of life.

FINANCIAL SUPPORT

Sao Paulo State Foundation for Research Support – FAPESP (number 08/54585-1)

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Liu L, Coker AL, Du XL, Cormier JN, Ford CE, Fang S: Long-term survival after radical prostatectomy compared to other treatments in older men with local/regional prostate cancer. J Surg Oncol. 2008; 97: 583-91.
2. Van Kampen M, De Weerdt W, Van Poppel H, De Ridder D, Feys H, Baert L: Effect of pelvic-floor re-education on duration and degree of incontinence after radical prostatectomy: a randomised controlled trial. Lancet. 2000; 355: 98-102.
3. Moore KN, Valiquette L, Chetner MP, Byrniak S, Herbison GP: Return to continence after radical retropubic prostatectomy: a randomized trial of verbal and written instructions versus therapist-directed pelvic floor muscle therapy. Urology. 2008; 72: 1280-6.
4. Filocamo MT, Li Marzi V, Del Popolo G, Cecconi F, Marzocco M, Tosto A, et al.: Effectiveness of early pelvic floor rehabilitation treatment for post-prostatectomy incontinence. Eur Urol. 2005; 48: 734-8.
5. Mariotti G, Sciarra A, Gentilucci A, Salciccia S, Alfarone A, Di Pierro G, et al.: Early recovery of urinary continence after radical prostatectomy using early pelvic floor electrical stimulation and biofeedback associated treatment. J Urol. 2009; 181: 1788-93.
6. Ribeiro LH, Prota C, Gomes CM, de Bessa J Jr, Boldarine MP, Dall'Oglio MF, et al.: Long-term effect of early postoperative pelvic floor biofeedback on continence in men undergoing radical prostatectomy: a prospective, randomized, controlled trial. J Urol. 2010; 184: 1034-9.
7. Bauer RM, Bastian PJ, Gozzi C, Stief CG: Postprostatectomy incontinence: all about diagnosis and management. Eur Urol. 2009; 55: 322-33.
8. Bales GT, Gerber GS, Minor TX, Mhoon DA, McFarland JM, Kim HL, et al.: Effect of preoperative biofeedback/pelvic floor training on continence in men undergoing radical prostatectomy. Urology. 2000; 56: 627-30.
9. Tobía I, González MS, Martínez P, Tejerizo JC, Gueglio G, Damia O, et al.: Randomized study on urinary continence after radical prostatectomy with previous kinesic perineal physiotherapy. Arch Esp Urol. 2008; 61: 793-8.

10. Van Kampen M, De Weerdt W, Van Poppel H, Baert L: Urinary incontinence following transurethral, transvesical and radical prostatectomy. Retrospective study of 489 patients. Acta Urol Belg. 1997; 65: 1-7.
11. Burgio KL, Goode PS, Urban DA, Umlauf MG, Locher JL, Bueschen A, et al.: Preoperative biofeedback assisted behavioral training to decrease post-prostatectomy incontinence: a randomized, controlled trial. J Urol. 2006; 175: 196-201; discussion 201.
12. Parekh AR, Feng MI, Kirages D, Bremner H, Kaswick J, Aboseif S: The role of pelvic floor exercises on post-prostatectomy incontinence. J Urol. 2003; 170: 130-3.

Correspondence address:
Dr. Carlos Alberto Ricetto Sacomani
Hospital A.C. Camargo
Rua Antonio Prudente, 211, Liberdade
São Paulo, SP, Brazil
E-mail: drsacomani@gmail.com

ORIGINAL ARTICLE

Vol. 39 (2): 189-194, March - April, 2013
doi: 10.1590/S1677-5538.IBJU.2013.02.06

Comparison of radiographic and pathologic sizes of renal tumors

Wei Chen, Linhui Wang, Qing Yang, Bing Liu, Yinghao Sun

Department of Urology, Changhai Hospital, Second Military Medical University, Shanghai, China

ABSTRACT

Purpose: The determination of the size of a renal tumor is important for staging, prognosis and selection of the appropriate surgical treatment. We investigated the difference of radiographic and pathologic size of renal tumors in a contemporary cohort of patients who underwent nephron sparing surgery and evaluated its clinical implications.

Materials and Methods: The records of 169 patients who received nephron sparing surgery for renal lesions suspicious for malignancy between January 2006 and December 2010 were reviewed retrospectively. Radiographic tumor size, defined as the largest diameter of tumor measured by CT images, and pathologic size, the largest diameter of tumor measured in the surgical specimen, were compared and analyzed.

Results: Among all subjects, mean radiographic and pathologic tumor size were 3.25 ± 1.78 cm and 3.03 ± 1.91 cm, respectively ($P < 0.001$), with a discrepancy of just 0.22 cm. When the patients were categorized according to radiographic tumor size in the 1 cm range, the mean radiographic tumor size was significantly greater than pathologic tumor size in the following groups: 2 to 3 cm ($P < 0.001$), 3 to 4 cm ($P < 0.001$), and 4 to 5 cm ($P = 0.028$). When radiographic and pathologic tumor sizes were compared according to the pathologic tumor subtype, a significant difference was observed only among those with clear cell renal carcinoma ($P < 0.001$).

Conclusions: Renal tumor size was overestimated by radiography as compared with pathology. The difference was just 0.22 cm with little clinical significance, suggesting that CT provides an accurate method to estimate renal tumor size preoperatively.

ARTICLE INFO

Key words:
Nephrons; Surgery; Pathology; Radiography; Carcinoma, Renal Cell

Int Braz J Urol. 2013; 39: 189-94

Submitted for publication:
July 09, 2012

Accepted after revision:
January 10, 2013

INTRODUCTION

The determination of the size of a renal tumor is important for staging, prognosis and selection of the appropriate surgical treatment. Several reports in the literature have concluded that the prognosis of renal tumor, including renal cell carcinoma (RCC), was related to the pathologic size of the tumor (1,2). Meanwhile, the increased use of modern imaging techniques such as computed tomography (CT) has led to an increase in the incidental discovery of smaller renal masses. As a result, nephron sparing surgery (NSS) has been developed and has become a standard surgical treatment for small renal masses. The oncologic outcome is similar to that achieved with radical nephrectomy (RN) (3,4). The decision to perform NSS is mainly determined by the radiographic size, but not the pathologic size, of the renal mass as measured by preoperative CT. Therefore, it is necessary to define the consistency between pathologic and radiographic sizes.

Several previous reports have shown a certain degree of discrepancy between the preopera-

tive size of renal tumors as measured by CT and the pathologic size as determined from surgical specimens (5-7). A difference in tumor size can alter patients' status regarding tumor stage and prognosis. Also, such discrepancy may result in inadvertent exclusion of a significant number of patients from the opportunity to receive NSS. As maximum preservation of kidney function as well as adequate cancer control is important for the management of RCC, such potential discrepancy should be identified. In this study, we compared the radiographic and pathologic renal tumor sizes of patients in our department who received open NSS or laparoscopic NSS. The main aim of our study was to determine if radiographic size is equal to pathologic size among renal tumors and, if not, whether radiography overestimates or underestimates tumor size and by how much.

MATERIALS AND METHODS

Upon securing approval from the institutional review board of our hospital, we reviewed the records of 169 patients who received open NSS or laparoscopic NSS for renal lesions suspected of malignancy from January 2006 to December 2010. Only the patients who underwent preoperative CT scans at our institution less than 4 weeks before undergoing surgery were included.

The size of renal tumors on contrast--enhanced CT scans was measured in three axes including the anterior-to-posterior, superior-to--inferior, and left-to-right axes. The radiographic size was accepted as the largest of these three diameters. Pathologic size was defined as the largest diameter of the tumor as determined by pathologic examination. In patients with multifocal renal tumors, the tumor with the largest diameter was evaluated. The measurement of tumor size by CT scan and pathologic size were performed by one radiologist and one pathologist.

The clinical informations, including each patient's age, gender, tumor side, histologic subtype and primary tumor classification, were recorded. The primary tumor classification was established according to the AJCC 7th edition of RCC TNM-staging system. In our study, patients were categorized according to radiographic tumor size and pathological diagnoses. The mean values of radiographic and pathologic tumor size, along with differences in these sizes, were calculated for each category. The correlation between radiographic and pathologic tumor size was also analyzed. All categorical variables were analyzed by either a two-tailed Fisher's exact test or a Chi-square test, as appropriate. All continuous variables were analyzed by either a two-tailed Student's t test or a one-way analysis of variance, as appropriate. Statistical Package for Social Sciences 17.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. P values < 0.05 were considered statistically significant.

RESULTS

A total of 169 patients underwent NSS and were included in our study. A summary of the patient demographics is shown in Table-1. The patients included 106 males and 63 females with an overall median age of 48.7 years. The majority pathologic subtypes were clear cell renal cell carcinoma and angiomyolipoma, accounting for 50.3% and 27.2% of all subjects, respectively. Among all the patients, there were about 134 patients with T1a clinical stage and 28 with T1b clinical stage, accounting for 79.3% and 16.6%, respectively. Only 7 patients with T2 clinical stage received NSS. All tumors had no positive margins.

Among all subjects, mean radiographic tumor size and mean pathologic tumor size were 3.25 $\pm$ 1.78 cm and 3.03 $\pm$ 1.91 cm, respectively ($P < 0.001$), which indicated that the mean radiographic tumor size was greater than the mean pathologic size (Table-2). However, the difference between radiographic and pathologic size was just 0.22 cm with little clinical significance. The relationship between both measurements of tumor size is depicted in Figure-1 and indicates the existences of a strong correlation ($r = 0.956$, $P < 0.001$).

When all the patients were categorized according to radiographic tumor size (in 1 cm ranges), mean radiographic tumor size was greater than pathologic tumor size for all ranges of tumor size, except for the ≥ 7 cm range (Table-2). However, mean radiographic tumor size was significantly greater than pathologic tumor size only in the

Table 1 - Patient Characteristics.

Variables	Median or n (%)
No. of total subjects	169
Age (years)	48.7 (16-80)
Gender	
Male	106 (62.7)
Female	63 (37.3)
Tumor side	
Left	82 (48.5)
Right	87 (51.5)
Histology	
Clear cell	85 (50.3)
Papillary	8 (4.7)
Chromophobe	2 (1.2)
RCC other	6 (3.6)
Oncocytoma	5 (3.0)
Angiomyolipoma	46 (27.2)
Benign other	17 (10.0)
Primary tumor classification	
T1a	134 (79.3)
T1b	28 (16.6)
T2a	4 (2.4)
T2b	3 (1.7)

RCC, renal cell carcinoma

ranges of ≥ 2 cm and < 3 cm, ≥ 3 cm and < 4 cm, ≥ 4 cm and < 5 cm.

When radiographic and pathologic tumor sizes were compared according to pathologic tumor subtypes, a significant difference between radiographic and pathologic tumor size was observed only among those with clear cell RCC ($P < 0.001$) (Table-3). Among the 85 patients with clear cell histology, tumor size was overestimated by 0.27 cm on CT. The tumor sizes were underestimated only in those with chromophobic RCC but overestimated in other pathologic subtypes.

DISCUSSION

The discrepancy between radiographic and pathologic renal tumor size has been discussed in previous reports (5-8). To the best of our knowledge, we present the largest comparison of radiographic and pathologic tumor size for patients with a renal mass treated by NSS. Consistent with some previous observations, in our study, radiographic size overestimated the pathologic size when comparing all patients, but the overall difference between radiographic and pathologic sizes was only 0.22 cm. Although it was statistically significant, we do not think this disparity represents a clinically significant result.

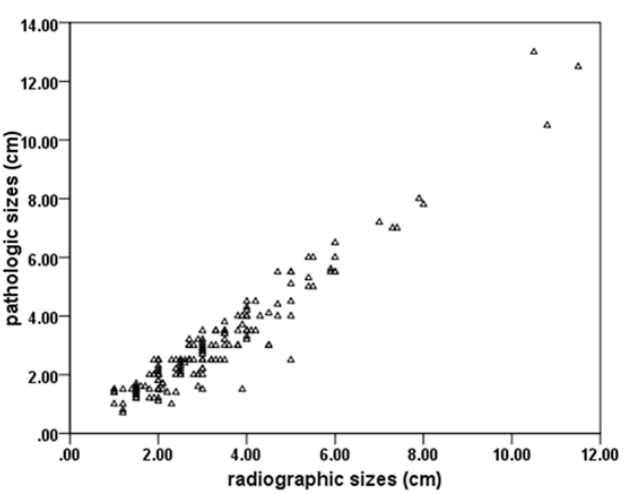
In our series, subgroup analysis showed that the discrepancy between radiographic and pathologic size increased with tumor size in the range of 0 to 4 cm. However, the discrepancy decreased with increased tumor size, when the tumors were larger than 4 cm. The radiographic size overestimated the pathologic size in all groups except the tumors with sizes exceeding 7 cm, but the discrepancy had little significance because only 8 patients were included in this group. The largest gap between the two measurements occurred in tumors of 3 to 4 cm in size, which was different from some previous studies (5-7). When evaluating the subgroups according to 1 cm intervals, Schlomer et al. (5) and Kurta et al. (6) found that the largest differences in size were in patients with tumors of 4 to 5 cm, while Lee et al. (7) considered that the largest differences occurred in patients with tumors of < 1 cm in size.

Tumor size has been widely used when recommending NSS for patients in elective-surgery scenarios (9). Traditionally, a 4 cm cutoff has been recommended, although more recent observations suggested that a threshold of > 4 cm and even 7 cm for appropriately selected patients was safe and effective (10,11). In our study, the tumors with radiographic size less than 4 cm were overestimated by CT, but the discrepancy would not affect the decision between RN and NSS. However, the

Table 2 - The mean radiologic and pathologic tumor size (in the 1-cm radiologic category and clinical stage).

RS range (cm)	n	RS (cm)	PS (cm)	Mean difference (95% CI) (cm)	t	P-value
1 to < 2	31	1.47 ± 0.29	1.45 ± 0.36	0.02 (-0.10,0.13)	0.281	0.781
2 to < 3	54	2.36 ± 0.32	2.12 ± 0.52	0.24 (0.12,0.36)	3.992	< 0.001
3 to < 4	40	3.32 ± 0.32	2.93 ± 0.59	0.39 (0.21,0.56)	4.482	< 0.001
4 to < 5	19	4.23 ± 0.28	3.89 ± 0.62	0.34 (0.04,0.63)	2.395	0.028
5 to < 6	13	5.31 ± 0.34	5.04 ± 0.94	0.27 (-0.23,0.77)	1.164	0.267
6 to < 7	4	6.00 ± 0.00	5.87 ± 0.48	0.13 (-0.64,0.89)	0.522	0.638
≥ 7	8	8.80 ± 1.82	9.13 ± 2.51	-0.33 (-1.15,0.50)	-0.930	0.383
Total	169	3.25 ± 1.78	3.03 ± 1.91	0.22 (0.13,0.30)	5.040	< 0.001

Figure 1 - Relationship between radiologic and pathologic tumor sizes.



situation is different if the size of the tumor was larger than 4 cm. In the group with tumor sizes ranging from 4 to 5 cm, pathologic size was smaller than radiographic size. In some centers, a tumor size of 4 cm is still regarded as the cutoff between RN and NSS. According to our findings, a portion of patients with renal tumors slightly larger than 4 cm measured by CT, with actually pathologic size less than 4 cm, should receive NSS instead of RN. Recent studies have shown that PN for renal tumors provides superior intermediate--term preservation of renal function compared with RN (12,13). In addition, chronic renal failure is more prevalent than previously thought among patients with a renal mass and more than 25% of all patients with a renal mass have at least Grade 3 chronic kidney disease at presentation (14). It is therefore necessary to perform NSS for renal tumors to preserve renal function. Based on our results, we suggest that the threshold of tumor size of 4 cm for NSS should been expanded to some extent, and patients with tumors slightly larger than 4 cm could be offered elective NSS with proper informed consent, which is in agreement with previous studies (5,7). In our study, all tumors had no positive margins. Without doubt, whether tumors were smaller than 4 cm or slightly larger than 4 cm, it is necessary to keep the margin negative. Obviously, preoperative planning for NSS for a renal lesion also requires consideration of its location (exophytic vs. intrarenal, central vs. peripheral, hilar vs. polar) and relation to surrounding structures (main renal vessels, collecting system, colon).

Histological subtype is also an important prognostic indicator for patients with renal tumors. Several studies have showed that there is correlation among tumor size, histology, and

Table 3 - The mean radiologic and pathologic tumor sizes according to histological subtype.

HS	n	RS (cm)	PS (cm)	Mean difference (95% CI) (cm)	t	P-value
Clear cell	85	2.73 ± 0.94	2.46 ± 1.01	0.27 (0.16,0.38)	4.963	< 0.001
Papillary	8	2.91 ± 1.18	2.78 ± 0.87	0.13 (-0.26,0.53)	0.819	0.440
Chromophobe	2	1.90 ± 0.14	2.05 ± 0.07	-0.15 (-0.79,0.49)	-3.000	0.205
RCC other	6	3.02 ± 1.42	2.87 ± 1.29	0.15 (-0.17,0.47)	1.218	0.278
Oncocytoma	5	2.80 ± 1.44	2.40 ± 1.17	0.40 (-0.20,1.00)	1.845	0.139
Angiomyolipoma	46	4.48 ± 2.55	4.29 ± 2.87	0.19 (-0.03,0.41)	1.717	0.093
Benign other	17	3.06 ± 1.46	2.99 ± 1.40	0.07 (-0.13,0.26)	0.720	0.482

HS: histological subtype; **RS:** radiographic size; **PS:** pathologic size; **CI:** confidence interval; **RCC:** renal cell carcinoma

metastatic potential (15,16). Tumors with histology other than clear cell carcinoma appear to have a favorable prognosis and to be suitable for NSS, regardless of tumor size (17). Kurta et al. (6) evaluated the difference between mean CT tumor size and mean pathological size within each histological subgroup, and they found that there were statistically significant differences in the clear cell and papillary types, but the differences were small and unlikely to be clinically significant. Lee et al. (7) found that a significant difference was observed among those with clear cell RCC and papillary RCC, and pathologic tumor size was overestimated in clear cell RCC while underestimated in papillary RCC. In our series, statistically significant differences between radiographic and pathologic tumor size were observed only for clear cell tumors. The difference was small (0.27 cm) with no clinical significance, which was similar to the result obtained by Kurta et al. (6).

The present study has several limitations. Firstly, our study was a retrospective, single-institution analysis of patients. A standardized, prospective study would more definitively characterize the relationship between the radiographic and pathologic size of renal tumors. Secondly, although the time from the CT examination to the operation was limited to 4 weeks, it was not certain that the size of the renal tumor had remained the same throughout this period. Thirdly, the parameters measured in CT scans or specimens may be inaccurate, which would influence the analysis. Much of some potential errors, such as measurement errors, differences in transverse diameter orientation, would have been avoided in a prospective study, where the methods of measurement would have to follow a definite procedure. Finally, formalin fixation may shrink the pathologic specimen to some extent.

CONCLUSIONS

In summary, we found a statistically significant overestimation of renal tumor size when comparing radiographic with pathologic size. Nevertheless, the overall difference was only 0.22 cm. Among the tumors with sizes ranging from 4 to 5 cm, radiographic tumor size was significantly larger than pathologic size. This result may affect decisions to perform NSS in some patients with a radiographic tumor size slightly larger than 4 cm. In spite of slight overestimation of radiographic size compared with pathologic size, and with the expansion of indication for NSS, we believe that CT scans would be appropriate for staging and selection of treatment approaches for renal tumors.

ACKNOWLEDGEMENTS

This study was supported by the Municipal Hospital's Project for Emerging and Frontier Technology of Shanghai China (No. SHDC12010115).

ABBREVIATIONS

RCC = Renal Cell Carcinoma
CT = Computed Tomography
NSS = Nephron Sparing Surgery
RN = Radical Nephrectomy

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Lughezzani G, Jeldres C, Isbarn H, Perrotte P, Shariat SF, Sun M, et al.: Tumor size is a determinant of the rate of stage T1 renal cell cancer synchronous metastasis. J Urol. 2009; 182: 1287-93.
2. Ku JH, Moon KC, Kwak C, Kim HH: Metachronous metastatic potential of small renal cell carcinoma: dependence on tumor size. Urology. 2009; 74: 1271-5.
3. Roos FC, Brenner W, Müller M, Schubert C, Jäger WJ, Thüroff JW, et al.: Oncologic long-term outcome of elective nephron-sparing surgery versus radical nephrectomy in patients with renal cell carcinoma stage pT1b or greater in a matched-pair cohort. Urology. 2011; 77: 803-8.
4. Weight CJ, Lieser G, Larson BT, Gao T, Lane BR, Campbell SC, et al.: Partial nephrectomy is associated with improved overall survival compared to radical nephrectomy in patients with unanticipated benign renal tumours. Eur Urol. 2010; 58: 293-8.
5. Schlomer B, Figenshau RS, Yan Y, Bhayani SB: How does the radiographic size of a renal mass compare with the pathologic size? Urology. 2006; 68: 292-5.
6. Kurta JM, Thompson RH, Kundu S, Kaag M, Manion MT, Herr HW, et al.: Contemporary imaging of patients with a renal mass: does size on computed tomography equal pathological size? BJU Int. 2009; 103: 24-7.
7. Lee SE, Lee WK, Kim DS, Doo SH, Park HZ, Yoon CY, et al.: Comparison of radiographic and pathologic sizes of renal tumors. World J Urol. 2010; 28: 263-7.
8. Herr HW, Lee CT, Sharma S, Hilton S: Radiographic versus pathologic size of renal tumors: implications for partial nephrectomy. Urology. 2001; 58: 157-60.
9. Coffin G, Hupertan V, Taksin L, Vaessen C, Chartier-Kastler E, Bitker MO, et al.: Impact of elective versus imperative indications on oncologic outcomes after open nephron-sparing surgery for the treatment of sporadic renal cell carcinomas. Ann Surg Oncol. 2011; 18: 1151-7.
10. Roos FC, Brenner W, Jäger W, Albert C, Müller M, Thüroff JW, et al.: Perioperative morbidity and renal function in young and elderly patients undergoing elective nephron-sparing surgery or radical nephrectomy for renal tumours larger than 4 cm. BJU Int. 2011; 107: 554-61.
11. Becker F, Roos FC, Janssen M, Brenner W, Hampel C, Siemer S, et al.: Short-term functional and oncologic outcomes of nephron-sparing surgery for renal tumours ≥ 7 cm. Eur Urol. 2011; 59: 931-7.
12. Deklaj T, Lifshitz DA, Shikanov SA, Katz MH, Zorn KC, Shalhav AL: Laparoscopic radical versus laparoscopic partial nephrectomy for clinical T1bN0M0 renal tumors: comparison of perioperative, pathological, and functional outcomes. J Endourol. 2010; 24: 1603-7.
13. Lane BR, Fergany AF, Weight CJ, Campbell SC: Renal functional outcomes after partial nephrectomy with extended ischemic intervals are better than after radical nephrectomy. J Urol. 2010; 184: 1286-90.
14. Huang WC, Levey AS, Serio AM, Snyder M, Vickers AJ, Raj GV, et al.: Chronic kidney disease after nephrectomy in patients with renal cortical tumours: a retrospective cohort study. Lancet Oncol. 2006; 7: 735-40.
15. Remzi M, Ozsoy M, Klingler HC, Susani M, Waldert M, Seitz C, et al.: Are small renal tumors harmless? Analysis of histopathological features according to tumors 4 cm or less in diameter. J Urol. 2006; 176: 896-9.
16. Skolarus TA, Serrano MF, Berger DA, Bullock TL, Yan Y, Humphrey PA, et al.: The distribution of histological subtypes of renal tumors by decade of life using the 2004 WHO classification. J Urol. 2008; 179: 439-43; discussion 443-4.
17. Onishi T, Oishi Y, Yanada S, Abe K, Hasegawa T, Maeda S: Prognostic implications of histological features in patients with chromophobe cell renal carcinoma. BJU Int. 2002; 90: 529-32.

Correspondence address:
Dr. Yinghao Sun
Department of Urology,
Changhai Hospital,
168 Changhai Road,
Shanghai 200433, China.
Fax: + 86 21 3503-0006
E-mail: syhchanghai@126.com

ORIGINAL ARTICLE

Vol. 39 (2): 195-202, March - April, 2013
doi: 10.1590/S1677-5538.IBJU.2013.02.07

Minimally Invasive Pyeloplasty in Horseshoe Kidneys with Ureteropelvic Junction obstruction: A case series

Stephen Faddegon, Candace Granberg, Yung K Tan, Patricio C. Gargollo, Jeffrey A. Cadeddu

Department of Urology, University of Texas Southwestern Medical Center, Dallas, TX, USA

ABSTRACT

Background and Purpose: Horseshoe kidney is an uncommon renal anomaly often associated with ureteropelvic junction (UPJ) obstruction. Advanced minimally invasive surgical (MIS) reconstructive techniques including laparoscopic and robotic surgery are now being utilized in this population. However, fewer than 30 cases of MIS UPJ reconstruction in horseshoe kidneys have been reported. We herein report our experience with these techniques in the largest series to date.

Materials and Methods: We performed a retrospective chart review of nine patients with UPJ obstruction in horseshoe kidneys who underwent MIS repair at our institution between March 2000 and January 2012. Four underwent laparoscopic, two robotic, and one laparoendoscopic single-site (LESS) dismembered pyeloplasty. An additional two pediatric patients underwent robotic Hellstrom repair. Perioperative outcomes and treatment success were evaluated.

Results: Median patient age was 18 years (range 2.5-62 years). Median operative time was 136 minutes (range 109-230 min.) and there were no perioperative complications. After a median follow-up of 11 months, clinical (symptomatic) success was 100%, while radiographic success based on MAG-3 renogram was 78%. The two failures were defined by prolonged $t_{1/2}$ drainage, but neither patient has required salvage therapy as they remain asymptomatic with stable differential renal function.

Conclusions: MIS repair of UPJ obstruction in horseshoe kidneys is feasible and safe. Although excellent short-term clinical success is achieved, radiographic success may be lower than MIS pyeloplasty in heterotopic kidneys, possibly due to inherent differences in anatomy. Larger studies are needed to evaluate MIS pyeloplasty in this population.

ARTICLE INFO

Key words:
Kidney; Laparoscopy; Robotics; Surgical Procedures, Minimally Invasive

Int Braz J Urol. 2013; 39: 195-202

Submitted for publication:
April 24, 2012

Accepted after revision:
December 21, 2012

INTRODUCTION

Horseshoe kidney is a renal fusion anomaly characterized by renal malrotation, variable blood supply, high insertion of the ureter, and a propensity to form ureteropelvic junction (UPJ) obstruction in up to one third of cases (1). Since first being introduced in 1993 (2), minimally invasive pyeloplasty has become the gold standard for the treatment of UPJ obstruction (3). In heterotopic kidneys, success rates range between 90 and 100% (2,4,5). However, there are limited published series of MIS pyeloplasty for UPJ obstruction in horseshoe kidneys. Since the first case report in 1996 (6), fewer than 30 cumulative cases have been reported in the literature, with the largest individual case series consisting of 5 patients (7). These sparse reports may reflect the relative rarity of horseshoe UPJ obstruction, but may also relate to a perceived increase in surgical difficulty due to unfamiliar and variable anatomy.

Currently, there is no consensus regarding the optimal surgical approach for the treatment of horseshoe UPJ obstruction. We herein report our experience with various MIS approaches, which to our knowledge is the largest series to date.

MATERIALS AND METHODS

Patients

We conducted a retrospective review of consecutive patients with a horseshoe UPJ obstruction treated with MIS techniques at our institution from March 2000 to January 2012. Twelve patients were identified, but three patients were excluded because postoperative renography studies were not available as they moved away shortly following their surgeries and despite intense efforts, could not be reached. The study received Institutional Review Board approval. Four pediatric patients (≤ 18 years old) were treated by a pediatric urologist and the remainder by an adult minimally invasive surgeon. Indication for surgery was ipsilateral flank pain in eight patients, and urinary tract infection in one patient, with confirmation of UPJ obstruction by lasix renography in each case. Additional preoperative evaluation included a CT scan (Figure-1), IVP and/or renal ultrasound.

The surgical approach was determined by surgeon preference and body habitus. All robotic pyeloplasties were performed by one pediatric urologist, while the laparoscopic cases were performed by one adult urologist. The case of LESS pyeloplasty was chosen based on the favorable BMI of the patient.

Surgical techniques

A transperitoneal approach was used in each case. Five adult patients were treated including four that underwent conventional laparoscopic dismembered pyeloplasty, and one that underwent conventional laparoendoscopic single-site (LESS) dismembered pyeloplasty. Four pediatric patients were treated via a robotic approach including two that underwent dismembered pyeloplasty and two that underwent Hellstrom repair (Table-1).

For each approach, patients were placed in a flexed, lateral decubitus position. For conventional

Figure 1 - CT scan demonstrating right UPJ obstruction of a horseshoe kidney.

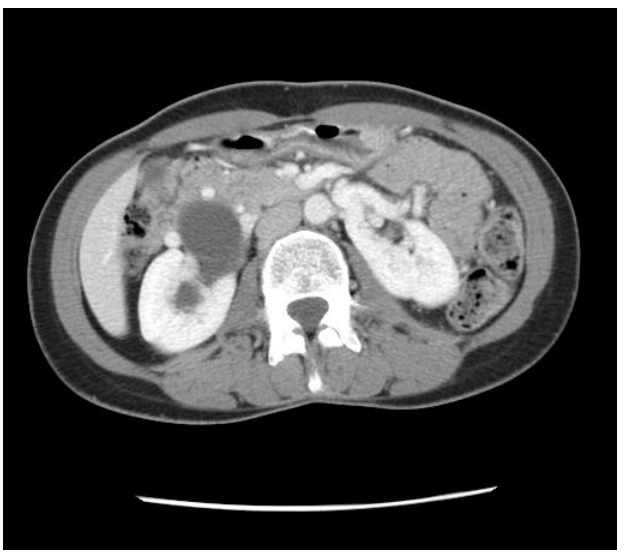


Table 1 - Patient Characteristics.

Age (Median + Range)	18 years [2.5, 62]
Male	8/9 (89%)
Race	
Caucasian	7/9 (78%)
Hispanic	2/9 (22%)
BMI (Median + Range)	23 [17, 32.5]
Left side	8/9 (89%)
Crossing vessel	7/9 (78%)
Primary UPJO	8/9 (89%)
Calculus present	2/9 (22%)
Prior abdominal surgery	2/9 (22%)
Surgical technique (N)	
Conventional Lap dismembered	4
Robotic dismembered	2
Robotic Hellstrom vascular hitch	2
c-LESS dismembered	1

laparoscopy, 3 ports including a 10/12 mm umbilical camera port, a mid-clavicular subcostal 10/12 mm port, and a mid-clavicular 5 mm lower quadrant port were used. The working ports were positioned more inferiorly on the abdomen than heterotopic cases. The robotic approach required 3-4 ports: a 10/12 mm camera port at the umbilicus, two 8 mm robotic ports, and an optional assistant port in the lower quadrant. The LESS pyeloplasties were performed through a 2.5 cm incision at the umbilicus through which three ports (2x5 mm, 1x10 mm) were positioned in a triangular fashion through the anterior abdominal fascia. During LESS, a 5 mm 45 degree laparoscope was used along with articulating instruments (Real Hand, Novare, CA) that crossed within the abdomen. The laparoscope was placed through the most medial trocar and positioned anteriorly in the abdomen, looking down onto the surgical field. For right-sided cases, a 3-5 mm xyphoid instrument was used, if necessary, for liver retraction.

The principles of dissection were uniform regardless of approach utilized. The colon was reflected medially and the ureter isolated distally. Due to the low renal position in horseshoe kidneys, the ureter was isolated in closer proximity to the iliac and other pelvic vessels. The ureter and renal pelvis were dissected within Gerota's fascia. Direct manipulation of a crossing vessel, if present, was avoided to prevent vessel injury and potential compromise of lower pole parenchyma. Instead, traction on the ureter and pelvis was alternated to expose the attachments to the vessel until the ureter would slide freely beneath it.

Indwelling JJ ureteral stents were placed in eight of the nine cases. We prefer antegrade stent placement for both the laparoscopic and robotic approaches to avoid the additional operative time required for cystoscopy and retrograde placement at the beginning of the case. For LESS surgery, however, we prefer retrograde stent placement because of the lack of an upper quadrant port that provides the optimal angle for antegrade placement.

The anastamosis was performed using running 3-0 polyglactin suture. During LESS pyeloplasty, a 5 mm accessory mid-axillary port (subsequently used for the surgical drain) was placed to facilitate triangulation during the anastomosis. Postoperatively Foley catheter drainage was continued for 24-48 hours. Drain output was monitored after Foley removal and if stable, the drain was discontinued. Ureteral stents were removed 4-6 weeks postoperatively.

Two pediatric patients were treated with a 'vascular hitch' (Hellstrom) procedure. After complete mobilization of the ureter from the vessels, normal peristalsis across the UPJ and the absence of an obvious intrinsic stricture was confirmed visually. The lower pole vessels were then affixed in a cephalad position away from the UPJ by imbricating the redundant pelvic wall over the vessels with 2-3 absorbable sutures, similar to the approach described by Gundeti et al. (8).

Treatment success was determined with both clinical and radiographic follow-up. Clinical (symptomatic) success was defined as an absence of recurrent flank pain or other clinical condition attributable to UPJ obstruction (e.g. recurrent urinary tract infections or recurrent renal calculi). Radiographic success was defined as stable differential function (no greater than 10% decrease) and $t_{1/2}$ drainage of less than 20 minutes on postoperative lasix renogram. One exception was made to this definition in a patient undergoing robotic dismembered pyeloplasty. Despite having a postoperative $t_{1/2}$ of 27.3 minutes, his preoperative $t_{1/2}$ was greater than 50 minutes and the differential function of the ipsilateral moiety improved from 38% to 62%, demonstrating clearly that an obstruction no longer existed. The prolonged $t_{1/2}$ was presumably secondary to a redundant pelvis.

Descriptive statistics for demographic, clinical and postoperative outcomes are reported. By nature of the small sample size, our data is non--normally distributed so we report non-parametric tests (median, range). There was insufficient statistical power for a robust statistical analysis.

RESULTS

Median patient age was 18 years (range 2.5-62). Median body mass index (BMI) was 25 (range 17-32.5). Eight were male, 89% had left sided UPJ obstruction, and 78% had crossing vessel pathophysiology. Eight patients had a primary UPJ obstruction, while one patient's obstruction was thought to be secondary to stone disease (Table-1).

The overall median operative time was 136 minutes (range 109-230) with a median blood loss of 12.5 cc (range 5-50). There were no perioperative complications. All except one patient had a ureteral stent placed intraoperatively; one pediatric patient underwent unstented Hellstrom repair. The median length of stay was 1.5 days (range 1-4 days) (Table-2).

Clinical success was 100% after a median follow-up of 11 months (range 3-45). Follow--up imaging consisted of a minimum of one lasix MAG-3 renogram obtained at least 3 months from the surgery date. Six patients underwent a single postoperative renogram, while two patients had four renograms and one patient had two. The median differential function at last follow-up was 48% (range 24-62%), unchanged from 51% (range 28-71%) preoperatively. The median drainage $t_{1/2}$ decreased substantially from 45 minutes (range 20-50) to 13 minutes (range 3.5-41) (Table-3). At last follow-up, seven of nine patients (78%) with postoperative renograms showed radiographic success. The two patients who failed by renographic follow-up had undergone laparoscopic pyeloplasty. The first patient had unobstructed drainage on a renogram obtained at 9 months ($t_{1/2}$ = 11 minutes), but at last follow-up (30 months), demonstrated evidence of re-obstruction ($t_{1/2}$ = 31.6 minutes). The second patient demonstrated a persistently prolonged $t_{1/2}$ of 40.8 minutes at three months ($t_{1/2}$ was not reached on the preoperative study). Nevertheless, both patients remained asymptomatic with stable differential function and have not required any further intervention.

Table 2 - Perioperative outcomes by MIS approach.

	Laparoscopy	Robot	Robot	c-LESS	Overall
Technique	Dismembered	Dismembered	Hellstrom	Dismembered	
N	4	2	2	1	9
Year of Surgery	2008- 2012	2010	2011	2008	2008-2012
BMI (Median & range)	29.9 [27.1, 32.5]	21 [19, 23]	19.5 [17, 22]	19.1	25 [17, 32.5]
OR time, mins (Median & range)	149.5 [109, 230]	138 [120, 155]	126 [115,137]	204	136 [109, 230]
Blood loss, mL (Median & range)	50 [20, 50]	5 [5,5]	5 [5,5]	50	12.5 [5, 50]
Length of Stay, days (Median & range)	3 [2, 4]	1 [1,1]	1 [1,1]	3	1.5 [1, 4]
Perioperative complication	None	None	None	None	None
Follow-up Duration, months (Median & range)	25.5 [3, 45]	15 [12.5, 17]	7 [6, 8]	31	11 [3, 45]
Clinical success	100%	100%	100%	100%	100%
Radiographic success	50%	100%	100%	100%	78%

Table 3 - Functional Outcomes.

	Preoperative	Postoperative (At last follow-up)
Differential function, %* (Median + Range)	51 [28, 71]	48 [24, 62]
$t_{1/2}$ drainage,* mins (Median + Range)	45 [20, 50]	13 [3.5, 41]
% Symptomatic	100%	0%

*Refers to Ipsilateral Renal Moiety

Outcomes were also analyzed according to surgical approach. Median BMI for the laparoscopy group was higher than that for the robotic or LESS groups. Median OR time for robotic and conventional laparoscopy was shorter than the LESS group, while median hospital length of stay was shorter for patients treated via a robotic approach. While these differences are noted, there are insufficient patient numbers to allow an appropriate statistical comparison (Table-2).

DISCUSSION

The largest series to date on pyeloplasty for horseshoe UPJ obstruction report outcomes for open Foley Y-V plasty and they generally lack reporting of both radiologic and clinical follow up which is now standard (9). Nevertheless, open surgery success rates in horseshoe kidneys were less favorable than in orthotopic kidneys, ranging from 55 to 80% (9,10) compared to over 90% respectively (11,12). In comparison, success rates of endopyelotomy in horseshoe kidneys range from 66-75% based on three small series, each containing just 3-4 patients (1,13,14). Again, these success rates are less favorable than for endopyelotomy in orthotopic kidneys, which range from 77 to 89% (15,16) with absent crossing vessels, and less than 70% in the presence of a crossing vessel. The overall radiographic success rate of 78% in this study is thus comparable to success rates reported for open pyeloplasty in horseshoe kidneys (9,10). Smaller series of horseshoe MIS pyeloplasty are limited to cohorts of five or fewer patients, and report success rates ranging from 66% to 100% (1,7,17) (Table-4). Together, these data would suggest that horseshoe UPJO has a greater propensity for failure than orthotopic kidneys.

Although 78% is inferior to the greater than 90% success rate for MIS pyeloplasty in orthotopic kidneys (18,19), the MIS approach offers the same advantages for both anatomical variants: an equivalent success rate to the open approach but with shorter convalescence. In orthotopic kidneys, MIS pyeloplasty has emerged as the 'gold standard' treatment for UPJ obstruction, leading to the evolution of robotic and LESS applications over the past decade (2,3,20). These approaches will likely become more utilized for horseshoe UPJO into the future.

The primary technical challenges of pyeloplasty in this population relate to aberrant lower pole vessels, unfamiliar caudal position of the kidney, and the renal isthmus (21). Despite these anatomical challenges we believe that proficiency obtained during MIS repair in orthotopic kidneys reasonably translates into competency in horseshoe MIS pyeloplasty. To optimize success, anatomic complexities can be anticipated with appropriate preoperative imaging. A CT angiogram can accurately delineate the vasculature and collecting system (21). Magnetic resonance urography (MRU) can also be utilized for simultaneous evaluation of renal function and anatomy (22). One post-mortem study identified

Table 4 - Comparison to other case series.

	Bove et al. (7)	Chammas et al. (17)	Lallas et al. (1)	Current Study
Technique	Conventional Laparoscopic	Robotic	2 Laparoscopic 1 Robotic	4 Laparoscopic 4 Robotic 1 LESS
N	5	3	3	9
OR time, min	195 (mean)	148.3 (mean)	330 (mean)	136 [109, 230]
Blood loss, mL	122 (mean)	< 100 (mean)	< 25 (mean)	12.5 [5, 50]
Length of Stay, days	3.2 (mean)	7.6 (mean)	12.33 (mean)	1.5 [1, 4]
Follow-up Duration, months	4, 13 (range)	21 (mean)	21.2 (mean)	11 [3, 45]
Clinical success	100%	100%	66.6%	100%
Radiographic success	100%	100%	66.6%	78%

three groups of vessels in a horseshoe kidney that should be kept in mind; the main renal vessels, aberrant vessels to the isthmus, and accessory vessels to the poles (23). For standard cases, we prefer to obtain a CT scan with contrast and a baseline renogram to assess the pertinent anatomy and baseline function and drainage.

Laparoscopic pyeloplasty has been performed at our institution since 2000, while our first LESS pyeloplasty was performed in 2008. Robotic pyeloplasty in children was instituted within the past 2 years. This exemplifies the strong influence of the era of surgery as well as surgeon preference in choice of surgical approach. Perioperative outcomes mirrored those for laparoscopic, robotic and LESS pyeloplasty in orthotopic kidney (1,17,24). As expected, the LESS approach required the most time while laparoscopy took slightly longer than robotics. A recent systematic review in orthotopic kidneys similarly suggested a 10 minutes operative time reduction for the robotic approach compared to laparoscopy, although this was not statistically significant (24). It is possible that the time saved from easier intracorporeal suturing with the robot is lost by the longer time required for robot docking and setup.

The majority of our patients underwent a dismembered pyeloplasty while two pediatric patients successfully underwent a Hellstrom vascular hitch repair, as first described in 1949 (25). Horseshoe UPJ obstruction caused by an intrinsic stenosis, high ureteral insertion, or abnormal course of the ureter across the isthmus can be addressed by dismembered pyeloplasty (21). A crossing vessel causing obstruction in isolation can theoretically be treated with either a vascular hitch or dismembered pyeloplasty. Prior studies have suggested the best candidates for a Hellstrom repair are those with a crossing vessel and a normal caliber ureter with good peristalsis across the UPJ (8). In a series of 20 patients undergoing an MIS vascular hitch procedure, a success rate of 95% was reported at a mean follow up of 22 months (8). While our experience and the experience of others (26,27) suggests it is a reasonable approach in well selected patients of this unique population, its application remains controversial.

Robotic-LESS pyeloplasty may prove to be a valuable technique in the future. In a hor-

seshoe kidney, the renal pelvis is located at the level of the umbilicus, directly below the single incision platform. This short working distance means very little lateral movement of instruments is required, making the procedure even more favorable than r-LESS in an orthotopic kidney. Furthermore, unlike c-LESS, an accessory lateral port is never required for suturing thanks to the robotic articulation that allows suturing through the single incision with relative ease.

This case series is limited by its small number, even smaller when divided by surgical approach, making impossible a meaningful statistical comparison to pyeloplasty in orthotopic kidneys. Due to the rarity of horseshoe kidney, large case series will take time to occur, which limits our ability to interpret the outcomes and/or advantages of various techniques.

CONCLUSIONS

Success rates of MIS pyeloplasty in horseshoe kidneys may be inferior to those in heterotopic kidneys, although larger studies are required to allow for more accurate comparison. The choice of MIS approach may be influenced by surgeon experience, patient expectations, BMI and resource allocation, but each approach appears to be safe and technically feasible.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Lallas CD, Pak RW, Pagnani C, Hubosky SG, Yanke BV, Keeley FX, et al.: The minimally invasive management of ureteropelvic junction obstruction in horseshoe kidneys. World J Urol. 2011; 29: 91-5.
2. Schuessler WW, Grune MT, Tecuanhuey LV, Preminger GM: Laparoscopic dismembered pyeloplasty. J Urol. 1993; 150: 1795-9.
3. Erdeljan P, Caumartin Y, Warren J, Nguan C, Nott L, Luke PP, et al.: Robot-assisted pyeloplasty: follow-up of first Canadian experience with comparison of outcomes between experienced and trainee surgeons. J Endourol. 2010; 24: 1447-50.
4. El-Shazly MA, Moon DA, Eden CG: Laparoscopic pyeloplasty: status and review of literature. J Endourol. 2007; 21: 673-8.
5. Winfield HN: Management of adult ureteropelvic junction obstruction--is it time for a new gold standard? J Urol. 2006; 176: 866-7.
6. Janetschek G, Peschel R, Altarac S, Bartsch G: Laparoscopic and retroperitoneoscopic repair of ureteropelvic junction obstruction. Urology. 1996; 47: 311-6.
7. Bove P, Ong AM, Rha KH, Pinto P, Jarrett TW, Kavoussi LR: Laparoscopic management of ureteropelvic junction obstruction in patients with upper urinary tract anomalies. J Urol. 2004; 171: 77-9.
8. Gundeti MS, Reynolds WS, Duffy PG, Mushtaq I: Further experience with the vascular hitch (laparoscopic transposition of lower pole crossing vessels): an alternate treatment for pediatric ureterovascular ureteropelvic junction obstruction. J Urol. 2008; 180: 1832-6; discussion 1836.
9. Pitts WR Jr, Muecke EC: Horseshoe kidneys: a 40-year experience. J Urol. 1975; 113: 743-6.
10. Das S, Amar AD: Ureteropelvic junction obstruction with associated renal anomalies. J Urol. 1984; 131: 872-4.
11. Nguyen DH, Aliabadi H, Ercole CJ, Gonzalez R: Nonintubated Anderson-Hynes repair of ureteropelvic junction obstruction in 60 patients. J Urol. 1989; 142: 704-6.
12. Persky L, Krause JR, Boltuch RL: Initial complications and late results in dismembered pyeloplasty. J Urol. 1977; 118: 162-5.
13. Jabbour ME, Goldfischer ER, Stravodimos KG, Klima WJ, Smith AD: Endopyelotomy for horseshoe and ectopic kidneys. J Urol. 1998; 160: 694-7.
14. Nakamura K, Baba S, Tazaki H: Endopyelotomy in horseshoe kidneys. J Endourol. 1994; 8: 203-6.
15. Preminger GM, Clayman RV, Nakada SY, Babayan RK, Albala DM, Fuchs GJ, et al.: A multicenter clinical trial investigating the use of a fluoroscopically controlled cutting balloon catheter for the management of ureteral and ureteropelvic junction obstruction. J Urol. 1997; 157: 1625-9.
16. Shalhav AL, Giusti G, Elbahnasy AM, Hoenig DM, McDougall EM, Smith DS, et al.: Adult endopyelotomy: impact of etiology and antegrade versus retrograde approach on outcome. J Urol. 1998; 160: 685-9.
17. Chammas M Jr, Feuillu B, Coissard A, Hubert J: Laparoscopic robotic-assisted management of pelvi-ureteric junction obstruction in patients with horseshoe kidneys: technique and 1-year follow-up. BJU Int. 2006; 97: 579-83.
18. Janetschek G, Peschel R, Bartsch G: Laparoscopic Fenger plasty. J Endourol. 2000; 14: 889-93.
19. Siqueira TM Jr, Nadu A, Kuo RL, Paterson RF, Lingeman JE, Shalhav AL: Laparoscopic treatment for ureteropelvic junction obstruction. Urology. 2002; 60: 973-8.
20. Peters CA: Robotic pyeloplasty--the new standard of care? J Urol. 2008; 180: 1223-4.
21. Yohannes P, Smith AD: The endourological management of complications associated with horseshoe kidney. J Urol. 2002; 168: 5-8.

22. Mostafavi MR, Prasad PV, Saltzman B: Magnetic resonance urography and angiography in the evaluation of a horseshoe kidney with ureteropelvic junction obstruction. Urology. 1998; 51: 484-6.
23. Janetschek G, Kunzel KH: Percutaneous nephrolithotomy in horseshoe kidneys. Applied anatomy and clinical experience. Br J Urol. 1988; 62: 117-22.
24. Braga LH, Pace K, DeMaria J, Lorenzo AJ: Systematic review and meta-analysis of robotic-assisted versus conventional laparoscopic pyeloplasty for patients with ureteropelvic junction obstruction: effect on operative time, length of hospital stay, postoperative complications, and success rate. Eur Urol. 2009; 56: 848-57.
25. Hellstrom J, Giertz G, Lindblom K: Pathogenesis and treatment of hydronephrosis. J Belge Urol. 1951; 20: 1-6.
26. Meng MV, Stoller ML: Hellström technique revisited: laparoscopic management of ureteropelvic junction obstruction. Urology. 2003; 62: 404-8; discussion 408-9.
27. Simforoosh N, Javaherforooshzadeh A, Aminsharifi A, Soltani MH, Radfar MH, Kilani H: Laparoscopic management of ureteropelvic junction obstruction in pediatric patients: a new approach to crossing vessels, crossing vein division, and upward transposition of the crossing artery. J Pediatr Urol. 2010; 6: 161-5.

Correspondence address:
Dr. Jeffrey A. Cadeddu
Professor of Urology and Radiology
Department of Urology
University of Texas Southwestern Medical Center
5323 Harry Hines Blvd., J8.106, Dallas, TX 75390, USA
Fax: + 1 214 648-8786
E-mail: jeffrey.cadeddu@utsouthwestern.edu

ORIGINAL ARTICLE

Vol. 39 (2): 203-208, March - April, 2013
doi: 10.1590/S1677-5538.IBJU.2013.02.08

Emergency double-J stent insertion following uncomplicated Ureteroscopy: risk-factor analysis and recommendations

Yousef S. Matani, Mohammed A. Al-ghazo, Rami S. Al-azab, Osamah Bani-hani, Daher K. Rabadi

Department of General Surgery and Urology (YSM, MAA, RSA, OB) and Department of Anaesthesia (DKR) Jordan University of Science and Technology, Faculty of Medicine, King Abdullah University Hospital, Irbid-Jordan

ABSTRACT

Purpose: Emergency double-J (DJ) stenting following "uncomplicated" ureteroscopic (UURS) stone treatment is both morbid and costly. Our study aims at identifying those patients who are more likely to require such an extra procedure. Handling of this complication will also be highlighted.

Materials and Methods: 319 cases of UURS cases were selected out of 903 patients, who were admitted for URS stone treatment at King Abdullah University Hospital during the period from May, 2003 to December, 2010. Thirty-eight of them (11.9%) had emergency post-URS DJ stenting within 24 hours of discharge. The medical records of all UURS cases were retrospectively reviewed. Comparison in demographic and stone-related variables was made using 2-paired t-test with $P < 0.05$. Operative findings of 38 stented patients were outlined.

Results: Significant risk factors for emergency stenting were noted in males with larger (> 1.5 cm) and proximal stones (38 stented vs. 281 unstented). Operative risk factors among the 38 patients were: initial procedure time > 45 minutes (42.1%), ureteral wall edema (21.1%), repeated access for stones > 1.5 cm (21.1%), impacted stone (10.5%) and ignored or missed stones/fragments (4.6%).

Conclusions: The need for emergency DJ stenting following UURS stone treatment is not uncommon. The routine insertion is impractical and weakly-supported. With risk-factor stratification, selective and individualized DJ stenting policy is recommended.

ARTICLE INFO

Key words:
Ureteroscopy; Calculi; Stents; Emergencies

Int Braz J Urol. 2013; 39: 203-8

Submitted for publication:
June 06, 2012

Accepted after revision:
January 15, 2013

INTRODUCTION

Fragmentation and clearance of ureteric stones can either be achieved by extracorporeal shock wave lithotripsy (ESWL) or ureteroscopy stone treatment (URS). URS stone removal has been found to carry a better overall stone-free rate compared to ESWL. The Current European guidelines recommend primary use of URS in treatment of most ureteric stones (1).

The insertion of double-J (DJ) stent during URS stone extraction is controversial. Since the pioneering report by Hosking et al. (2) and the radical characterization by Moon (3), urologists started to adopt a more selective policy.

Complications of DJ stent insertion include disturbing storage lower urinary tract symptoms, pain, hematuria, infection and poorer qua-

lity of life (4,5). It is, however, thought to reduce post URS obstruction, facilitate clearance of stone fragments and decrease stricture rate (6,7).

The definition of uncomplicated URS (UURS) is both lacking and weakly standardized. Denstedt et al. defined UURS as a procedure with "no evidence of perforation or lack of clinically important edema". Free flow of contrast into the bladder on retrograde pyelography is exclusive of edema (8). Other studies used an endoscopic, non-validated grading of ureteric edema on a scale of 0 (mild) to 2 (severe) (6,9).

Our study will look at risk-group stratification of patients who might require stenting during their initial "UURS" and address the concept of "prophylactic" DJ stent use.

MATERIALS AND METHODS

From May 2003 to December 2010, 903 patients had undergone semirigid URS with Holmium laser (365 micron; 0.5-1.4J/5-10 Hz) lithotripsy. All patients were admitted to the urology department at King Abdullah University Hospital and their medical records retrospectively analyzed. All patients had a preoperative consent. Imaging studies included kidney-ureter-bladder (KUB) X-ray and non-enhanced computed tomography (NECT).

Among a total of 903 patients, 319 underwent primary "straightforward" UURS which was defined based on the following selection criteria:

1. All had single and unilateral ureteric stone;
2. Intra-operative perforation was not documented;
3. DJ stent and/or ureteric catheter were not inserted;
4. Ureteric dilatation and/or usage of access catheter were not used;
5. Children and pregnant ladies were excluded;
6. Stone free after the procedure was documented (defined as complete removal and/or residual stone fragments < 3 mm in diameter).

URS was performed, using 8/9.8 semirigid ureteroscope (Richard Wolf, Germany), under general anesthesia in all patients. Urine cultures were negative. Prophylactic antibiotic was given at induction as a single 1 g IV ceftriaxone. Subsequently, 500mg oral ciprofloxacin tablets were given twice daily for 24 hours.

The stone size and location were determined by KUB and NECT films. They were divided into proximal, middle and distal third ureteral stones. URS stone extraction was achieved by Dormia basket and/or forceps.

Thirty-eight out of 319 UURS had emergency stent insertion within 24 hours of initial URS due to intolerable colic and significant discomfort. Diagnostic URS was performed, prior to stenting, for defining a possible etiology or injury. A height-matched length 6F DJ stent was used. Discharge was made within 24 hours and the stent was removed after 1-2 weeks.

The demographic features, stone-related factors and operative URS findings were analyzed and tabulated. Comparison between those stented and un-stented (38 vs. 281) groups was made using 2-tailed t-test statistics. A $P < 0.05$ was taken as the level of significance. The analysis was performed with computer software (Statistical Package for the Social Sciences, version 16.0).

RESULTS

Thirty eight of the 319 UURS (11.9 %) patients had emergency stent insertion. The procedure was complication-free. The mean operative time was 25 minutes. The demographic and stone-related variables of the study group are listed in Table-1. Twenty seven patients were men and 11 women (2.5:1), with a mean age of 38.2 years (range 28-62). The stones included 9 proximal (23.7%), 11 mid-ureter (28.9%), and 18 distal stones (47.4%). Average stone diameter was 10.2 cm (range 7-23 mm).

Significant preoperative risk variables included male sex ($P = 0.037$) and proximal stones ($P = 0.018$). Average ages were comparable (38.2 vs. 39.1 years, $P = 0.30$). Average stone diameter was 1.2 cm and 0.94 cm in the stented and un-stented groups, respectively ($P = 0.06$).

The URS findings are listed in Table-2. Relevant risk factors included: operative time superior to 45 minutes in 16 patients (42.1%), repe-

Table 1 - Demographics and stone features.

No. of patients	38
sex(M:F)	2.5:1(27/11)
Mean age in years(Range)	38.2 (28-62)
Stone	
Mean size in mm (range)	10.2(7-23)
Location	
Upper	9(23.7%)
Middle	11(28.9%)
Lower	18(47.4%)

Table 2 - URS findings prior to DJ stenting (Risk factors).

No. of patients	38
Mean operative time(minutes)	25
Risk factors	
Operative time > 45 minutes	16(42.1%)
localized wall edema	8(21.1%)
Repeated access for stones >1.5 cm	8(21.1%)
Impacted stone	4(10.5%)
Ignored small calyceal stone	1(2.3%)
Residual stone fragment < 3 mm	1(2.3%)

ated access through the ureteral orifice for larger stones (> 1.5 cm) in 8 (21.1%), localized wall edema in 8 (21.1%), handling of impacted stones in 4 (10.5%), ignored small calyceal stone in 1 patient (2.3%) and residual stone fragments < 3mm in 1 patient (2.3%). Control and comparison of these risk factors with the unstented group would have been contributory but clearly unethical.

DISCUSSION

URS was first reported in 1982 (10) by Perez-Castro in cooperation with Karl Storz. The use of stents during this period was not only strange, but also unfavorable. Eisenberger referred to stents as ''Steckerin'' (Bavarian for small sticks) (11). DJ stent was first described by Finney et al. in 1978 (12). Criticism to its role, however, appeared in the late 90s (2,3). Nowadays, the urologists remain, sharply, divided on the need for stenting following UURS treatment of lithiasis. Both routine and selective use has been practiced. Selective use, in particular, should depend on a variety of variables related to patients, stones, technology and experience.

Nabi et al. meta-analyzed 9 trials and concluded that stents have significantly higher rate of storage lower urinary symptoms (LUTS), infection, analgesia use, and ureteric stricture. Stenting, on the other hand, did not influence rates of stone clearance. The authors, however, criticized data inconsistency and lack of standardization. They, therefore, kept the issue of stenting open (5). Similar conclusions were reached by three recent evaluations (13-15). They, basically, advised against routine DJ stenting and were not satisfied by homogeneity and pooling of materials. An excellent review of this dilemma was expressed by Keeley and Timoney (16) who identified the pros and cons of stenting and advised for more meaningful studies.

The use of an alternative and temporary drainage procedure has, recently, been considered. It utilizes short-term insertion of ureteric catheters. This accessory procedure may overcome edema, reduce pain, decrease outpatient visits, avoid secondary endoscopy and limit costs. Djaladat et al. were able to show that pain, storage LUTS and outpatient visits were significantly reduced in the catheter group. Urinary tract infection (UTI) was established in 7 and 4 % in catheter and non-catheter groups respectively. Readmission and stone clearance rates were comparable in a 2-week follow up (17). Reduction in pain and international prostate symptom scores was noted in one-day post-URS catheterization (18).

Baseless avoidance of stenting carries measureable morbidity and cost. DJ stenting is beneficial when obstruction secondary to edema and/or inflammation was anticipated (19). It is, also, effective in reducing pain and promoting drainage in hydronephrosis (20). Cheung et al. highlighted the value of selection in reducing overall stenting rate

without altering stone-free outcome. Their stenting rate was 39% and limited to impacted stones, severe preoperative obstruction and residual poor postoperative drainage (21). Stents were, additionally, found useful in pregnant ladies (22), in upper urinary tract diseases (urolithiasis) (23) and when ureteral access sheath was used (24). Factors that contribute to DJ--associated morbidity include stent design, size, positioning, associated UTI, and duration (25). Recent use of drug eluting stents (26) and alpha blockers (27) were reported to cause less pain and discomfort.

In this analysis, higher risk of emergency stenting was noted among males with larger and more proximal ureteric stones. The impact of stone location and size might be explained by increased manipulation, repeated access and development of wall edema. Increased risk in males is, however, difficult to justify. Comparable Intraoperative risk factors were reported in a similar recent study by Tanriverdi et al. (28). Summary of variables is listed in Table-3. In their analysis, about 2/3 of cases had prolonged procedure, repeated access and ureteral

Table 3 - Summary of data: current and reference no. 28 studies.

	Current	Ref. 28
No. of patients (%)	38/319 (11.9)	23/276 (8.3)
Sex(M:F)	2.5:1(27:11)	1.5:1(14:9)
Mean age in years(Range)	38.2 (28-62)	41.5
Stone:		
Mean size in mm (range)	10.2(7-23)	12(9-22)
Location (%)		
Upper	9(23.7)	6(26.1)
Middle	11(28.9)	6(26.1)
Lower	18(47.4)	11(47.8)
Mean operative time(minutes)	25	14
Etiology & Risk factors: (%)		
Operative time (minutes)	> 45 in 16(42.1)	> 60 in 9(39.1)
Localized wall edema	8(21.1)	10(43.5)
Repeated access for stones > 1.5 cm	8(21.1)	6(26.1)
Impacted stone	4(10.5)	4(17.4)
Small calyceal stone	Ignored in 1(2.3)	Unrecognized in 4(17.4)
Left/retained stone fragments	< 3 mm in 1(2.3)	< 4mm in 3(13.1)
Obstructing blood clots	0	4(17.4)
Kinking of ureter	0	2(8.7)
Relatively narrowed segment	0	2(8.7)
UTI	0	1(4.4)
Nonspecific findings	-	9(39.1)

wall edema. Additional causes of postoperative obstruction were linked to residual or missed stones, blood clots, kinking or narrowed segments and UTI.

We do agree with the arguments raised against routine DJ stenting in UURS stone treatment (5,13-15,28,29). Insertion should better be individualized. Relative indications might include prolonged procedure (> 45 minutes), "significant" wall edema, repeated access, impacted stone, larger stones (> 1.5 cm), use of access sheath, ureteric dilatation and pregnancy. Complete removal and clearance of stone(s)/fragments are highly recommended. The use of ureteric catheterization was not tested in our analysis.

CONCLUSIONS

Insertion of DJ stents during UURS treatment of stones is neither Angel nor Evil. Its role has not yet been decisively outlined. Stent insertion remains "optional" and a consensus is still remote. A risk-based selection may prove to be a better practice. In difficult and lengthy URS procedures with significant stone burden, DJ stenting should be seriously considered.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Preminger GM, Tiselius HG, Assimos DG, Alken P, Buck AC, Gallucci M, et al.: 2007 Guideline for the management of ureteral calculi. Eur Urol. 2007; 52: 1610-31.
2. Hosking DH, McColm SE, Smith WE: Is stenting following ureteroscopy for removal of distal ureteral calculi necessary? J Urol. 1999; 161: 48-50.
3. Moon TD: Ureteral stenting--an obsolete procedure? J Urol. 2002; 167: 1984.
4. Netto NR Jr, Ikonomidis J, Zillo C: Routine ureteral stenting after ureteroscopy for ureteral lithiasis: is it really necessary? J Urol. 2001; 166: 1252-4.
5. Nabi G, Cook J, N'Dow J, McClinton S: Outcomes of stenting after uncomplicated ureteroscopy: systematic review and meta-analysis. BMJ. 2007; 334: 572.
6. Ryan PC, Lennon GM, McLean PA, Fitzpatrick JM: The effects of acute and chronic JJ stent placement on upper urinary tract motility and calculus transit. Br J Urol. 1994; 74: 434-9.
7. Lumma PP, Schneider P, Strauss A, Plothe KD, Thelen P, Ringert RH, et al.: Impact of ureteral stenting prior to ureterorenoscopy on stone-free rates and complications. World J Urol. 2011. Oct 29. [Epub ahead of print]
8. Denstedt JD, Wollin TA, Sofer M, Nott L, Weir M, D'A Honey RJ: A prospective randomized controlled trial comparing nonstented versus stented ureteroscopic lithotripsy. J Urol. 2001; 165: 1419-22.
9. Cheung MC, Lee F, Leung YL, Wong BB, Tam PC: A prospective randomized controlled trial on ureteral stenting after ureteroscopic holmium laser lithotripsy. J Urol. 2003; 169: 1257-60.
10. Pérez-Castro Ellendt E, Martínez-Piñeiro JÁ: Ureteral and renal endoscopy. A new-approach. Eur Urol. 1982; 8: 117-20.
11. Rassweiler J: A landmark paper for endourology. Eur Urol. 2006; 50: 395.
12. Finney RP: Experience with new double J ureteral catheter stent. J Urol. 1978; 120: 678-81.
13. Pengfei S, Yutao L, Jie Y, Wuran W, Yi D, Hao Z, et al.: The results of ureteral stenting after ureteroscopic lithotripsy for ureteral calculi: a systematic review and meta-analysis. J Urol. 2011; 186: 1904-9.
14. Tang L, Gao X, Xu B, Hou J, Zhang Z, Xu C, et al.: Placement of ureteral stent after uncomplicated ureteroscopy: do we really need it? Urology. 2011; 78: 1248-56.
15. Song T, Liao B, Zheng S, Wei Q: Meta-analysis of postoperatively stenting or not in patients underwent ureteroscopic lithotripsy. Urol Res. 2012; 40: 67-77.
16. Keeley FX Jr, Timoney AG: Routine stenting after ureteroscopy: think again. Eur Urol. 2007; 52: 642-4.
17. Djaladat H, Tajik P, Payandemehr P, Alehashemi S: Ureteral catheterization in uncomplicated ureterolithotripsy: a randomized, controlled trial. Eur Urol. 2007; 52: 836-41.
18. Moon KT, Cho HJ, Cho JM, Kang JY, Yoo TK, Moon HS, et al.: Comparison of an Indwelling Period Following Ureteroscopic Removal of Stones between Double-J Stents and Open-Ended Catheters: A Prospective, Pilot, Randomized, Multicenter Study. Korean J Urol. 2011; 52: 698-702.
19. Wang CJ, Huang SW, Chang CH: Indications of stented uncomplicated ureteroscopic lithotripsy: a prospective randomized controlled study. Urol Res. 2009; 37: 83-8.
20. Mustafa M: The role of stenting in relieving loin pain following ureteroscopic stone therapy for persisting renal colic with hydronephrosis. Int Urol Nephrol. 2007; 39: 91-4.
21. Cheung MC, Yip SK, Lee FC, Tam PC: Outpatient ureteroscopic lithotripsy: selective internal stenting and factors enhancing success. J Endourol. 2000; 14: 559-64.
22. Akpinar H, Tüfek I, Alici B, Kural AR: Ureteroscopy and holmium laser lithotripsy in pregnancy: stents must be used postoperatively. J Endourol. 2006; 20: 107-10.
23. Hao P, Li W, Song C, Yan J, Song B, Li L: Clinical evaluation of double-pigtail stent in patients with upper urinary tract diseases: report of 2685 cases. J Endourol. 2008; 22: 65-70.

24. Rapoport D, Perks AE, Teichman JM: Ureteral access sheath use and stenting in ureteroscopy: effect on unplanned emergency room visits and cost. J Endourol. 2007; 21: 993-7.
25. El-Nahas AR, El-Assmy AM, Shoma AM, Eraky I, El-Kenawy MR, El-Kappany HÁ: Self-retaining ureteral stents: analysis of factors responsible for patients' discomfort. J Endourol. 2006; 20: 33-7.
26. Krambeck AE, Walsh RS, Denstedt JD, Preminger GM, Li J, Evans JC, et al.: A novel drug eluting ureteral stent: a prospective, randomized, multicenter clinical trial to evaluate the safety and effectiveness of a ketorolac loaded ureteral stent. J Urol. 2010; 183: 1037-42.
27. Yakoubi R, Lemdani M, Monga M, Villers A, Koenig P: Is there a role for α-blockers in ureteral stent related symptoms? A systematic review and meta-analysis. J Urol. 2011; 186: 928-34.
28. Tanriverdi O, Yencilek F, Koyuncu H, Yencilek E, Sarica K: Emergent stenting after uncomplicated ureteroscopy: evaluation of 23 patients. Urology. 2011; 77: 305-8.
29. Hollenbeck BK, Schuster TG, Seifman BD, Faerber GJ, Wolf JS Jr: Identifying patients who are suitable for stentless ureteroscopy following treatment of urolithiasis. J Urol. 2003; 170: 103-6.

Correspondence address:
Dr. Yousef S. Matani
Associate professor of Urology
Department of General Surgery and Urology,
Faculty of Medicine
Jordan University of Science and Technology
Irbid, Jordan
Um Al-sommaq Post Office, PO Box 2171
11821 Amman, Jordan
Telephone: +96 279 557-2751
E mail: ymatani@gmail.com

ORIGINAL ARTICLE

Vol. 39 (2): 209-213, March - April, 2013
doi: 10.1590/S1677-5538.IBJU.2013.02.09

Occupational Hazard: Radiation Exposure for the Urologist – Developing a Reference Standard

Seth A. Cohen, Sriram S. Rangarajan, Tony Chen, Kerrin L. Palazzi, J. Scott Langford, Roger L. Sur

Department of Surgery and Division of Urology, U C San Diego Health Science System, San Diego, CA.

ABSTRACT

Introduction: To date, there is a paucity of literature offering practicing urologists a reference for the amount of radiation exposure received while surgically managing urolithiasis. This study examines the cumulative radiation exposure of an urologist over 9 months.
Materials and Methods: We present a case series of fluoroscopic exposures of an experienced stone surgeon operating at an academic comprehensive stone center between April and December 2011. Radiation exposure measurements were determined by a thermoluminescent dosimeter worn on the outside of the surgeon's thyroid shield. Estimations of radiation exposure (mrem) per month were charted with fluoroscopy times, using scatter plots to estimate Spearman's rank correlation coefficients.
Results: The total 9-month radiation exposure was 87 mrems for deep dose equivalent (DDE), 293 mrem for lens dose equivalent (LDE), and 282 mrem for shallow dose equivalent (SDE). Total fluoroscopy time was 252.44 minutes for 64 ureteroscopies (URSs), 29 percutaneous nephrolithtomies (PNLs), 20 cystoscopies with ureteral stent placements, 9 shock wave lithotripsies (SWLs), 9 retrograde pyelograms (RPGs), 2 endoureterotomies, and 1 ureteral balloon dilation. Spearman's rank correlation coefficients examining the association between fluoroscopy time and radiation exposure were not significant for DDE (p = 0.6, Spearman's rho = 0.2), LDE (p = 0.6, Spearman's rho = 0.2), or SDE (p = 0.6, Spearman's rho = 0.2).
Conclusions: Over a 9-month period, total radiation exposures were well below annual accepted limits (DDE 5000 mrem, LDE 15,000 mrem and SDE 50,000 mrem). Although fluoroscopy time did not correlate with radiation exposure, future prospective studies can account for co-variates such as patient obesity and urologist distance from radiation source.

ARTICLE INFO

Key words:
Urolithiasis; Nephrolithiasis; Kidney Calculi; Radiation Injuries; Urologic Surgical Procedures; Neoplasms

Int Braz J Urol. 2013; 39: 209-13

Submitted for publication:
September 03, 2012

Accepted after revision:
January 30, 2013

INTRODUCTION

Recent literature has introduced the risks of radiation exposure for patients. Studies have found a 600% increase in medical radiation exposure to the United States (U.S.) population since 1980 (1). Given the importance of imaging to kidney stone diagnosis and treatment, efforts have been made to standardize recommendations in order to balance radiographic imaging with its inherent long term risks, such as those seen with repeat computerized tomography (CT) for nephrolithiasis (2). Parallel movements have occurred to efficiently use fluoroscopy in the operating room to decrease patient exposure (3-5).

Although understanding patient radiation exposure risks is clearly critical, urologic health care worker exposure has also been investigated recently to determine risks in the work environment (6). In a 2011 survey sent to members of the Endourological Society, compliance with chest

and pelvic shields was reported to be 97%; however, usage of thyroid shields, dosimeters, lead-impregnated glasses, and gloves were only 68%, 34.3%, 17.2%, and 9.7% respectively (7). Most reports of urologic health care worker radiation exposure risks include data on procedure-specific radiation scatter, i.e., how much radiation scatter occurs during an average ureteroscopy or percutaneous case. The current literature lacks data on long-term radiation exposure that urologists receive for all "general" endourologic cases. This study examines the cumulative radiation exposure of an urologist over 9 months, taking into account radiation exposure for all endourologic procedures [ureteroscopy (URS), shock wave lithotripsy (SWL), percutaneous nephrolithotomy (PNL), cystoscopy, retrograde pyelograms, etc.].

MATERIALS AND METHODS

We retrospectively analyzed data from our Institutional Review Board-approved database documenting a case series of fluoroscopic exposures of a single right-handed, experienced stone surgeon operating at an academic comprehensive kidney stone center. A waiver of consent was obtained as the study presented no more than minimal risk to human subjects and involved no procedures for which written consent was normally required, outside of the context of the investigation. All cases utilizing fluoroscopy between April and December 2011 were included in the dataset. Radiation exposure measurements were determined by a single thermoluminescent dosimeter (TLD) worn on the outside of the surgeon's thyroid shield. All fluoroscopic imaging was performed with one of two available under-couch X-ray emitter and over-couch image intensifiers (GE OEC 9800 & 9900). The urologist wore a 0.5 mm lead thyroid shield, lead apron, and lead-impregnated glasses during all endourologic procedures requiring fluoroscopy. Radiation exposure for both lens dose equivalent (LDE) and shallow dose equivalent (SDE) were obtained directly from the single TLD. To account for lead being worn, TLD readings were multiplied by 0.3 to yield deep dose equivalent (DDE) radiation exposure values. All readings were expressed in millirem (mrem) which is one-thousandth of a rem (Roentgen equivalent man). The monthly fluoroscopy times for all surgeries were recorded as well. Estimations of radiation exposure (mrem) per month were then charted with fluoroscopy times, using scatter plots to estimate Spearman's rank correlation coefficients with Type I error alpha = 0.05.

RESULTS

A total of 137 surgical procedures using fluoroscopy were identified over this 9-month period. Complete fluoroscopy time data was available for 134 procedures; 3 procedures without complete fluoroscopy time data were excluded from analysis. The total 9-month radiation exposure was 87 mrems for deep dose equivalent (DDE), 293 mrem for lens dose equivalent (LDE), and 282 mrem for shallow dose equivalent (SDE). Total fluoroscopy time during this period was 252.44 minutes for: 64 URS, 29 PNL, 20 cystoscopies with ureteral stent placements, 9 SWL, 9 RPGs, 2 endoureterotomies, and 1 ureteral balloon dilation (Table-1 and Figure-1). Spearman's rank correlation coefficients examining the association between fluoroscopy time and radiation exposure were not significant for DDE ($p = 0.6$, Spearman's rho = 0.2), LDE ($p = 0.6$, Spearman's rho = 0.2), or SDE ($p = 0.6$, Spearman's rho = 0.2).

DISCUSSION

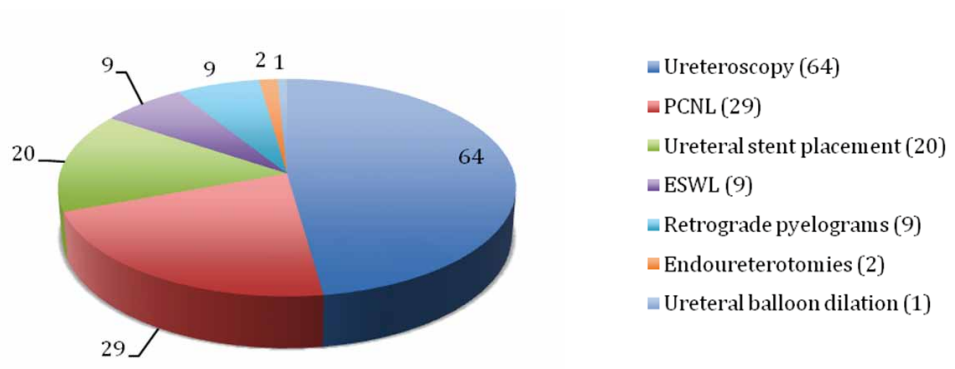
The risks posed by a urological career's worth of low-dose ionizing radiation to practicing surgeons remain unclear (8). The 2007 International Commission on Radiation Protection (ICRP) guidelines recommend an occupational dose limit of no more than 50 mSv (5,000 mrem) per year or more than 100 mSv (10,000 mrem) averaged over 5 years (9). U.S. regulations (Title 10, part 20 of the Code of Federal Regulations) mandate annual accepted limits (DDE 5000 mrem, LDE 15,000 mrem and SDE 50,000 mrem). To ensure practitioners are within these guidelines, there exists a need for a controlled measurement of radiation exposure experienced by practicing U.S. urologists over a period of time - an aim that formed the basis of this study.

The applicability of recent literature looking at radiation exposure to urologists has been limited

Table 1 - Cumulative Radiation Exposure and Time.

Month	Time (min.)	DDE (mrem)	LDE (mrem)	SDE (mrem)
April	30.49	9	31	30
May	43.09	5	17	16
June	17.04	0	0	0
July	18.35	4	14	13
August	39.01	7	22	22
September	36.39	15	51	51
October	38.53	21	70	66
November	11.16	12	41	38
December	18.38	14	47	46
Sum	**252.44**	**87**	**293**	**282**

Figure 1 - Endourological Surgeries Requiring Fluoroscopy Over 9 Months.



by two factors: every major study from the past 25 years has been 1) conducted abroad and/or 2) focused specifically on the radiation doses of individual procedures, particularly PNL (6,10-13). The most recent exposure data from North American institutions occurred in the distant past: a 1986 radiation exposure report of 7 PNLs and a 1996 radiation exposure report of 5 unspecified urologic procedures while analyzing the efficacy of a newly designed fluoroscopic drape (14,15).

Most recently, a German study reported data of 235 pooled and averaged dose exposure TLD readings from five different urologic procedures performed by 12 surgeons over 6 months (16). While radiation exposure data from European institutions have contributed to our understanding of the radiation risks faced by urologists, inherent characteristics of these investigations make it difficult for urologists in North America to generalize the data to their environments.

For example, exposures may differ secondary to varying practice patterns among international countries versus those in North America. This may be secondary to differences in training and equipment, but also secondary to the acknowledged higher rates of obesity of the North American population, more specifically, the U.S. (17). In addition, an accurate appraisal of the radiation exposure faced by an urologist demands an incorporation of data from the entire spectrum of urology procedures performed using fluoroscopy, not just a subset of procedures such as PNLs. To our knowledge, this is the first published investigation to report cumulative radiation exposure data for a single urologist from a North American institution. It is also one of the only datasets that incorporates a number of different urological procedures, such as SWL, which have been excluded from many of the prior publications. This variety of endourological surgeries and procedures more accurately reflects an endourologist's practice, and may come closer to estimating true radiation exposure over a given time period. This is the only study to achieve a semblance of broad generalization and realistic application of such data. Our data presents a summation of exposures across a 9 month period as opposed to averaged doses of selected cases, allowing practitioners a more comprehensive reference standard for an understanding of radiation exposure.

Regarding study design, we incorporated a single TLD placed outside the thyroid shield, yielding mrem values for DDE, LDE, and SDE, giving a reasonably accurate estimate of total upper-body exposure radiation exposure. Although the study only utilized this singular location for placement of the TLD, this is thought to be consistent with the current practices of most North American urologists. We nevertheless acknowledge there are limitations - the study's applicability to individual urologists is limited by factors which may vary between practitioners, including operating facility and equipment, fellowship status, experience, and position in the operating room. In addition, inherent to any case series is a lack of randomization and controls, which limits our ability to account for differences in stone burden, surgical complexity, and patient body habitus. We also found that we could draw no significant correlations between increasing fluoroscopy time (minutes) and increasing radiation exposure (mrems). Although this would appear to make sense intuitively, the data did not yield such results. This could be secondary to any of the confounding factors listed above, and may also draw attention to TLDs as, perhaps, limited instruments in their ability to measure accurate radiation exposure. Such findings may deserve further review in future studies.

Using current devices and measures, our findings demonstrate that the quantity of radiation an academic urologist with a high-case volume is exposed to over the course of 9 months would appear to be below ICRP recommendations. Efforts to improve radiation safety, however, continue to be of utmost importance. The continued effort of the urologic community to reduce the fluoroscopy time required for a given procedure is essential. In conjunction with these efforts, we hope that our results will serve as a foundation for a reference standard for North American urologists from which they may extrapolate their respective radiation exposures. Importantly, we hope that such data will heighten awareness of radiation risk to practicing urologists in North America and encourage practitioners to continue safe radiation practices.

It remains essential to emphasize that there is no "safe" level of radiation exposure, and even small amounts could potentially cause a stochastic effect, such as cancer. This is why keeping the radiation dose as low as reasonably achievable (ALARA), a concept designated as optimization by the IRCP, is so essential to keep in mind during practice (9). Optimization requires indentifying parameters and using procedures/protocols to yield the necessary clinical information, while keeping radiation doses as low as possible (1).

CONCLUSIONS

Over a 9 month period, total radiation exposure for an endourology practice appears to be within accepted limits, as suggested by the IRCP (DDE 5000 mrem, LDE 15,000 mrem and SDE 50,000 mrem). Although fluoroscopy time did not correlate with radiation exposure, future prospec-

tive studies can account for co-variates such as patient obesity and urologist distance from radiation source.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Linet MS, Slovis TL, Miller DL, Kleinerman R, Lee C, Rajaraman P, et al.: Cancer risks associated with external radiation from diagnostic imaging procedures. CA Cancer J Clin. 2012; Epub.
2. Ferrandino MN, Bagrodia A, Pierre SA, Scales CD Jr, Rampersaud E, Pearle MS, et al.: Radiation exposure in the acute and short-term management of urolithiasis at 2 academic centers. J Urol. 2009; 181: 668-72; discussion 673.
3. Mancini JG, Raymundo EM, Lipkin M, Zilberman D, Yong D, Bañez LL, et al.: Factors affecting patient radiation exposure during percutaneous nephrolithotomy. J Urol. 2010; 184: 2373-7.
4. Ngo TC, Macleod LC, Rosenstein DI, Reese JH, Shinghal R: Tracking intraoperative fluoroscopy utilization reduces radiation exposure during ureteroscopy. J Endourol. 2011; 25: 763-7.
5. Greene DJ, Tenggadjaja CF, Bowman RJ, Agarwal G, Ebrahimi KY, Baldwin DD: Comparison of a reduced radiation fluoroscopy protocol to conventional fluoroscopy during uncomplicated ureteroscopy. Urology. 2011; 78: 286-90.
6. Safak M, Olgar T, Bor D, Berkmen G, Gogus C: Radiation doses of patients and urologists during percutaneous nephrolithotomy. J Radiol Prot. 2009; 29: 409-15.
7. Elkoushy MA, Andonian S: Prevalence of orthopedic complaints among endourologists and their compliance with radiation safety measures. J Endourol. 2011; 25: 1609-13.
8. Liu SZ: Biological effects of low level exposures to ionizing radiation: theory and practice. Hum Exp Toxicol. 2010; 29: 275-81.
9. [No authors listed]: The 2007 Recommendations of the International Commission on Radiological Protection. ICRP publication 103. Ann ICRP. 2007; 37: 1-332.
10. Rao PN, Faulkner K, Sweeney JK, Asbury DL, Sambrook P, Blacklock NJ: Radiation dose to patient and staff during percutaneous nephrostolithotomy. Br J Urol. 1987; 59: 508-12.
11. Hellawell GO, Mutch SJ, Thevendran G, Wells E, Morgan RJ: Radiation exposure and the urologist: what are the risks? J Urol. 2005; 174: 948-52; discussion 952.
12. Kumari G, Kumar P, Wadhwa P, Aron M, Gupta NP, Dogra PN: Radiation exposure to the patient and operating room personnel during percutaneous nephrolithotomy. Int Urol Nephrol. 2006; 38: 207-10.
13. Majidpour HS: Risk of radiation exposure during PCNL. Urol J. 2010; 7: 87-9.
14. Lowe FC, Auster M, Beck TJ, Chang R, Marshall FF: Monitoring radiation exposure to medical personnel during percutaneous nephrolithotomy. Urology. 1986; 28: 221-6.
15. Giblin JG, Rubenstein J, Taylor A, Pahira J: Radiation risk to the urologist during endourologic procedures, and a new shield that reduces exposure. Urology. 1996; 48: 624-7.
16. Ritter M, Krombach P, Martinschek A, Siegel FP, Schmitt M, Weiss C, et al.: Radiation exposure during endourologic procedures using over-the-table fluoroscopy sources. J Endourol. 2012; 26: 47-51.
17. Finucane MM, Stevens GA, Cowan MJ, Danaei G, Lin JK, Paciorek CJ, et.: National, regional, and global trends in body-mass index since 1980: systematic analysis of health examination surveys and epidemiological studies with 960 country-years and 9.1 million participants. Lancet. 2011; 377: 557-67.

Correspondence address:
Dr. Roger L. Sur
U.C. San Diego Comprehensive Kidney Stone Center
Department of Surgery and Division of Urology
200 W. Arbor Drive #8897
San Diego, CA 92130-8897, USA
Fax: + 1 619 543-6557
E-mail: rlsur@ucsd.edu

ORIGINAL ARTICLE

Vol. 39 (2): 214-221, March - April, 2013
doi: 10.1590/S1677-5538.IBJU.2013.02.10

Effects of Serenoa Repens, Selenium and Lycopene (Profluss®) on chronic inflammation associated with Benign Prostatic Hyperplasia: results of "FLOG" (Flogosis and Profluss in Prostatic and Genital Disease), a multicentre Italian study

Giuseppe Morgia, Sebastiano Cimino, Vincenzo Favilla, Giorgio Ivan Russo, Francesco Squadrito, Giuseppe Mucciardi, Lorenzo Masieri, Letteria Minutoli, Giuseppe Grosso, Tommaso Castelli

Department of Urology (GM, SC, VF, GIR, TC) and Department of Hygiene and Public Health, University of Catania (GG), Catania, Department of Urology (LM) and Department of Clinical and Experimental Medicine and Pharmacology – Section of Pharmacology (GM), University of Messina Messina and Department of Urology, University of Florence (LM), Florence, Italy

ABSTRACT

Objective: To evaluate the efficacy of Profluss® on prostatic chronic inflammation (PCI).
Materials and Methods: We prospectively enrolled 168 subjects affected by LUTS due to bladder outlet obstruction submitted to 12 cores prostatic biopsy for suspected prostate cancer + 2 cores collected for PCI valuation. First group consisted of 108 subjects, with histological diagnosis of PCI associated with BPH and high grade PIN and/or ASAP, randomly assigned to 1:1 ratio to daily Profluss® (group I) for 6 months or to control group (group Ic). Second group consisted of 60 subjects, with histological diagnosis of BPH, randomly assigned to 1:1 ratio to daily Profluss® + α-blockers treatment (group II) for 3 months or to control group (group IIc). After 6 months first group underwent 24 cores prostatic re-biopsy + 2 cores for PCI while after 3 months second group underwent two-cores prostatic for PCI. Specimens were evaluated for changes in inflammation parameters and for density of T-cells (CD3, CD8), B-cells (CD20) and macrophages (CD68).
Results: At follow-up there were statistical significant reductions of extension and grading of flogosis, mean values of CD20, CD3, CD68 and mean PSA value in group I compared to Ic, while extension and grading of flogosis in group II were inferior to IIc but not statistical significant. A statistically significant reduction in the density of CD20, CD3, CD68, CD8 was demonstrated in group II in respect to control IIc.
Conclusions: Serenoa repens+Selenium+Lycopene may have an anti-inflammatory activity that could be of interest in the treatment of PCI in BPH and/or PIN/ASAP patients.

ARTICLE INFO

Key words:
Phytotherapy; Serenoa; Inflammation; Prostatitis; Prostatic Hyperplasia

Int Braz J Urol. 2013; 39: 214-21

Submitted for publication:
October 10, 2012

Accepted after revision:
January 15, 2013

INTRODUCTION

Benign prostatic hyperplasia (BPH) is a common cause of bothersome lower urinary tract symptoms in man, representing a growing entity in terms of healthcare costs and morbidity.

Approximately 50% of men aged between 50 and 60 years, 60% of men aged between 60

and 70 years, and up to 90% of men aged > 80 years have some degree of benign prostatic enlargement (BPE) (1).

Medical therapies can provide adequate alleviation of BPE symptoms (LUTS): The two primary classes of oral medications that are prescribed for the treatment of symptoms are alpha-blockers and 5-alpha-reductase inhibitors (5ARIs). Despite medical therapy improves LUTS, some adverse events (ejaculatory dysfunction, loss of libido, erectile dysfunction) are caused by the treatment, worsening an already compromised sexual function (2).

The aetiology of BPH is still far from being fully understood but multiple partially overlapping and complementary theories have been proposed (3). There are some evidences that prostatic inflammation could be a key component in BPE and BPH progression.

Two of the major clinical studies on BPH (MTOPS and Reduce study) recently demonstrated the link between histological prostatic inflammation and prostate enlargement or symptoms scores (4,5). Numerous major key players in chronic inflammation have been studied in BPH: varieties of growth factors and cytokines have been shown to be involved both in the inflammatory process and in the epithelial/stromal prostatic cells interactions (6). These mediators are released in the prostatic gland by inflammatory cells that can be found on most of the surgery-derived BPH specimens (7).

The inflammatory cells may trigger a sophisticated and well-orchestrated inflammatory cascade, resulting in excessive oxidative stress, activation of the transcription factor nuclear factor--kappa B (NF-κB), production of several cytokines and overexpression of inducible-cyclooxygenase (COX-2), inducible-nitric-oxide-synthase (iNOS) and 5-lipoxygenase (5-LOX), leading, in turn, to the release of prostaglandins, nitrates, and leukotrienes. Furthermore, inflammatory cells produce growth factors, such as vascular endothelial growth factor (VEGF) and transforming growth factor-β (TGF-β), which may support fibromuscular growth in BPH (8).

Plant extracts have been used in the medical management of BPH-induced LUTS with the aim to relieve symptoms without adverse events related to treatment (9).

Serenoa Repens (SeR), derived from the berries of the saw palmetto tree, is the most popular naturally derived medication for BPH (10). Several mechanisms of action have been proposed to explain its therapeutic efficacy, including inhibition of 5α-reductase and dihydrotestosterone binding to androgen receptors, a weak α1-adrenergic receptor antagonism, inhibition of growth factors--induced prostate cell proliferation and inhibition of COX-2 and 5-LOX (11).

Besides SeR, both lycopene (LY), a dietary carotenoid synthesized by plants, fruits, and microorganisms with a strong antioxidant activity, and selenium (Se), an essential trace element mainly functioning through seleno-proteins and able to promote an optimal antioxidant/oxidant balance, have been shown to exert beneficial effects in BPH (12-16).

The aim of this study is to evaluate the efficacy of Profluss® (SeR-Se-LY) in reducing chronic inflammation in patient with benign prostatic hyperplasia and/or PIN/ASAP.

MATERIALS AND METHODS

Study design, patients selection criteria and allocation

The "Flogosis And Profluss in Prostatic and Genital Disease" (FLOG) study was a multicentre study involving 9 urological Italian centres between January 2009 and December 2010 that analysed prospectively collected data of two category of patients affected by BPH and/or PIN/ASAP. The inclusion criteria for the first group were: presence of LUTS due to bladder outlet obstruction (BOO) secondary to clinical BPH, assessed by urodynamic and pressure flow evaluation, PSA > 4ng/mL and/or DRE abnormality and/or abnormal findings on transrectal ultrasound, 12 cores prostatic biopsy performed for suspected prostate cancer with two more cores collected from the left and right lobes (for chronic inflammation evaluation), histological diagnosis of prostatic chronic inflammation associated with BPH and high grade PIN and/or ASAP. Exclusion criteria were: treatment with NSAIDs or corticosteroids in the previous 6 months, urinary infection, treatment with finasteride or dutasteride, phytotherapy in the previous 6 months; diagnosis of prostatic cancer

(PCa). Fourty patients were excluded after PCa was diagnosed and finally 108 patients were analysed in this study. Subjects were randomly assigned to 1:1 ratio to SeR 320mg+LY5mg+Se50mcg/day treatment (group I) for 6 months or to control group (group Ic) and then underwent 24 cores prostatic re-biopsy with two more needle biopsies (for chronic inflammation evaluation) collected from the left and right lobes in the same previous areas allowing similar histopathological analysis.

Inclusion criteria for second group were: presence of LUTS due to bladder outlet obstruction (BOO) secondary to clinical BPH, assessed by urodynamic and pressure flow evaluation, PSA > 4ng/mL and/or DRE abnormality and/or abnormal findings on transrectal ultrasound, 12 cores prostatic biopsy performed for suspected prostate cancer with two more cores collected from the left and right lobes (for chronic inflammation valuation), histological diagnosis of prostatic chronic inflammation associated with BPH and indication for surgical treatment. Exclusion criteria were: treatment with NSAIDs or corticosteroids in the previous 6 months; treatment with finasteride or dutasteride, phytotherapy in the previous 6 months, urinary infection, diagnosis of PCa. Sixty-six consecutive patients met inclusion criteria. Six patients dropped out for various reasons: 2 lost to follow-up and 4 excluded due to concomitant drug medication which was not allowed by the criteria we had established. Finally, a total of 60 patients were included in the analysis and they were enrolled and randomly assigned to 1:1 ratio to SeR 320mg+LY5mg+Se50mcg/day and α-blockers treatment (group II) for 3 months or to control group (group IIc). At month 3 two-cores prostatic biopsy was performed in the same areas of the previous allowing similar histopathological analysis and then underwent Transurethral Resection of Prostate (TURP).

Principles outlined in the Declaration of Helsinki were followed and all patients signed a written informed consent form.

Histopathological evaluation

Prostate specimens were fixed with 10% buffered formalin for 8-12 hours (biopsy), 12-24 hours (TURP) and sent for central review by a blinded pathologist.

Coloration of specimens was performed by hematoxylin-eosin. On the main representative area of each histological specimen, parameters of flogosis like extensions and grading of flogosis were evaluated, according to the score of Nickel (17).

On paraffin sections of 5 micron were assessed, by immunohistochemical technique using monoclonal antibodies (Dako company), B-lymphocytes (CD20), T-lymphocytes (CD3, CD8), and macrophages (CD68). The detection system was the universal kit LSAB of the Dako company. All the immunohistochemical procedures were performed using the automated immunostaining Optimax Plus system (Biogenex, San Ramon, USA).

The immunostaining specimens were assessed using the Axioplan Zeiss microscope with Axiovision software. For each specimen with positive immunostaining for CD20, CD3, CD8 and CD68, only the main representative areas were selected, using a zoom 10x.

Subsequently the positive cells were counted in three fields with lens 20x, within this area, the medium value was considered as expression of B and T lymphocyte density and as expression of the macrophages density present in the specimen.

Statistical analysis

Baseline characteristics are presented as frequencies of occurrence or mean and standard deviation as appropriate. Statistical differences among groups of frequencies were tested by Chi-square test. Given the not normal distribution of continuous data, we tested differences among independent samples by Mann-Whitney U-test.

All statistical tests were two-tailed and p-value < 0.05 were considered significant. Data were entered into Microsoft Excel for Windows (Microsoft Corporation, Redmond, WA). Statistical analysis was performed using SPSS for Windows release 17.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Clinical results

Baseline demographic and clinical characteristics of patients analyzed are listed in Table-1. No differences in terms of parameters collected, such

Table 1 - Baseline clinical and histopathological characteristics of population and comparision of groups at inclusion.

Variables	PIN/ASAP patients		P-value	BPH patients		P-value
	I group (Profluss®) (n=54)	Ic (control group) (n = 54)		II group (Profluss®) (n=30)	IIc (control group) (n=30)	
Age, y, mean ± SD	64.6 ± 4.0	65.5 ± 2.3	0.15	67.4 ± 5.7	69.8 ± 4.4	0.18
Weight, Kg, mean ± SD	74 ± 6.4	74.2 ± 5.3	0.24	75 ± 5.7	75 ± 6.55	0.43
BMI, Kg/m^2, mean ± SD	26.5 ± 2.4	26.3 ± 2.1	0.35	27.1 ± 2.9	26.9 ± 1.5	0.52
Total number of infiltrates, mean ± SD	39.3 ± 4.5	38.5 ± 3.42	0.65	75.4 ± 3.4	74.4 ± 2.9	0.41
B lymphocytes (CD20), mean ± SD	14.8 ± 4.3	14.8 ± 8.8	0.84	41.5 ± 2.2	42.0 ± 1.3	0.54
T lymphocytes (CD3), mean ± SD	6.4 ± 2.8	6.5 ± 3.2	0.76	19.5 ± 2.3	20.6 ± 4.0	0.12
T lymphocytes (CD8), mean ± SD	12.5 ± 3.7	11.5 ± 3.4	0.16	6.2 ± 3.2	6.1 ± 2.0	0.15
Macrophages (CD68), mean ± SD	5.92 ± 2.2	5.7 ± 1.3	0.54	7.1 ± 1.5	6.9 ± 2.1	0.15
Prostate volume, cc, mean ± SD	50.5 ± 19.9	52.4 ± 18.2	0.12	47.3 ± 14.8	53.4 ± 1.0	0.14
PSA, ng/mL, mean ± SD	5.5 ± 1.2	5.21 ± 2.1	0.35	5.5 ± 0.8	5.31 ± 0.9	0.65

as age, prostate volume, PSA values, urodynamic findings and other general health status variants were observed between groups.

Histopathological findings and results

At 6-month histopathological evaluation, a significant difference of flogosis was demonstrated between Groups I and Ic, with a reduction both of extension ($P < 0.001$) and grading ($P < 0.001$) of flogosis among treated patients (Figure-1).

Histopathological evaluation performed at 3 months in patients enrolled in Group II revealed no significant difference of extension and grading of flogosis in respect to group control IIc (Figure-1). However, a slight reduction of extension and grading could be observed in the treated group.

A significant reduction of total interstitial mononuclear cells, B lymphocytes, T lymphocytes and macrophages in Group I compared with control group (Ic) was observed at 6-month evaluation ($P < 0.001$) (Table-2).

Among patients with BPH (Group II), total interstitial mononuclear cells, B lymphocytes, T lymphocytes and macrophages were significantly reduced at 3 month evaluation compared with control group ($P < 0.001$) (Table-3).

According with the reduction of extension and grade of flogosis in Group I, mean PSA values was significantly lower than in control group ($P < 0.0001$) (Table-2).

No statistical significant reduction of PSA value was demonstrated between Groups II and IIc (Table-3).

DISCUSSION

Almost all surgery-derived BPH specimens show inflammatory infiltrates at histological examination (18,19); yet most of these patients neither have clinical signs of infection nor any correlation with bacterial or other foreign antigens.

Figure 1 - Extension and grading of flogosis on surgical specimens of patients treated with (Profluss®) and control group with PIN/ASAP (I Group) and BPH (II Group).

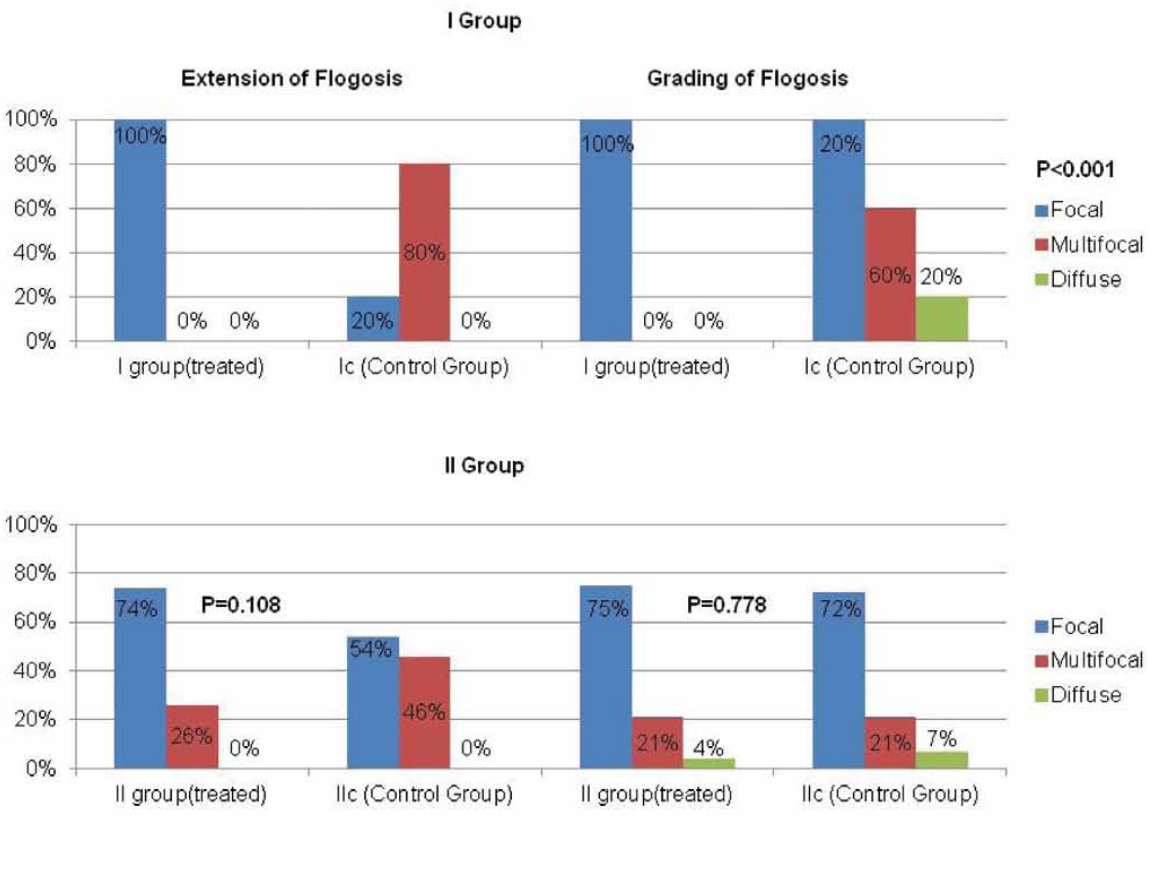


Table 2 - Total number of infiltrates, inflammatory cells markers included in the study and serum PSA with average findings in PIN/ASAP patients at 6-month evaluation.

Variables	I group (Profluss®) (n = 54) (Mean ± SD)	Ic (Control Group) (n = 54) (Mean ± SD)	P-values
Total number of infiltrates	29.6 ± 4.12	38.2 ± 3.24	< 0.001
B lymphocytes (CD20)	14.20 ± 4.94	14.61 ± 4.87	< 0.001
T lymphocytes (CD3)	6 ± 3.50	6.38 ± 2.06	< 0.001
T lymphocytes (CD8)	5 ± 1.80	8.87 ± 4.21	< 0.001
Macrophages (CD68)	4.40 ± 1.61	5.64 ± 2.45	< 0.001
PSA, ng/mL	4.16 ± 0.89	5.62 ± 1.04	< 0.001

Table 3 - Total number of infiltrates, inflammatory cells markers included in the study and serum PSA with average findings in BPH patients at 3-month evaluation.

Variables	II Group (Profluss®) (n=30) (Mean ± SD)	IIc (Control Group) (n=30) (Mean ± SD)	P-values
Total number of infiltrates	47.9 ± 2.56	71.9 ± 2.87	< 0.001
B lymphocytes (CD20)	25.8 ± 1.97	40.6 ± 1.92	< 0.001
T lymphocytes (CD3)	12.7 ± 2.98	18.6 ± 2.31	< 0.001
T lymphocytes (CD8)	4.1 ± 1.09	6 ± 1.17	< 0.001
Macrophages (CD68)	5.3 ± 1.05	6.7 ± 1.2	< 0.001
PSA, ng/mL	2.54 ± 0.768	2.54 ± 1.02	0.584

In a study by Robert et al. it was found that most patients, treated by surgery for complicated and/or symptomatic BPH, had inflammatory cells infiltrating BPH tissues: 81% had T-lymphocytes markers, 52% had B-lymphocytes markers, and 82% had macrophages markers (20). Therefore, in patients with high-grade prostatic inflammation IPSS score and prostate volume were significantly higher. These findings were confirmed by Mishra et al. (21), who compared pathology specimens in 374 patients who underwent transurethral resection of the prostate (TURP) for either LUTS or urinary retention. They found 70% of men with urinary retention have acute and/or chronic inflammation versus 45% of men without LUTS.

These data may support the hypothesis that an anti-inflammatory therapy may act to relief BPH symptoms and may also condition prostate tissue growth (22).

Among all phytotherapics, the lipidosterolic extract of Serenoa repens, a compound used to relief symptoms of BPH, had shown an anti-inflammatory activity modifying the production of leukotrienes and 5 hydroxyeicosatetraenoic acid, via the inhibition of the oxidative enzyme 5 lypoxygenase rather than phospholipase A2 or cell viability. Since the infiltration of inflammatory cells appears to have a role in BPH, inhibition of the production of chemotactic leukotrienes and other 5 lypoxygenase metabolites by Serenoa repens could be useful in BPH treatment (23).

Serenoa Repens, in a multicenter, open pilot study, was also evaluated for its effects on inflammatory markers. Tumor necrosis factor-α and IL-1b were dramatically lower in the Serenoa repens-treated group; both biological markers have been used as indicators of prostatic inflammation in cases of chronic prostatitis (23).

It has been hypothesized that SeR, Ly, and Se, administered together, might amplify their therapeutic efficacy on the proliferative and inflammatory component of BPH (24).

The efficacy of this association was recently confirmed in an in vitro and in vivo comparison study performed on rats with partial bladder outlet obstruction: prostate pro-inflammatory phenotype, as well as hyperplasia, was reduced more efficiently than the single compounds (25).

Analyzing specimens from patients with high grade PIN and/or ASAP, a statistical significant reduction in the extension, and in the grading of the inflammatory cells infiltrate was demonstrated in the patients treated with SeR+Ly+Se (Group I).

Therefore, it was confirmed by immune-histochemical technique using monoclonal antibodies, that mononuclear cell infiltration (B-lymphocytes CD20, T-lymphocytes CD3-CD8 and

macrophages CD68) is the most common pattern. SeR+Ly+Se combination therapy resulted in a statistical significant reduction of this inflammatory infiltrate.

The inflammatory infiltrates are responsible for the secretion of cytokines, which are involved in the paracrine and autocrine regulation of stromal and epithelial cell growth. This mechanism has been considered to influence the development of prostate cancer and BPH (26): SeR+Se+Ly association acting on these pathway may be considered to chemoprevent both conditions.

According with the reduction of extension, grade of flogosis and CD20, CD3, CD8,CD68, mean PSA values decreased from 5.62 ± 1.04 to 4.16 ± 0.89 ($p < 0.001$), suggesting that PSA value could be an useful marker of prostate inflammation. This result was underlined also in a study from Li Gui--Zhong et al. Thus, if an elevated PSA level is considered in association with histological inflammation with detailed grading in a high number of biopsy specimens, it might prevent unnecessary repeated biopsies (27).

The aim of the analysis performed in the Group II was to verify whether in patients with bladder outlet obstruction, waiting for TURP, SeR+Se+LY association determined the same effects observed in Group I.

Comparing the results with control group, there were no statistically significant differences in terms of reducing the extension and grading of inflammation.

As reported in several studies, the extent of inflammation and grade correlated positively with the serum PSA level (28,29). Probably in the Group II we have selected patients with lower extension and grade of flogosis: this finding is also supported by the fact that between the two subsets there were no statistical significant differences in terms of total PSA.

Analyzing the inflammatory infiltrate, surprisingly it has been documented a statistical significant reduction of CD20, CD3, CD8, CD68. In the study by Vela Navarrete et al., SeR alone showed a reduction of the biological markers (TNFα and IL-1β): however, the histological findings resulted in a reduction only in the number of lymphocytes B (CD20) (23).

Possible limits of this research could be represented by the lack of placebo controlling. A multicentre and randomized, double-blinded, placebo-controlled study could fill this gap and offer results that better clarify the activity of this category of treatment.

CONCLUSIONS

Our data support the anti-inflammatory activity of the combination of SeR+Se+LY in patients with histological inflammation detected on biopsy specimens. Patients with bladder outlet obstruction could benefit from this therapy on the inflammatory component of BPH. More data supporting these findings may confirm these evidences.

ACKNOWLEDGMENTS

Authors are grateful to University of Catania, University of Firenze, University of Roma Torvergata, University of Messina, Hospital of Bronte (Catania), Hospital “Buccheri La Ferla” (Palermo), Hospital of Napoli, Hospital of Avellino, and Israelitic Hospital (Rome) to have been part of the study. Authors also wish to thank Stefano Marventano for editing the manuscript.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Berry SJ, Coffey DS, Walsh PC, Ewing LL: The development of human benign prostatic hyperplasia with age. J Urol. 1984; 132: 474-9.
2. Miner M, Rosenberg MT, Perelman MA: Treatment of lower urinary tract symptoms in benign prostatic hyperplasia and its impact on sexual function. Clin Ther. 2006; 28: 13-25.
3. Tang J, Yang J: Etiopathogenesis of benign prostatic hypeprlasia. Indian J Urol. 2009; 25: 312-7.
4. Roehrborn CG, Kaplan SA, Noble WD: The impact of acute or chronic inflammation in baseline biopsy on the risk of clinical progression of BPE: Results from the MTOPS study. AUA Meeting 2005. J Urol, suppl., 2005; 173: 346, abstract 1277.

5. Nickel JC, Roehrborn CG, O'Leary MP, Bostwick DG, Somerville MC, Rittmaster RS: The relationship between prostate inflammation and lower urinary tract symptoms: examination of baseline data from the REDUCE trial. Eur Urol. 2008; 54: 1379-84.
6. Kramer G, Mitteregger D, Marberger M: Is benign prostatic hyperplasia (BPH) an immune inflammatory disease? Eur Urol. 2007; 51: 1202-16.
7. Di Silverio F, Gentile V, De Matteis A, Mariotti G, Giuseppe V, Luigi PA, et al.: Distribution of inflammation, pre-malignant lesions, incidental carcinoma in histologically confirmed benign prostatic hyperplasia: a retrospective analysis. Eur Urol. 2003; 43: 164-75.
8. Sciarra A, Di Silverio F, Salciccia S, Autran Gomez AM, Gentilucci A, Gentile V: Inflammation and chronic prostatic diseases: evidence for a link? Eur Urol. 2007; 52: 964-72.
9. Comhaire F, Mahmoud A: Preventing diseases of the prostate in the elderly using hormones and nutriceuticals. Aging Male. 2004; 7: 155-69.
10. Suzuki M, Ito Y, Fujino T, Abe M, Umegaki K, Onoue S, et al.: Pharmacological effects of saw palmetto extract in the lower urinary tract. Acta Pharmacol Sin. 2009; 30: 227-81.
11. Buck AC: Is there a scientific basis for the therapeutic effects of serenoa repens in benign prostatic hyperplasia? Mechanisms of action. J Urol. 2004; 172: 1792-9.
12. Diwadkar-Navsariwala V, Prins GS, Swanson SM, Birch LA, Ray VH, Hedayat S, et al.: Selenoprotein deficiency accelerates prostate carcinogenesis in a transgenic model. Proc Natl Acad Sci U S A. 2006; 103: 8179-84.
13. Schwarz S, Obermüller-Jevic UC, Hellmis E, Koch W, Jacobi G, Biesalski HK: Lycopene inhibits disease progression in patients with benign prostate hyperplasia. J Nutr. 2008; 138: 49-53.
14. Kandaş NO, Randolph C, Bosland MC: Differential effects of selenium on benign and malignant prostate epithelial cells: stimulation of LNCaP cell growth by noncytotoxic, low selenite concentrations. Nutr Cancer. 2009; 61: 251-64.
15. Wertz K, Siler U, Goralczyk R: Lycopene: modes of action to promote prostate health. Arch Biochem Biophys. 2004; 430: 127-34.
16. Obermüller-Jevic UC, Olano-Martin E, Corbacho AM, Eiserich JP, van der Vliet A, Valacchi G, et al.: Lycopene inhibits the growth of normal human prostate epithelial cells in vitro. J Nutr. 2003; 133: 3356-60.
17. Nickel JC, True LD, Krieger JN, Berger RE, Boag AH, Young ID: Consensus development of a histopathological classification system for chronic prostatic inflammation. BJU Int. 2001; 87: 797-805.
18. Kohnen PW, Drach GW: Patterns of inflammation in prostatic hyperplasia: a histologic and bacteriologic study. J Urol. 1979; 121: 755-60.
19. Nickel JC, Downey J, Young I, Boag S: Asymptomatic inflammation and/or infection in benign prostatic hyperplasia. BJU Int. 1999; 84: 976-81.
20. Robert G, Descazeaud A, Nicolaïew N, Terry S, Sirab N, Vacherot F, et al.: Inflammation in benign prostatic hyperplasia: a 282 patients' immunohistochemical analysis. Prostate. 2009; 69: 1774-80.
21. Mishra VC, Allen DJ, Nicolaou C, Sharif H, Hudd C, Karim OM, et al.: Does intraprostatic inflammation have a role in the pathogenesis and progression of benign prostatic hyperplasia? BJU Int. 2007; 100: 327-31.
22. Sebastiano C, Vincenzo F, Tommaso C, Giuseppe S, Marco R, Ivana C, et al.: Dietary patterns and prostatic diseases. Front Biosci (Elite Ed). 2012; 4: 195-204.
23. Vela Navarrete R, Garcia Cardoso JV, Barat A, Manzarbeitia F, López Farré A: BPH and inflammation: pharmacological effects of Permixon on histological and molecular inflammatory markers. Results of a double blind pilot clinical assay. Eur Urol. 2003; 44: 549-55.
24. Magri V, Trinchieri A, Perletti G, Marras E: Activity of Serenoa repens, lycopene and selenium on prostatic disease: evidences and hypotheses. Arch Ital Urol Androl. 2008; 80: 65-78.
25. Bonvissuto G, Minutoli L, Morgia G, Bitto A, Polito F, Irrera N, et al.: Effect of Serenoa repens, lycopene, and selenium on proinflammatory phenotype activation: an in vitro and in vivo comparison study. Urology. 2011; 77: 248.e9-16.
26. De Nunzio C, Kramer G, Marberger M, Montironi R, Nelson W, Schröder F, et al.: The controversial relationship between benign prostatic hyperplasia and prostate cancer: the role of inflammation. Eur Urol. 2011; 60: 106-17.
27. Gui-Zhong L, Libo M, Guanglin H, Jianwei W: The correlation of extent and grade of inflammation with serum PSA levels in patients with IV prostatitis. Int Urol Nephrol. 2011; 43: 295-301.
28. Ozden C, Ozdal OL, Guzel O, Han O, Seckin S, Memis A: The correlation between serum prostate specific antigen levels and asymptomatic inflammatory prostatitis. Int Urol Nephrol. 2007; 39: 859-63.
29. Kandirali E, Boran C, Serin E, Semercioz A, Metin A: Association of extent and aggressiveness of inflammation with serum PSA levels and PSA density in asymptomatic patients. Urology. 2007; 70: 743-7.

Correspondence address:
Dr. Giorgio Ivan Russo
University of Catania, Italy
Fax: + 39 095 378-2373
E-mail: giorgioivan@virgilio.it

ORIGINAL ARTICLE

Vol. 39 (2): 222-227, March - April, 2013
doi: 10.1590/S1677-5538.IBJU.2013.02.11

The histology of prostate tissue following prostatic artery embolization for the treatment of benign prostatic hyperplasia

George Camara-Lopes, Romulo Mattedi, Alberto A. Antunes, Francisco C. Carnevale, Giovanni G. Cerri, Miguel Srougi, Venancio A. Alves, Katia R. M. Leite

Department of Pathology (GCL, RM, VAA), Department of Radiology (AAA, MS, KRML) and Department of Urology FCC, GGC), Laboratory of Medical Research, LIM55, University of Sao Paulo Medical School, SP, Brazil

ABSTRACT

Objective: Prostatic artery embolization (PAE) for the treatment of patients with symptomatic benign prostatic hyperplasia (BPH) is believed to be a safe procedure with a low risk of adverse side effects. Artery embolization is a viable treatment option in patients who are refractory to the classic noninvasive treatments. Knowledge of the histological characteristics of prostate tissue following the procedure is still limited. In this study, we describe the microscopic aspects of the prostate following PAE for BPH.

Materials and Methods: Two patients underwent transurethral resections of the prostate (TURP) after PAE. Embolizations were performed under local anesthesia with an initial pelvic angiography to evaluate the iliac vessels and the prostate arteries using a 2.8 French microcatheter. The prostate was embolized with 300-500µm Microspheres (Embosphere®), using complete blood stasis as the end point. The prostate tissues were analyzed histologically to characterize the effects of the embolization.

Results: The embolic material within the prostate tissue was easily identified as homogeneous, bright eosin-red spheroids filling the vessel lumens. Ischemic necrosis surrounded or not by chronic inflammatory reactions containing macrophages were considered as a result of the artery embolization. Also, some aspects related to the healing process were observed being fibrotic nodules surrounded by glands with squamous metaplasia of the epithelial lining the most important. In the remaining sections, due to the precocious surgical intervention, the classic findings of BPH were still present with the glandular and stromal hyperplasia associated with nonspecific chronic prostatitis.

Conclusions: This is the first description of prostate histology in BPH patients treated by PAE, a new procedure that is being used increasingly as a therapeutic intervention. The recognition of the changes caused by this new modality of treatment has become a very important differential in a chronic granulomatous reaction of the prostate tissue.

ARTICLE INFO

Key words:
Prostatic Hyperplasia; Therapeutics; Arteries; Histology; Embolization, Therapeutic; Prostate

Int Braz J Urol. 2013; 39: 222-7

Submitted for publication:
July 07, 2012

Accepted after revision:
February 04, 2013

INTRODUCTION

Benign prostatic hyperplasia (BPH) is the most common benign neoplasm in men (1,2). It typically occurs in the sixth and seventh decades, and more than 40% of men diagnosed with BPH present with the clinical manifestations (3-6). BPH causes obstructive urinary symptoms such as hesitancy, a decreased urinary stream, intermittency, sensation of incomplete emptying, nocturia, and

an increase in frequency and urgency (3). Voiding difficulties attributable to BPH can be quantified with the International Prostatic Symptom Score. Various medications, specifically 5-α-reductase inhibitors and selective α-blockers, can decrease the severity of voiding symptoms caused by BPH (3-6). Transurethral resection of the prostate (TURP) continues to be considered the best therapy for patients with BPH. Due to the relative morbidity of TURP, minimally invasive techniques have been developed as alternative treatments for BPH, including transurethral microwave thermotherapy and other laser-ablation therapies. However, open surgery, or TURP, represents the most common option for treating BPH (7-10). Taking into account the patients' comorbidities, surgical intervention in this group may be considered high-risk (6). Prostate artery embolization (PAE) has been used primarily to control massive hemorrhage after a prostatectomy or a prostate biopsy (11-15). PAE appears to be an acceptable and safe option to treat BPH, and it is now emerging as an innovative treatment modality (16,17). Here, we present the first description of prostate tissue histology in two patients who underwent TURP after PAE.

MATERIALS AND METHODS

Two male patients aged 67 and 68-years-old, with cardiovascular disease presented to the urology department with acute urinary retention due to BPH. They underwent transurethral catheter drainage, and the digital rectal examinations revealed large prostates of greater than 50 g in both patients. The prostate specific antigen (PSA) levels in these patients were 7.1 ng/ml and 12.9 ng/ml, respectively. Transrectal US showed an enlarged, heterogeneous prostate in each man. The prostates were 51 g and 63 g in volume, and the intravesical prostate protrusion measurements were 9 and 16 mm, respectively. Both patients underwent prostate biopsies and each patient had 12 tissue cores removed, which upon examination exhibited the features of benign prostatic tissue.

In order to provide good orientation to the prostate site and related structures in the pelvis, we filled a Foley balloon in the bladder of each patient with contrast medium (a mixture of 10 mL of 50% iodinated contrast medium plus 50% normal saline solution). Patients underwent angiography and embolization in the interventional radiology suite using the FD20 DSA unit (Philips, Netherlands) and with non-ionic Visipaque® contrast medium (Iodixanol 320 mg I/mL, GE, Healthcare, Europe). A 400 mg intravenous dose of ciprofloxacin was given prior to the procedure followed by 500 mg orally twice a day for seven days after PAE. Patients also received non-opioid analgesic and nonsteroidal anti-inflammatory medication after embolization, if necessary. Intervention was performed under local anesthesia through the right transfemoral approach. Initial pelvic angiography was performed to evaluate iliac vessels and the prostate arteries during arterial and late phases. Selective digital subtraction angiography of the right and left internal iliac arteries was performed with a 5-French Cobra 2 or Vertebral catheter to assess the blood supply to the prostate. Bilateral selective catheterization of the inferior vesical artery, superior vesical artery, obturator artery, middle rectal artery and internal pudendal artery was then performed using a microcatheter (Embocath®; Biosphere Medical, USA or Progreat® 2.8, Terumo, Japan). Angiography was performed by manually injecting 3-5 mL of contrast medium to identify any blood supply to the prostate and to ensure that the tip of the microcatheter was inside or at the ostium of the prostatic arteries. When spasm occurred nitroglycerin was used. Tris-acryl microspheres (Embosphere® Microspheres, Biosphere Medical, Roissy, France) 300-500µm in diameter were used for embolization. We diluted the 2.0 mL Embosphere® Microspheres syringe to a total volume of 22 mL using equal amounts of contrast medium and saline. The microsphere mixture was slowly injected under fluoroscopic guidance. Embolization of the prostatic arteries was performed to stasis, without reflux of the mixture to undesired arteries. Follow-up angiography was performed manually with the microcatheter using the power injector with the 5-F catheter at the anterior branch of the internal iliac artery to check for any further blood supply to the prostate. Embolization was then performed on the contralateral side by using the same technique.

One patient failed bilateral PAE twice. This patient was unable to void spontaneously when the indwelling urinary catheter was removed and the patient was referred to TURP after only 5% reduction

in the prostate size. The other patient was excluded from the PAE study protocol due to urodynamic findings and was submitted to TURP.

The two respective post-PAE specimens were fixed in formalin and submitted for histological evaluation according with the 2012 CAP protocol for the examination of TURP specimens (18,19). All the tissue was submitted for evaluation in the first patient and 8 slides was reviewed. The tissue from the second patient was partially submitted and 10 slides were reviewed. The slides were stained with hematoxylin and eosin and were examined using an optical microscope by our pathology department staff on the first sign out. A clinical history of embolization was provided to the pathologist at the initial time of pathologic examination in both cases and the history of negative previous biopsies from both patients were also noticed at the time. Gross pathology reports were reviewed, and pertinent data were recorded. Routine sections from gross specimens were microscopically examined and reviewed by other 2 pathologists.

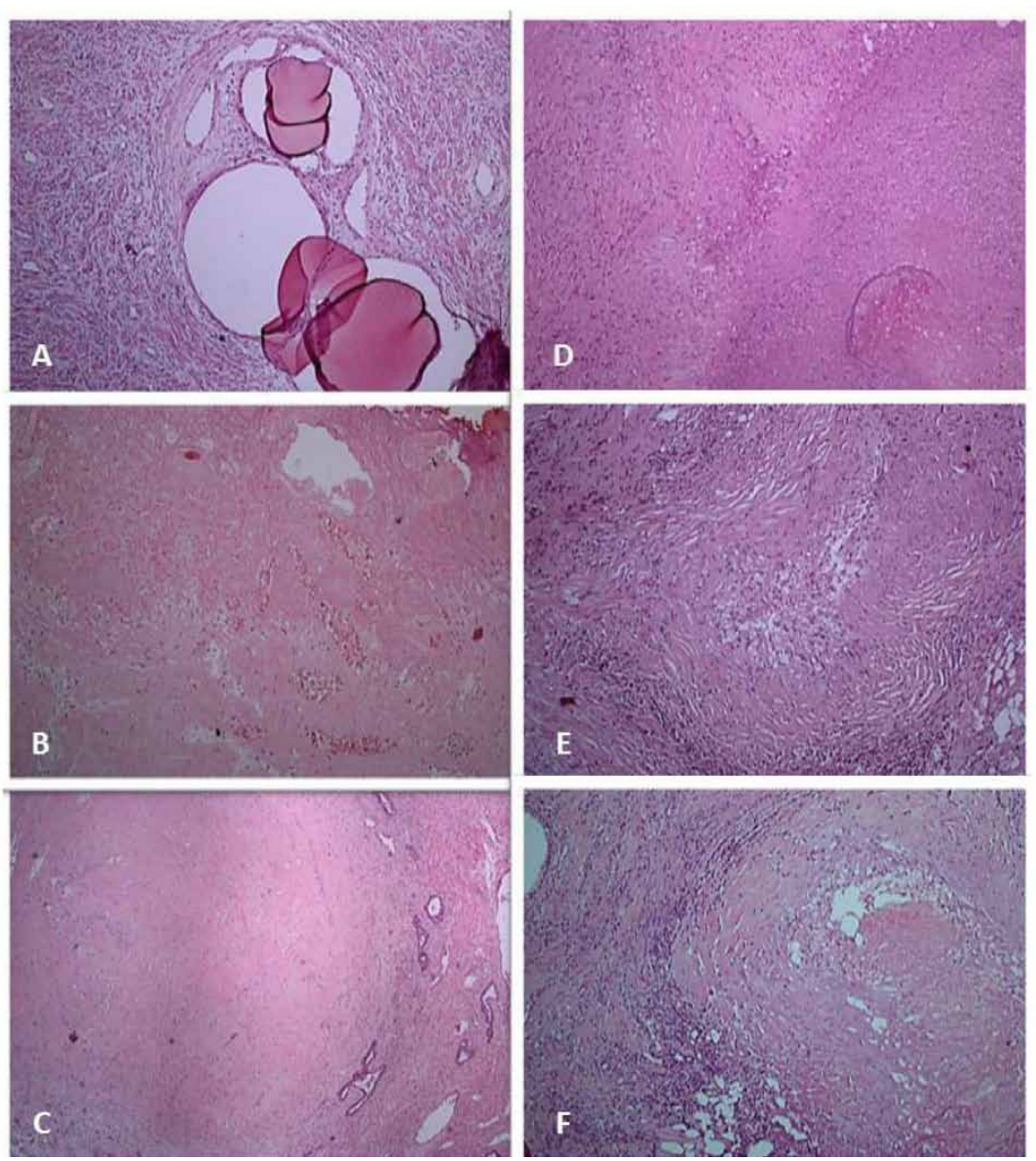
RESULTS

Surgical specimens were received in buffered formalin 10%, consisting in prostate tissue measuring 6 and 8 cm, weighting 14.5 and 36.0 grams for patients 1 and 2, respectively. The histological findings of the prostate samples were very similar for both patients and are illustrated in Figure-1. The embolic material was easily identified in both cases using standard hematoxylin and eosin staining as 2 to 3 mm homogeneous-appearing, brightly eosin--red spheroids. Despite the spheroids are the only specific finding on the specimens, we readily identified changes on the surrounding tissue due to the embolization, such as ischemic necrosis, that was surrounded or not by histiocytes, remembering a foreign body reaction rarely containing giant multinucleated cells. In the surrounding prostate tissue near the microspheres, there was identifiable vascular ectasia and moderate quantities of stromal lymphocytes. The transition between necrotic and normal prostate tissue was generally abrupt, and it was possible to detect a ribbon of neuthrophils, lymphocytes and proliferated fibroblasts delimitating the two areas. Also, a nodular fibrosis with hyalinization as a consequence of the healing process was present in some areas associated with squamous metaplasia of the epithelium lining the surrounding glands. All these alterations were found in about 5% of the tissue examined in both cases. The remaining 95% of the prostate tissue exhibited the classic findings of BPH, glandular and stromal hyperplasia, as well as mild, nonspecific chronic prostatitis. No high-grade intraepithelial neoplasia or adenocarcinoma was found in the slides of either patient.

DISCUSSION

Our report is the first to describe the histological features of the prostate after PAE. Although there are several reports that describe the post-treatment alterations in cancerous or non--cancerous prostate tissue following hormonal therapy, use of 5-α-reductase inhibitors or radiation (20), no post-PAE histological findings have been mentioned in the literature to date. With the continuing advances in the techniques and emerging therapies in BPH and cancer treatments, pathologists will play a central role in documenting the effects of these new treatment options on prostate tissue as well as a role in helping to improve these new methods. Although these findings are nonspecific and the only finding that confirms the attempted treatment BHP with PAE are the identification of the spheroids, the pathologist´s role in this scenario is to inform the clinicians the histological finding of the spheroid, try to quantify the amount of necrosis to give a notion and feedback of the effectiveness of the modality of treatment and make differential diagnosis with other conditions that could present as an chronic granulomatous reaction, such as acute infarct of hyperplastic nodules, fibrosis in advanced stages of infarct in nodular hyperplasia surrounded by squamous metaplasia, granulomas following any kind of surgical intervention or instrumentation, granulomas secondary to the use of BCG in treatment of urothelial neoplasm, and idiopathic or infectious granulomatous prostatitis. All these diagnostic entities should be ruled out in a setting of chronic granulomatous inflammatory reaction. Certainly the clinical history and clinical information of each one of these conditions

Figure 1 - Microscopic aspects of prostate tissue following PAE. (A) Embolic material used to occlude vessels represented by homogeneous, densely eosinophilic spheres. (B) Ischemic necrosis with no inflammatory reaction. (C) Fibrotic nodule surrounded by glands presenting squamous metaplasia of epithelial lining. (D-F) Extensive necrosis surrounded by histiocytes forming epithelioid granulomas.190x215 mm (96 x 96 DPI).



should be informed for the pathologist so that one can make a more accurate diagnostic. Arterial embolization has previously been used in cases of prostate hemorrhage following surgery or prostate biopsy (11-15), and recently PAE has been proposed as a new option to treat BPH (16,17). Studies on pigs have reported decreases in prostate gland size following PAE with no side effects, providing preliminary evidence that this approach is efficient and safe (21). We have previously described two cases of BPH successfully treated with PAE that resulted in a reduction in prostate size at 6 months of 47% in a patient undergoing a bilateral PAE and of 28% in a patient undergoing unilateral

PAE (16). A continued reduction in prostate volume was observed during the 18 months follow-up with magnetic resonance imaging (MRI) showing a reduction of 54% in the patient with the bilateral PAE. For the patient undergoing unilateral PAE, the reduction was stable at 6 months follow-up, and the patient experienced only mild urinary symptoms (22). Organ histology following embolization has been described in uterine leiomyomas. In these cases, the authors describe hyaline necrosis, dense fibrosis and a lack of inflammation (23). Further complications have been reported, the most dangerous including hematoma formation, fistulas, necrosis with secondary infection and septicemia and pseudoaneurysms responsible for significant hemorrhage. Although we do not know the long-term consequences of therapeutic artery embolization in the prostate, urologists and radiologists should remember the complications observed in the uteri as more patients undergo this new form of therapy (24,25). Arterial embolization procedures have the potential to become increasingly common as a result of being minimally invasive. In order to avoid misdiagnoses, pathologists should be aware of the histological characteristics that distinguish post-PAE prostate tissue from BPH, including extensive necrosis and granulomatous reaction. Further, the observation of these histological characteristics could prevent the possible complications that result from the vascular interruption in prostate tissue resulting in ischemia and necrosis.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Barry MJ: Measuring and assessing BPH in population groups. In Kirby RS, McConnell JD, Fitzpatrick Roehrborn JM, Roehrborn CG, Boyle P (ed.), Textbook of benign prostatic hyperplasia. Taylar & Francis, London. 2005; pp. 141-50.
2. Issa MM, Fenter TC, Black L, Grogg AL, Kruep EJ: An assessment of the diagnosed prevalence of diseases in men 50 years of age or older. Am J Manag Care. 2006; 12: S83-9.
3. Emberton M, Andriole GL, de la Rosette J, Djavan B, Hoefner K, Vela Navarrete R, et al.: Benign prostatic hyperplasia: a progressive disease of aging men. Urology. 2003; 61: 267-73.
4. Ziada A, Rosenblum M, Crawford ED: Benign prostatic hyperplasia: an overview. Urology. 1999; 3 (Suppl 3a): 1-6.
5. Guess HA, Arrighi HM, Metter EJ, Fozard JL: Cumulative prevalence of prostatism matches the autopsy prevalence of benign prostatic hyperplasia. Prostate. 1990; 17: 241-6.
6. AUA Practice Guidelines Committee: AUA guideline on management of benign prostatic hyperplasia (2003). Chapter 1: Diagnosis and treatment recommendations. J Urol. 2003; 170: 530-47.
7. Roehrborn CG, Preminger G, Newhall P, Denstedt J, Razvi H, Chin LJ, et al.: Microwave thermotherapy for benign prostatic hyperplasia with the Dornier Urowave: results of a randomized, double-blind, multicenter, sham-controlled trial. Urology. 1998; 51: 19-28.
8. Wheelahan J, Scott NA, Cartmill R, Marshall V, Morton RP, Nacey J, et al.: Minimally invasive laser techniques for prostatectomy: a systematic review. The ASERNIP-S review group. Australian Safety and Efficacy Register of New Interventional Procedures--Surgical. BJU Int. 2000; 86: 805-15.
9. Roehrborn CG, Issa MM, Bruskewitz RC, Naslund MJ, Oesterling JE, Perez-Marrero R, et al.: Transurethral needle ablation for benign prostatic hyperplasia: 12-month results of a prospective, multicenter U.S. study. Urology. 1998; 51: 415-21. Erratum in: Urology. 1998; 52: 159.
10. Cioanta I, Muschter R: Water-induced thermotherapy for benign prostatic hyperplasia. Tech Urol. 2000; 6: 294-9.
11. Mitchell ME, Waltman AC, Athanasoulis CA, Kerr WS Jr, Dretler SP: Control of massive prostatic bleeding with angiographic techniques. J Urol. 1976; 115: 692-5.
12. Appleton DS, Sibley GN, Doyle PT: Internal iliac artery embolisation for the control of severe bladder and prostate haemorrhage. Br J Urol. 1988; 61: 45-7.
13. Michel F, Dubruille T, Cercueil JP, Paparel P, Cognet F, Krause D: Arterial embolization for massive hematuria following transurethral prostatectomy. J Urol. 2002; 168: 2550-1.
14. Barbieri A, Simonazzi M, Marcato C, Larini P, Barbagallo M, Frattini A, et al.: Massive hematuria after transurethral resection of the prostate: management by intra-arterial embolization. Urol Int. 2002; 69: 318-20.
15. Rastinehad AR, Caplin DM, Ost MC, VanderBrink BA, Lobko I, Badlani GH, et al.: Selective arterial prostatic embolization (SAPE) for refractory hematuria of prostatic origin. Urology. 2008; 71: 181-4.
16. Carnevale FC, Antunes AA, da Motta Leal Filho JM, de Oliveira Cerri LM, Baroni RH, Marcelino AS, et al.: Prostatic artery embolization as a primary treatment for benign prostatic hyperplasia: preliminary results in two patients. Cardiovasc Intervent Radiol. 2010; 33: 355-61.

17. Carnevale FC, da Motta-Leal-Filho JM, Antunes AA, Baroni RH, Freire GC, Cerri LM, et al.: Midterm follow-up after prostate embolization in two patients with benign prostatic hyperplasia. Cardiovasc Intervent Radiol. 2011; 34: 1330-3.
18. Humphrey PA, Walther PJ: Adenocarcinoma of the prostate. I. Tissue sampling considerations. Am J Clin Pathol. 1993; 99: 746-59.
19. Merrimen JL, Jones G, Walker D, Leung CS, Kapusta LR, Srigley JR: Multifocal high grade prostatic intraepithelial neoplasia is a significant risk factor for prostatic adenocarcinoma. J Urol. 2009; 182: 485-90; discussion 490.
20. Evans AJ, Ryan P, Van derKwast T: Treatment effects in the prostate including those associated with traditional and emerging therapies. Adv Anat Pathol. 2011; 18: 281-93.
21. Sun F, Sánchez FM, Crisóstomo V, Lima JR, Luis L, García-Martínez V, et al.: Benign prostatic hyperplasia: transcatheter arterial embolization as potential treatment--preliminary study in pigs. Radiology. 2008; 246: 783-9.
22. Carnevale FC, da Motta-Leal-Filho JM, Antunes AA, Baroni RH, Freire GC, Cerri LM, et al.: Midterm follow-up after prostate embolization in two patients with benign prostatic hyperplasia. Cardiovasc Intervent Radiol. 2011; 34: 1330-3.
23. Kuzel D, Mara M, Horak P, Kubinova K, Maskova J, Dundr P, et al.: Comparative outcomes of hysteroscopic examinations performed after uterine artery embolization or laparoscopic uterine artery occlusion to treat leiomyomas. Fertil Steril. 2011; 95: 2143-5.
24. Takeda A, Kato K, Mori M, Sakai K, Mitsui T, Nakamura H: Late massive uterine hemorrhage caused by ruptured uterine artery pseudoaneurysm after laparoscopic-assisted myomectomy. J Minim Invasive Gynecol. 2008; 15: 212-6.
25. Aungst M, Wilson M, Vournas K, McCarthy S: Necrotic leiomyoma and gram-negative sepsis eight weeks after uterine artery embolization. Obstet Gynecol. 2004; 104: 1161-4.

Correspondence address:
Dr. Katia Ramos Moreira Leite
Department of Radiology
Laboratory of Medical Research, LIM55
University of Sao Paulo Medical School
Av. Dr. Arnaldo 455 / 2145
Sao Paulo, SP, 01246-903, Brazil
Telephone: +55 11 3061-7183
E-mail: katiaramos@uol.com.br

ORIGINAL ARTICLE

Vol. 39 (2): 228-239, March - April, 2013
doi: 10.1590/S1677-5538.IBJU.2013.02.12

Urine screening by Seldi-Tof, followed by biomarker identification, in a Brazilian cohort of patients with Renal Cell Carcinoma (RCC)

Gilda Alves, Denise A. Pereira, Vanessa Sandim, Antonio A. Ornellas, Niko Escher, Christian Melle, Ferdinand von Eggeling

Applied Genetic Laboratory, Hematology Division, National Institute of Cancer, (GA, DAP, VS); Division of Urology, National Institute of Cancer, (AAO); Division of Urology, Hospital Mário Kroeff, Rio de Janeiro, RJ, Brazil (AAO); Core Unit Chip Application, Institute of Human Genetics, Jena University Hospital, Jena, (NE, FE); Alere Technologies GmbH, Jena, (NE) and Biomolecular Photonics Group, Jena University Hospital, Germany (CM)

ABSTRACT

Purpose: To screen proteins/peptides in urine of Renal Cell Carcinoma (RCC) patients by SELDI-TOF (Surface Enhanced Laser Desorption Ionization - Time of Flight) in search of possible biomarkers.

Material and Methods: Sixty-one urines samples from Clear Cell RCC and Papillary RCC were compared to 29 samples of control urine on CM10 chip. Mass analysis was performed in a ProteinChip Reader PCS 4,000 (Ciphergen Biosystems, Fremont, CA) with the software Ciphergen Express 3.0. All chips were read at low and at high laser energy. For statistical analysis the urine samples were clustered according to the histological classification (Clear Cell and Papillary Carcinoma). For identification urine was loaded on a SDS PAGE gel and bands of most interest were excised, trypsinized and identified by MS/MS. Databank searches were performed in Swiss-Prot database using the MASCOT search algorithm and in Profound.

Results: Proteins that were identified from urine of controls included immunoglobulin light chains, albumin, secreted and transmembrane 1 precursor (protein K12), mannan--binding lectin-associated serine protease-2 (MASP-2) and vitelline membrane outer layer 1 isoform 1. Identification of immunoglobulins and isoforms of albumin are quite common by proteomics and therefore cannot be considered as possible molecular markers. K12 and MASP-2 play important physiological roles, while vitellite membrane outer layer 1 role is unknown since it was never purified in humans.

Conclusions: The down expression of Protein K-12 and MASP-2 make them good candidates for RCC urine marker and should be validated in a bigger cohort including the other less common histological RCC subtypes.

ARTICLE INFO

Key words:
Carcinoma, Renal Cell; Biological Markers; Proteomics;Proteins; MASP2 protein, human [Supplementary Concept]

Int Braz J Urol. 2013; 39: 228-39

Submitted for publication: May 10, 2012

Accepted after revision: November 11, 2012

INTRODUCTION

Urine analysis is a non-invasive method of clinical analysis and has been used primarily to monitor diseases of the urogenital tract. In a period of 24 hours, normal urine shows 150 milligrams of proteins and peptides that are derived from a variety of sources. Most of them come from the glomerular filtrate of plasma, while others from

the process of apoptosis and the cleavage of membrane proteins that are secreted. High molecular weight proteins (albumin, for example) are able to pass through the glomerular filtrate. Small proteins, or peptides (< 10 kDa) are easily filtered by the glomerulus and constitute an important source of information, even if the peptide is the result of proteolysis of larger proteins circulating in plasma (1-3). Urine is especially attractive for biomarker discovery in urological diseases since any change in concentration of proteins in plasma will reflect in the urine.

Mass Spectroscopy (MS) is an analytical technique that measures the mass-to-charge ratio of charged particles. It is used for determining the composition of a sample or molecule, and for elucidating the chemical structures of molecules, such as peptides and other chemical compounds. MS works by ionizing chemical compounds; the ions are then accelerated through a potential difference and focused into a beam. The ion beam passes through a magnetic field which bends the charged stream. Lighter components or components with more ionic charge will deflect in the field more than heavier or less charged components. The final element of a MS is the detector. The detector records either the charge induced or the current when an ion passes by or hits a surface. In a scanning instrument, the signals will generate a mass spectrum, a record of ions expressed as m/z (mass/charge).

MS/MS is the tandem mass spectrometry consisting of two mass spectrometers in series connected by a chamber known as a collision cell. The sample to be examined is essentially sorted and weighed in the first mass spectrometer, then broken into pieces in the collision cell, and a piece or pieces sorted and weighed in the second mass spectrometer. Identification by MS/MS is considered more accurate.

The technology of SELDI-TOF (Surface Enhanced Laser Desorption Ionization - Time of Flight) is a kind of mass spectrometer (MS). The instrument enables the separation of proteins and peptides by their physical properties (hydrophobic, hydrophilic, acidic, basic, with affinity for metal etc.) on a solid surface called Chip. The time-of-flight (TOF) analyzer measures the time they take to reach the detector. Ions velocities will depend on their masses and lighter ions will reach the detector first. The SELDI-TOF is very sensitive instrument and is able to detect proteins in fluids like urine without previous processing.

In spite of some new drugs for treatment (4), RCC is a disease that would benefit from improvement of early detection. More than 30% of patients show locally advanced or metastatic disease at diagnosis. Furthermore, approximately 40% of patients undergoing curative surgery for localized disease relapse. In search of possible biomarkers, we have screened proteins/peptides in individual samples of urine from 61 RCC Brazilian patients of the two most common histological subtypes (Clear Cell (CC) and Papillary).

MATERIAL AND METHODS

Patients and Controls

Patients and control individuals included in this study were adults of both genders, smokers and nonsmokers. This study was approved by the Ethics Committee of Instituto Nacional de Câncer (INCA), Rio de Janeiro, Brazil, registration # 38/05.

Urine samples were obtained from 61 untreated adult patients attending to the Departments of Urology of Instituto Nacional de Câncer and Hospital Mário Kröeff. Histological classification followed WHO guidelines. Table-1 shows the histological data as well as patient gender, Fuhrman grade, stage, follow-up and outcome. We were unable to establish the Fuhrman grade in a patient with papillary RCC type I. Patient follow-up data were gathered from medical charts and when necessary through contact with the patient family. We retrospectively reviewed the charts to determine dates of recurrence and death, as well as cause of death. Mean and median patient age was 59 and 55 years at diagnosis (range 27 to 84), respectively. Follow-up was evaluated in 61 patients. Median follow-up was 38 months (range 1 to 75). Patients with genetic syndromes or mental illness or immunodeficiency or who have been treated for cancer before were excluded from this study. Other exclusion criteria were the presence of proteinuria, microalbuminuria (diabetes mellitus), auto immune

Table 1 - Patient data, histopathological findings, tumor stage and survival analysis.

Pts.	Gender	Histological Type	Furhman Grade	Stage	Follow-up (months)	Deaths by Disease
1	Female	ccRCC	I	T1aNxM0	55	
2	Female	ccRCC	I	T2aNxMx	62	
3	Male	ccRCC	I	T1aNxMx	1	
4	Female	ccRCC	I	T1aNxM0	66	
5	Male	ccRCC	I	T2aN0M0	37	
6	Female	ccRCC	II	T1bNxM0	61	
7	Male	ccRCC	II	T1bNxM0	67	
8	Male	ccRCC	II	T3aNxMx	66	
9	Female	ccRCC	II	T1bNxMx	65	
10	Male	ccRCC	II	T1bNxMx	75	
11	Male	ccRCC	II	T1aN0M0	64	
12	Male	ccRCC	II	T1aNxMx	9	
13	Male	ccRCC	II	T3aNxMx	61	
14	Male	ccRCC	II	T3aNxMx	61	
15	Female	ccRCC	II	T1bNxMx	60	
16	Female	ccRCC	II	T1aNxMx	59	
17	Male	ccRCC	II	T1bN0Mx	47	
18	Female	ccRCC	II	T1bNxMx	54	
19	Male	ccRCC	II	T1bNxMx	36	
20	Male	ccRCC	II	T2aNxMx	36	
21	Female	ccRCC	II	T3bN0Mx		2

22	Female	ccRCC	II	T2aNxM0	29	
23	Male	ccRCC	II	T1bNxMx	32	
24	Female	ccRCC	II	T3aNxMx		9
25	Male	ccRCC	II	T3aNxMx	30	
26	Male	ccRCC	II	T1bNxM0	27	
27	Female	ccRCC	II	T3aNxMx	25	
28	Male	ccRCC	II	T3aNxMx		11
29	Male	ccRCC	II	T3aN2Mx	40	
30	Female	ccRCC	III	T3aNxMx		3
31	Male	ccRCC	III	T3aNxMx	64	
32	Female	ccRCC	III	T1N0M0	63	
33	Male	ccRCC	III	T3aNxM0		56
34	Male	ccRCC	III	T3aNxMx		1
35	Male	ccRCC	III	T3aNxMx	15	
36	Female	ccRCC	III	T2aNxMx	54	
37	Male	ccRCC	III	T3bN0Mx		22
38	Male	ccRCC	III	T3aNxMx		58
39	Male	ccRCC	III	T3aN1Mx		2
40	Male	ccRCC	III	T3bNx Mx	53	
41	Male	ccRCC	III	T3aNxMx		33
42	Male	ccRCC	III	T2aN0M0	32	
43	Male	ccRCC	III	T1bN1M0	28	
44	Female	ccRCC	III	T2aN0Mx	26	
45	Male	ccRCC	III	T3aN0M0	21	

46	Female	ccRCC	IV	T2bNxMx	64	
47	Male	ccRCC	IV	T1bN0M0	7	
48	Female	ccRCC	IV	T3aNxM0	30	
49	Female	ccRCC	IV	T2bNxMx		6
50	Male	ccRCC	IV	T3aN2Mx		22
51	Female	ccRCC	IV	T2bN2Mx		6
52	Male	ccRCC	IV	T2N0M0	1	
53	Female	ccRCC	IV	T1bN0M0	27	
54	Male	Papillary RCC type I	--	T1aNxM0		5
55	Female	Papillary RCC type I	II	T1bN0M0	30	
56	Female	Papillary RCC type I	II	T2aNxM0	50	
57	Female	Papillary RCC type I	II	T1NxM0	39	
58	Female	Papillary RCC type II	II	T3aN0M0	48	
59	Male	Papillary RCC type II	II	T2bN1M0	55	
60	Male	Papillary RCC type II	III	T2bNxMx		14
61	Female	Papillary RCC type II	IV	T2bNxM0		32

glomerulopathies, chronic kidney disease, urinary tract infections, lithiasis and prostatic diseases.

Twenty-nine control urine samples were obtained from over 40 years old healthy individuals from Laboratory of Molecular Biology, Department of Urology, Jena University Hospital, Germany. Control individuals had no indications of kidney abnormalities.

Urine Collection

The 10-20 mL first-void urine was discarded; the following 50-70 mL urine was collected, centrifuged at 1,000g for 10 minutes and the supernatant saved (5). Phenylmethanesulfonyl fluoride (PMSF) was added to the urine samples at final concentration of 1mM, aliquoted and frozen at -70ºC.

Binding conditions of urine proteins to the chips

Aliquots of urine were thawed in ice and were then vortexed at full power (6). Thereafter, all procedures were carried out at room temperature. All experiments were performed in duplicate. Three kinds of chips, IMAC30, Q-10 and CM10 (BIORAD), were tested.

The operating mechanism of the ProteinChip IMAC30 is the reversible binding of proteins to the surface through a coordinated metal interaction. The ProteinChip IMAC30 array incorporates nitrilotriacetic acid (NTA) groups and is capable of forming stable octahedral complexes with polyvalent metal ions, including Cu2+,Ni2+, Fe3+, and Ga3+. After loading the array surface with the desired metal ion, two free sites are available from the formed octahedral complex for interaction with specific amino acid residues (such as His) or posttranslational modifications such as phosphate groups. To generate selectivity, binding and washing buffers may contain increasing concentrations of competitors, such as imidazole, which compete with the coordinated metal on the NTA group for binding to the protein or peptide.

Q10 incorporates quaternized ammonium groups (positively charged) and thus acts as a strong anion exchanger. The Q10 surface binds peptides and proteins that are negatively charged at a given pH. By maintaining the pH of the binding or washing buffer at alkaline conditions (e.g., pH 8), an overall net negative charge is imparted on a greater number of proteins within the sample (therefore more binding). By decreasing the pH of the binding or washing buffer, an overall net positive charge is imparted on the proteins, resulting in less binding (i.e., more specificity).

CM10 incorporates a carboxylate chemistry (negatively charged) and thus acts as a weak cationic exchanger. CM10 surface binds proteins that are positively charged at a given pH. To generate selectivity, the pH of the binding buffer is increased or decreased, depending on the need. By decreasing the pH of the binding and washing buffers, an overall net positive charge is imparted on a greater number of proteins within the sample (therefore more binding). By increasing the pH of the binding and washing buffers, an overall net negative charge is imparted on the proteins, resulting in less binding (i.e., more specificity).

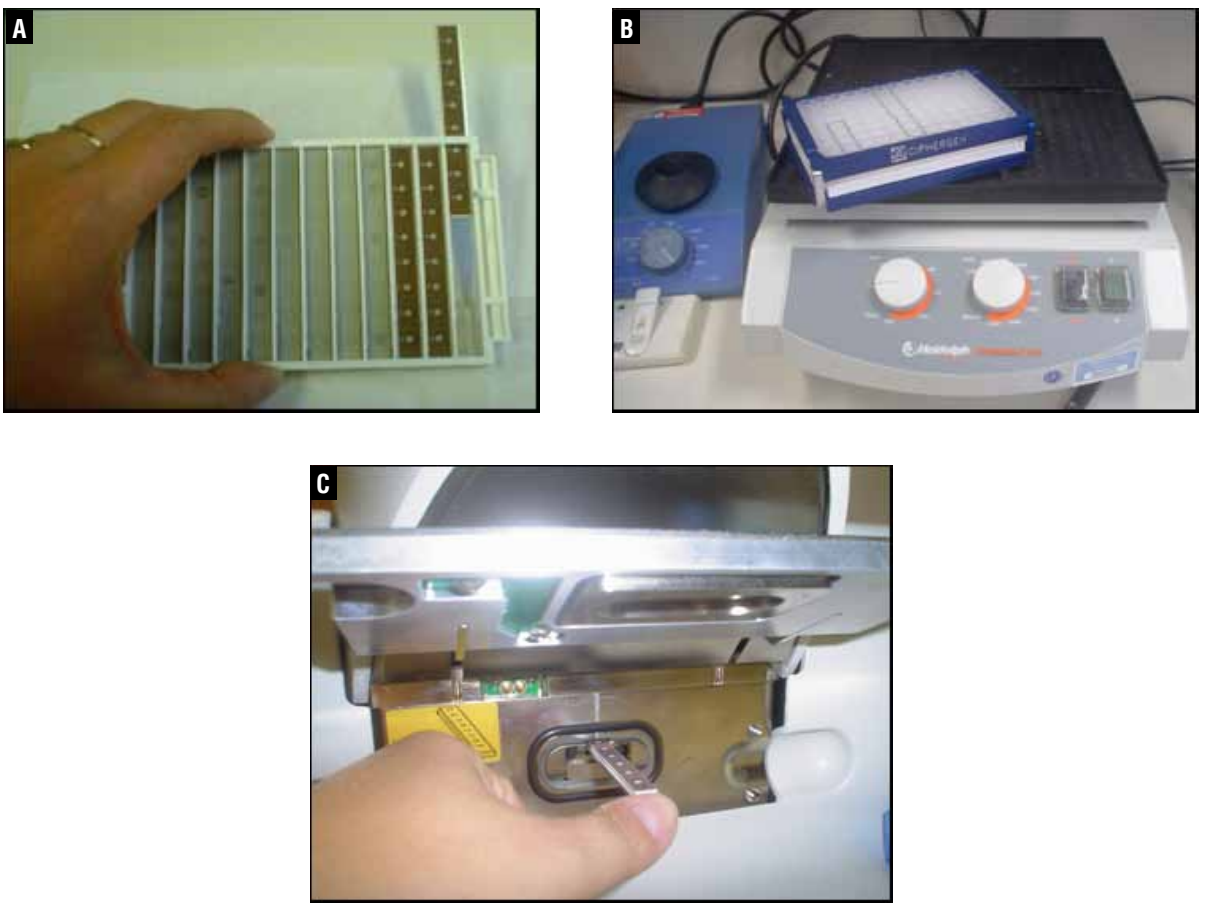
The effect of prefractioning of the urine samples was tested applying the Protein Chip Serum Fraction kit (BioRad, cat. K10-0007) according to manufacture's protocol. The increase of the peak intensity was also tested with the addition of a denaturing solution (9M urea, 2% CHAPS (3-[(3-Cholamidopropyl) dimethylammonio]-1--propanesulfonate hydrate), 50mM TRIS (Tris (hydroxymethyl)aminomethane)-HCl pH 9.0) to the urine before mixing to the binding buffer.

Alternatively, 50µL of pure urine were loaded with appropriate buffers in a bioprocessor (Ciphergen) for 90 minutes on a shaker. The chips were then washed, on a shaker, three times with the same buffers and two times with deionized water and air dried. A saturated solution of sinapinic acid (SPA) was prepared and 2x 0.5µL was applied in each spot. Figure-1a shows the assembling of chips into a frame that will go into a bioprocessor (Figure-1b); Figure-1c shows a chip being inserted into the SELDI-TOF).

Spectral data processing and statistical analysis

Pure urines from 29 cases of low grade ccRCC (Fuhrnann grade I + II), 24 from high grade ccRCC (Fuhrnann grade III + IV), 8 from were Papillary RCC (Fuhrnann grade II + III + IV) and 29 controls were individually analyzed in a ProteinChip Reader PCS 4,000 (Ciphergen Biosyste-

Figure 1 A-C - Assembling of chips into the bioprocessor and insertion of a chip into the SELDI-TOF.



ms, Fremont, CA) with the software Ciphergen Express Client 3.0. All chips were read at low laser energy (2,200 KJ) and at high laser energy (3,500KJ). Low laser energy and high laser energy focused mass range (m/z) of 1,000 to 20,000 Da and 20,000 to 200,000 Da, respectively. Peaks corresponding to albumin and hemoglobin were excluded from the analysis.

Urine pool fractionation with RP beads followed by SDS page gel

RP resins are a family of polymeric media useful for sample fractionation. RP beads are porous spherical beads, containing a surface area that is chemically stable to maximize extraction efficiency. The beads resin shows special characteristics such as: non-polar, non-ionic, ultra-clean, highly crossed-linked polymers that differ in particle size, pore size and surface area. The pore size large is selected according elements to enter into the pore and to adsorb onto the pore surface. Among the applications are exchange solvents, separate hydrophobic molecules by size and desalt samples.

Twenty mL of pooled urine from the control group were used for this experiment and mixed with 200µL of equilibrated RP beads (Polymer Laboratories Ltd., 15-20µm, 300Å, cat. 1412-2201) that were equilibrated in 10% acetonitrile/0.1% trichloroacetic acid. After 30 minutes incubation with mixing, the sample was centrifuged at 1,500g for 8 minutes and the supernatant was discarded.

The sample was fractionated into six fractions by sequential elution with 100µL aliquots of elution buffer with increasing concentrations of acetonitrile in 0.1% trichloroacetic acid (10-100% acetonitrile). Two µL of each fraction was directly spotted on the CM10 and analyzed by SELDI-TOF for selection. The fraction containing the protein of interest was concentrated in a vacuum centrifuge and loaded on a precast 12% SDS-PAGE gel (Invitrogen). Protein fractions co-migrated with the molecular maker Page Ruler Plus (Fermentas, cat. SM 1811). The gel was stained with Simply Blue Safe Stain (Enhanced Coomassie, Invitrogen).

Gel band trypsinization

The band of interest was excised from the stained gel and destained overnight with a mix of methanol 50% and acetic acid 10%. The gel piece was dehydrated in 100% acetonitrile for 30 minutes, dried in a vacuum centrifuge. Ten ng/mL of sequencing grade trypsin (Promega, UK) in 50 mM ammonium bicarbonate was added to dried gel piece and incubated at 37oC overnight.

MS/MS Analysis

The resulting peptides were analyzed in a MALDI TOF/TOF with a Bruker ultraflex device (Bruker Daltonics, Bremen, Germany). Briefly, samples were prepared in dried-droplet-preparation with α-hydroxy-cyano-cinnamic acid (CHCA). Peptide mass fingerprint and peptide fragmentation spectra of each sample were submitted for identification using MASCOT interface (MASCOT inhouse server 2.1.03, Matrix Science, London, UK) for search in the NCBI database. Hits were considered significant according to the MASCOT score (P = 0.05).

RESULTS

Binding conditions to the chips

Tests were performed with 3 different chips (IMAC30, Q-10 and CM10, BIORAD). The quantity of peaks that were produced in each condition was considered. The protocol applying fractions produced by the Protein Chip Serum Fraction kit did not improve the number of peaks and their intensity, no matter the chip. The protocol of pre-denaturing of the urine has not improved the results as well. The highest number of peaks were generated when pure urine samples were applied on CM10 chip therefore it was selected to perform the screening of RCC urines.

Peaks selection and identification

For analysis, the urine samples were clustered according to the histological classification and compared to 29 control samples. We observed 22 peaks (m/z) that were statistically overexpressed ($p < 0.05$) in the control group. The most significant peaks are listed at Table-2. An example of differently expressed peak is shown in Figure-2.

The selected RP fractions of the control urine pool were loaded in a SDS PAGE gel (Figure-3)

Table 2 – Peaks (m/z) found overexpressed in the Control urine group in comparison to different histological/grade types of Renal Cell Carcinoma (RCC) urines.

Groups compared	Peaks at Low Energy	Peaks at High Energy
Control x low grade ccRCC	9,772	23,622
Control x high grade ccRCC	4,751	23,615
	9,772	84,065
Control x low + high grade ccRCC	9,772	23,622
Control x Papillary RCC	9,097	23,615
	9,772	83,494

ccRCC – Clear Cell Renal Cell Carcinoma

Figure 2 - Spectra showing the peak of approximately 23,600 that is underexpressed in patients.

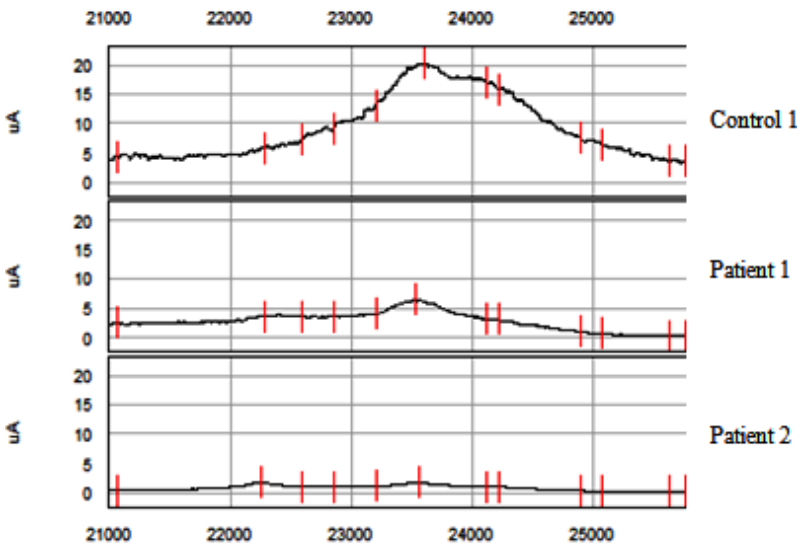


Figure 3 - Isolation of bands of interest from a Coomassie Blue stained 12% SDS PAGE gel from fraction eluted with 60% acetonitrile. Arrows indicate the bands of interest of approximately 9,770 (A) and 23,600 (B).

and bands of most interest (A and B) were excised for identification. Table 3 shows the results of the MS/MS analysis of bands A and B (Figure-3).

COMMENTS

The under detection of peaks in the urines of ccRCC and in Papillary RCC patients by SELDI--TOF screening was remarkable. This could be understandable by protein degradation in urine of these patients. The loss of proteins was noticed before by Sherief et al. (7) who reported that urinary extracellular matrix proteins laminin, collagen IV and fibronectin, which are known to be secreted by renal epithelial cells, were significantly decreased or absent in the urine specimens of patients with RCC. Corroborating to this, the metalloproteinase (MMP) activity was found higher in the urine of RCC patients (7). The elevated levels of MMP activity shall result in extracellular matrix proteins degradation that were either decreased or absent in the patients urines. Therefore, the explanation for the under detection of peaks reported herein could be due to protein degradation in the same way. Nevertheless, we cannot completely rule out that the results contain any bias due to the methods that were applied.

Table 3 - Protein identified by MS/MS.

Band	NCBI[1]	Human Protein	Score[2]	Average mass[3] (KDa)	Peptide[4]
A	gi|306999	Immunoglobulin light chain	81		R.LLIYGASTR.A -.EIVLTQSPGTLSLSPGER.A
A	gi|23241675	Albumin	195	46,442	K.FQNALLVR.Y K.LVNEVTEFAK.T K.KVPQVSTPTLVEVSR.N
B	gi|4506869	Secreted and transmembrane 1 precursor	113	27,307	R.DSHAGLYMWHLVGHQR.N R.AHGQESAIFNEVAPGYFSR.D
B	gi|3297879	MASP-2; mannan-binding lectin-associated serine protease-2	62	77,176	R.APGKDTFYSLGSSLDITFR.S
B	gi|32698964	Vitelline membrane outer layer 1 isoform 1	146	22,034	R.GLGDDTALNDAR.L R.VEAPTTLGDNTAANNVR.F K.VEPPQGIPGDDTALNGIR.L

[1]NCBI: National Center for Biotechnology Information: numbering refers to the protein in database

[2]Score: ideal value is above 35

[3]Theoretical molecular weight;

[4]Representative peptides: trypic peptides in which the analyses were based.

Proteins that were identified from urine of controls by MS/MS analysis were immunoglobulin light chain, albumin, secreted and transmembrane 1 precursor (protein K12), mannan-binding lectin-associated serine protease-2 (MASP-2) and vitelline membrane outer layer 1 isoform 1 (Table-3). Identification of immunoglobulins and isoforms of albumin are quite common by proteomics and therefore cannot be considered as possible molecular markers. On the other hand, the other 3 identified proteins should be taken into consideration. The peptides sequences (Table-3) from each one of these proteins were checked on uniprot data bank (www.uniprot.org) and they all corresponded 100% to the proteins assigned.

Expression of mRNA of K12 gene (SECTM1) was detected at the highest levels in peripheral blood leukocytes and breast cancer cell lines. Western blots showed that the K12 protein exists as a cluster of bands around 27 kDa, and extractions using nonionic detergents or high pH conditions demonstrate that it behaves as an integral membrane protein. Immunofluorescence localization studies reveal that K12 is not detectable on the cell surface, but instead is found in a perinuclear Golgi-like pattern and colocalizes with a well-known Golgi marker. In addition, an approximately 20-kDa soluble form of the K12 protein derived from the N-terminal domain is specifically secreted by cells into the culture medium. Immunohistochemical analysis of peripheral blood cells shows that K12 is found in leukocytes of the myeloid lineage, with the strongest staining observed in granulocytes and no detectable expression in lymphocytes. Based on its range of expression, it is possible that K12 is a protein

with potential importance in hematopoietic and/or immune system processes (8).

Mannan-binding lectin (MBL) is an oligomeric serum lectin that plays a role in innate immunity by activating the complement system. In human, two types of MBL-associated serine protease (MASP-1 and MASP-2) and a truncated protein of MASP-2 (small MBL-associated protein; sMAP or MAp19) are complexed with MBL (9). After activation by auto-catalytic cleavage, MASP-2 cleaves C2 and C4, leading to their activation and to the formation of C3 convertase.

Vitelline membrane outer layer 1 isoform 1 belongs to the family VMO1 that is conserved in the chicken, in the mouse and in human. In chickens, this protein participates in the construction of the vitelline membrane portion of the egg shell, a rigid structure required to maintain the shape of the egg (10). The role of this protein in humans is unknown, actually it was never purified. It was inferred from electronic annotation and probably is secreted.

Amazingly, the down expression of protein K-12 and MASP-2 were observed in two histological subtypes (ccRCC and Papillary) which show differentiated origin in the kidney and distinguished genetics alterations. Papillary RCCs are characterized by trisomy of chromosomes 3q, 7, 8, 12, 16, 17, and 20 and loss of the Y. On the other hand, chromosome 3p deletion is the most typical genetic alteration in ccRCC, present in 75.8% of cases which coincides with von Hippel-Lindau disease (11). This fact creates the possibility that a subtype independent RCC urine marker could be in the way. K-12 and MASP-2 should be tested in the urine of other RCC histological subtypes, although they are much less frequent than ccRCC and Papillary.

CONCLUSIONS

The down expression of Protein K-12 and MASP-2 make them good candidates for RCC urine makers and should be validated in a bigger cohort including the other less common histological RCC subtypes. Probably, these proteins were found in the control urine as a consequence of blood filtration role developed by the kidney. The lower abundance of these proteins in the patients' urine could be a consequence of their degradation or of kidney failure.

ACKNOWLEGMENTS

We thank Prof. Dr. Kestin Junker of Laboratory of Molecular Biology, Department of Urology, Jena University Hospital, 07740 Jena, Germany, for providing some of urine control samples and Annett Urbanek for technical assistance at SELDI-TOF.

This work was supported by PROBRAL CAPES/DAAD 293/08 and Programa de Oncobiologia (Rio de Janeiro, Brazil).

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Rindler MJ, Naik SS, Li N, Hoops TC, Peraldi MN: Uromodulin (Tamm-Horsfall glycoprotein/uromucoid) is a phosphatidylinositol-linked membrane protein. J Biol Chem. 1990; 265: 20784-9.
2. Pisitkun T, Shen RF, Knepper MA: Identification and proteomic profiling of exosomes in human urine. Proc Natl Acad Sci U S A. 2004; 101: 13368-73
3. Pisitkun T, Johnstone R, Knepper MA: Discovery of urinary biomarkers. Mol Cell Proteomics. 2006; 5: 1760-71.
4. Ather MH, Masood N, Siddiqui T: Current management of advanced and metastatic renal cell carcinoma. Urol J. 2010; 7: 1-9.
5. Schaub S, Rush D, Wilkins J, Gibson IW, Weiler T, Sangster K, et al.: Proteomic-based detection of urine proteins associated with acute renal allograft rejection. J Am Soc Nephrol. 2004; 15: 219-27.
6. Mataija-Botelho D, Murphy P, Pinto DM, Maclellan DL, Langlois C, Doucette AA: A qualitative proteome investigation of the sediment portion of human urine: Implications in the biomarker discovery process. Proteomics Clin Appl. 2009; 3: 95-105.
7. Sherief MH, Low SH, Miura M, Kudo N, Novick AC, Weimbs T: Matrix metalloproteinase activity in urine of patients with renal cell carcinoma leads to degradation of extracellular matrix proteins: possible use as a screening assay. J Urol. 2003; 169: 1530-4.

8. Slentz-Kesler KA, Hale LP, Kaufman RE: Identification and characterization of K12 (SECTM1), a novel human gene that encodes a Golgi-associated protein with transmembrane and secreted isoforms. Genomics. 1998; 47: 327-40.
9. Matsushita M, Thiel S, Jensenius JC, Terai I, Fujita T: Proteolytic activities of two types of mannose-binding lectin-associated serine protease. J Immunol. 2000; 165: 2637-42.
10. Guérin-Dubiard C, Pasco M, Mollé D, Désert C, Croguennec T, Nau F: Proteomic analysis of hen egg white. J Agric Food Chem. 2006; 54: 3901-10.
11. Algaba F, Akaza H, López-Beltrán A, Martignoni G, Moch H, Montironi R, et al.: Current pathology keys of renal cell carcinoma. Eur Urol. 2011; 60: 634-43.

Correspondence address:
Dr. Antonio Augusto Ornellas
Department of Urology
Instituto Nacional de Câncer
Praça da Cruz Vermelha, 23
Rio de Janeiro, RJ, 20230-130, Brasil
E-mail: ornellasa@hotmail.com

ORIGINAL ARTICLE

Vol. 39 (2): 240-247, March - April, 2013
doi: 10.1590/S1677-5538.IBJU.2013.02.13

Structural analysis of testicular appendices in patients with cryptorchidism

Guilherme D. Tostes, Suelen F. Costa, João P. de Carvalho, Waldemar S. Costa, Francisco J.B. Sampaio, Luciano A. Favorito

Urogenital Research Unit, Rio de Janeiro State University, Rio de Janeiro, Brazil

ABSTRACT

Objectives: Report the incidence and structure of testicular appendices (TAs) in patients with cryptorchidism, comparing their incidence with epididymal anomalies (EA) and patency of the vaginal process (PVP) and analyzes the structure of TAs.
Material and Methods: We studied 72 testes of patients with cryptorchidism (average of 6 years), and 8 testes from patients with hydroceles (average of 9 years). We analyzed the relations among the testis, epididymis and PVP and prevalence and histology of the TAs. The appendices of 10 patients with cryptorchidism and 8 with hydrocele were dissected and embedded in paraffin and stained with Masson trichrome; Weigert and Picro-Sirius Red with polarization and immunohistochemistry analysis of the collagen type III fibers to observe collagen. The stereological analysis was done with the software Image Pro and Image J, using a grid to determine volumetric densities (Vv). Means were statistically compared using the ANOVA and unpaired T test ($p < 0.05$).
Results: Of the 72 testes with cryptorchidism, 20 (27.77%) presented EA, 41 (56.9%) had PVP and 44 (61.1%) had TAs. Of the 44 testes with cryptorchidism and appendices, 30 (68.18%) presented PVP and 11 (25%) presented EA. There was no alteration of the epithelium in the appendices of patients in both groups. Stereological analysis documented the prevalence of ESFs (mean of 1.48%), prevalence of veins (mean of 10.11%) and decrease ($p = 0.14$) of SMCs in the TAs of patients with cryptorchidism (mean = 4.93%). Collagen III prevailed in the TAs of patients with cryptorchidism.
Conclusion: The testicular appendices presented significant structural alteration in the patients with cryptorchidism, indicating that TAs present a structural remodeling.

ARTICLE INFO

Key words:
Cryptorchidism; anatomy and histology; Epididymis; abnormalities

Int Braz J Urol. 2013; 39: 240-7

Submitted for publication:
October 03, 2012

Accepted after revision:
January 10, 2013

INTRODUCTION

Testicular and epididymal appendices have been considered congenital anomalies (1). The appendix testis or hydatid of Morgagni is believed to be the embryologic remnant of the cranial end of the Mullerian or paramesonephric duct (2). It is present in more than 90% of males, it varies in size from 1-10 mm in diameter and it is usually pedunculated, which predisposes to torsion. It is the most frequently twisted of the four testicular appendages (3-5). The other are the appendix of the epididymis, which is a remnant of the wolffian duct, the paradidymis and the vas aberrant. These vestigial structures have a similar histology, composed of gelatinous and vascular connective tissue covered with a columnar epithelium (6).

The functions of testicular appendices are controversial, but they may control the amount of serous fluid within the space of the vaginal

process (7). Another hypothesis suggests that the surface epithelium, subepithelial capillaries and lymphatic vessels of testicular appendices form a functional unit (8). Some studies have analyzed the embryology (9) and structure of the testicular and epididymal appendices (3,10).

Studies of the incidence of these structures in patients with cryptorchidism and comparison with individuals without testicular position anomalies are rare in the literature (11). There are no published studies analyzing the structure of testicular appendices in patients with cryptorchidism.

This work reports the incidence and structure of testicular appendices (TAs) in patients with cryptorchidism, comparing their incidence with epididymal anomalies and patency of the vaginal process and analyzes the structure of testicular appendices to assess whether their architecture is altered when cryptorchidism is present.

MATERIALS AND METHODS

The present work received institutional review committee and parent approval and has been carried out in accordance to the ethical standards of the responsible institutional committee on human experimentation.

We analyzed 72 testes from 55 patients with cryptorchidism (17 with bilateral cryptorchidism) and compared them with a control group of 8 testes from patients with hydroceles. The patients with cryptorchidism had ages from 1 to 13 years (average of 6 years), while those in the control group ranged from 1 to 14 years old (average of 9 years). In the two groups, we analyzed the relations among the testis, epididymis and patency of the vaginal process and the incidence and histology of the testicular appendices.

To analyze the relations between the testis and epididymis during surgery, we used a previous classification (12,13). By this classification, there are six types of relations between the testis and epididymis: Type I - epididymis attached to the testis at the head and tail; Type II - epididymis totally attached to the testis; Type III - epididymis attached to the testis only at the head; Type IV - epididymis attached to the testis only at the tail; Type V - no visible connection between the testis and epididymis; and Type VI - epididymal atresia. Type I and II relations are considered normal while the other types are considered to be anatomical anomalies (12). To analyze the vaginal process we considered two situations: (a) complete obliteration of the vaginal process between the internal inguinal ring and the superior pole of the testis; and (b) complete patency of the vaginal process.

In relation to the testicular appendices, we analyzed the following situations in the two groups during the surgeries: I) absence of testicular and epididymal appendices; II) presence of testicular appendix only; III) presence of epididymal appendix only; IV) presence of testicular and epididymal appendices; V) presence of two epididymal appendices and one testicular appendix; and VI) presence of paradidymis or vas aberrans of Haller.

The appendices of 10 patients with cryptorchidism and 8 patients with hydrocele were separated from the other structures and fixed in 10% buffered formalin, and routinely processed for paraffin embedding. Then 5 µm thick sections were obtained at 200 µm- intervals. Smooth muscle cells, connective tissue and elastic system fibers were studied by histochemical and immunolabeling methods.

The sections were stained with hematoxylin-eosin to assess the integrity of the tissue. We performed the following stainings: Masson's trichrome, in order to quantify connective tissue and smooth muscle, and Weigert resorcin fucsin with previous oxidation in order to observe elastic system fibers. Connective tissue, smooth muscle cells and elastic system fibers were quantified by a digital method (14). Five sections per specimen were stained, and five fields of each section were selected. All selected fields were photographed with an Olympus DP70 camera coupled to an Olympus BX51 microscope. The images were processed and saved with the Image Pro software. The tissue was quantified using the Image J software to determine the area density (Ad) of each component (14). Immunolabeling was performed to confirm the results. For smooth muscle cells (SMCs), Monoclonal Alfa Actin Antibody 08-0106 (Zymed Laboratories, San Francisco, CA, United States) was used. To confirm the results for elastic

fibers (EF), Monoclonal Elastin Antibody ab 9519 (Abcam Laboratories, Cambridge, MA, United States) was used.

Means were statistically compared using the unpaired t-test and linear regression was performed when applicable. Statistical relevance was accepted if $p < 0.05$.

RESULTS

Of the 72 testes with cryptorchidism, 7 were abdominal (9.7%), 54 (75%) were located in the inguinal canal and 11 (15.3%) were supra-scrotal. Of the testis with cryptorchidism, 20 (27.77%) presented epididymal anomalies, 41 (56.9%) had patent vaginal process and 44 (61.1%) had testicular appendices. The prevalence of the testicular appendices in the testis with cryptorchidism and their relation with the testicular position are reported in Table-1. There was no statistically significant difference in relation to the location of the appendices ($p = 0.0652$).

Of the 7 abdominal testes, all had patent vaginal process and 4 (57%) had testicular appendices. Of the 54 testes located in the canal, 31 (57%) had appendices, and of these, 21 (38.8%) presented patent vaginal process and 19 (35.8%) had epididymal anomalies. Of the 11 cases of supra-scrotal testes, 9 (82%) had appendices, 5 (55.5%) had patency of the vaginal process and 1 (11.1%) presented epididymal anomaly. Finally, of the 8 cases in the control group, 7 had appendices (87.5%), 1 (14.28%) had epididymal anomaly and 1 (14.28%) had patent vaginal process.

Of the 44 testes with cryptorchidism and appendices, 30 (68.18%) presented patent vaginal process and 11 (25%) showed epididymal anomalies. Of the 28 testes with cryptorchidism and without appendices, 11 (39.28%) presented patent vaginal process and 9 (32.14%) showed epididymal anomalies. The relation between the type of testicular appendix found and the patency of the vaginal process and the presence of epididymal anomalies is shown in Table-2. There was no statistically significant difference in relation to the patency of vaginal process between the patients with cryptorchidism and appendices with the control group ($p = 0.053$) and with the patients with cryptorchidism without appendices ($p = 0.252$). Also there was no statistically significant difference in relation to the epididimal anomalies between the patients with cryptorchidism and appendices with the control group ($p = 0.305$) and with the patients with cryptorchidism without appendices ($p = 0.6830$). Of the 55 patients, 17 had bilateral cryptorchidism, for a total of 34 testes. Of these, 1 was abdominal, 24 were located in the canal and 9 were supra-scrotal. Of the 34 testes of this group, 25 (73.5%) had testicular appendices, with 13 being right testes and 12 left ones, with no statistical difference.

Sections stained with Masson's trichrome from the testicular appendix demonstrated a vascular stroma lined with pseudocolumnar epithelium. The stroma consisted of loose connective tissue

Table 1 – Incidence of the testicular and epididymal appendices studied with respect to position of the testes with cryptorchidism and the testes in the control group. There was no statistically significant difference in relation to the location of the appendices (p = 0.0652).

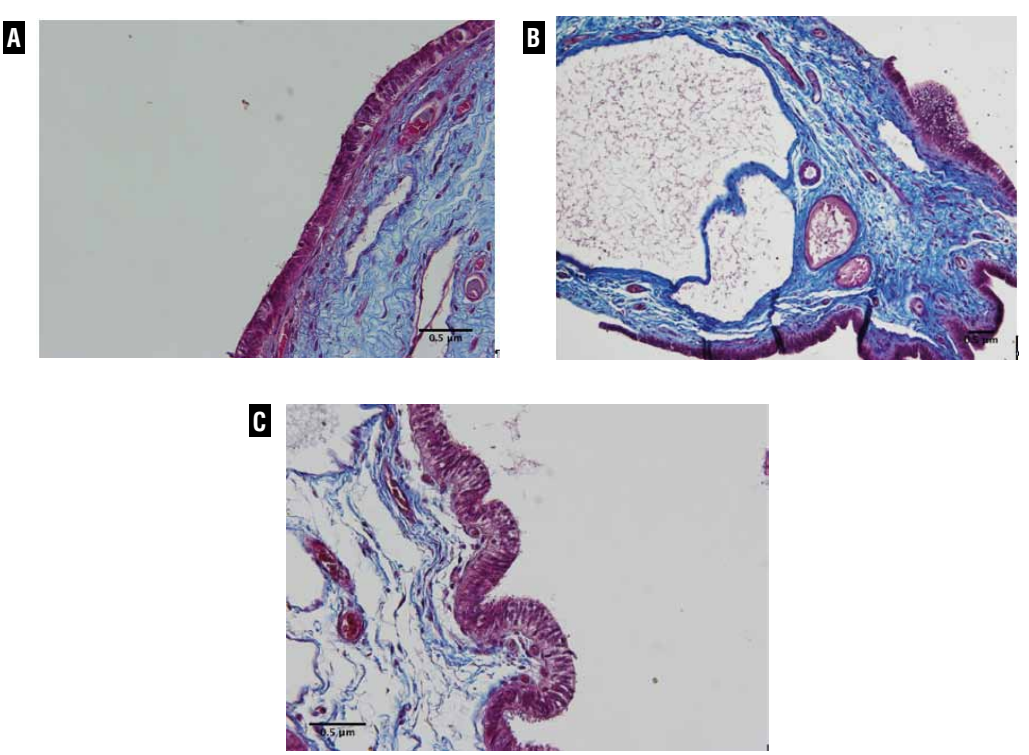
Appendices	Abdomen	Canal	Supra-Scrotal	Control
No appendix	3	23	2	1
Testicular appendix	1	23	7	5
Epididymal appendix	2	4	0	1
Testicular and epididymal appendix	1	4	2	1
Total	**7**	**54**	**11**	**8**

Table 2 – Relation between the type of appendix found and the patency of the vaginal process and presence of epididymal anomaly. There was no statistically significant difference in relation to the patency of vaginal process (p = 0.252) and epididymal anomalies (p = 0.6830) between the patients with cryptorchidism and appendices with the patients with cryptorchidism without appendices.

Appendices	Patent vaginal process	Epididymal anomaly
Testicular (31)	20	5
Epididymal (6)	5	4
Testicular and Epididymal (7)	5	2
Total (44-100%)	**30 (68.18%)**	**11(25%)**

containing blood vessels, fibroblasts and varying numbers of acini (gland-like structures), which were lined with a non-ciliated columnar epithelium (Figure-1A). The epididymal appendices were vesicular structures. The cavity of the vesicle was lined with a pseudostratified columnar epithelium (Figure-1B). There was no apparent alteration between the epithelium in the appendices of patients with cryptorchidism and in the control group, as seen in Figures 1A and 1C.

Figure 1 - Appendix epithelium. A) Patient with cryptorchidism with 3-years-old, showing the vascular stroma lined with pseudostratified epithelium of the testicular appendix. Masson's trichrome x200. B) Patient of the control group with 13-year-old, showing epididymal appendix with cavitation covered by pseudostratified epithelium. Masson's trichrome x 200. C) Patient of the control group with 13-years-old, showing pseudostratified epithelium of the entire testicular appendix. Masson's trichrome x200.



Stereological analysis documented a prevalence of elastic fibers in the testicular appendices of the patients with cryptorchidism (mean of 1.48%) in comparison to the appendices of the patients with a hydrocele testis (mean of 0.29%), a result that was not statistically different ($p = 0.22$). Figure-2 shows the testicular appendices in patients with cryptorchidism and in the control group stained by Weigert's resorcin-fucsin with oxidation. The testes of the patients with cryptorchidism contain a larger quantity of elastic fibers.

Stereological analysis also showed a greater incidence of vessels in the testicular appendices of the patients with cryptorchidism (mean of 10.11%, standard deviation of 6.88) in relation to the control group (mean of 4.77%, standard deviation of 1.9). This difference was statistically significant ($p < 0.001$). Figure-3 shows the testicular appendices in patients with cryptorchidism and those of the control group, stained by Masson's trichrome. A greater number of vessels can be observed in the testes of patients with cryptorchidism.

Stereology analysis showed a decrease without significance ($p = 0.14$) of smooth muscle cells in the appendices of patients with cryptor-

Figure 2 - Elastic system fibers of the testicular appendix. A) Testicular appendix of 13-year-old patient with cryptorchidism. Weigert x400. B) Testicular appendix of 13-year-old patient of the control group. Weigert x400. Note the marked increase of elastic fibers (brown) in the patient with cryptorchidism when compared to the control.

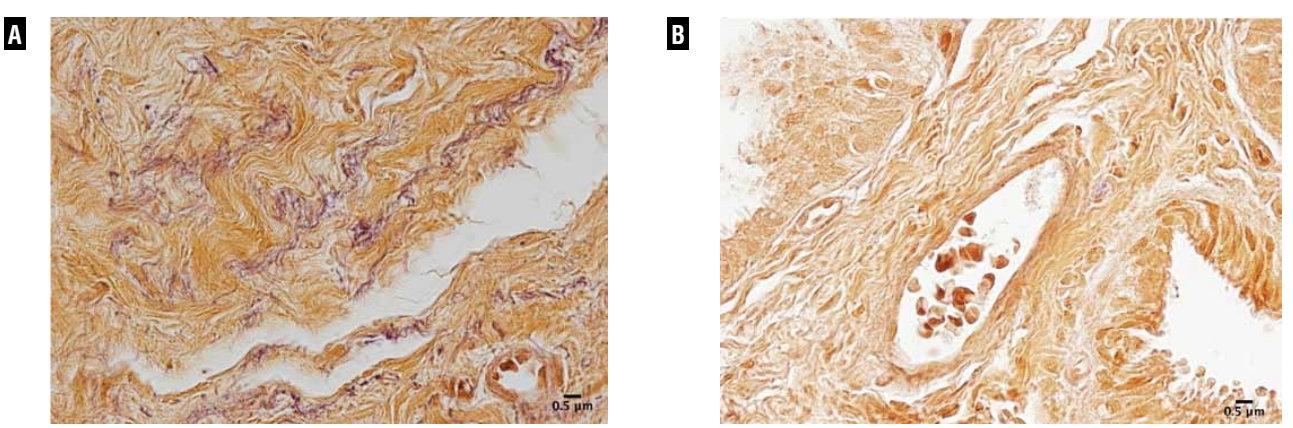
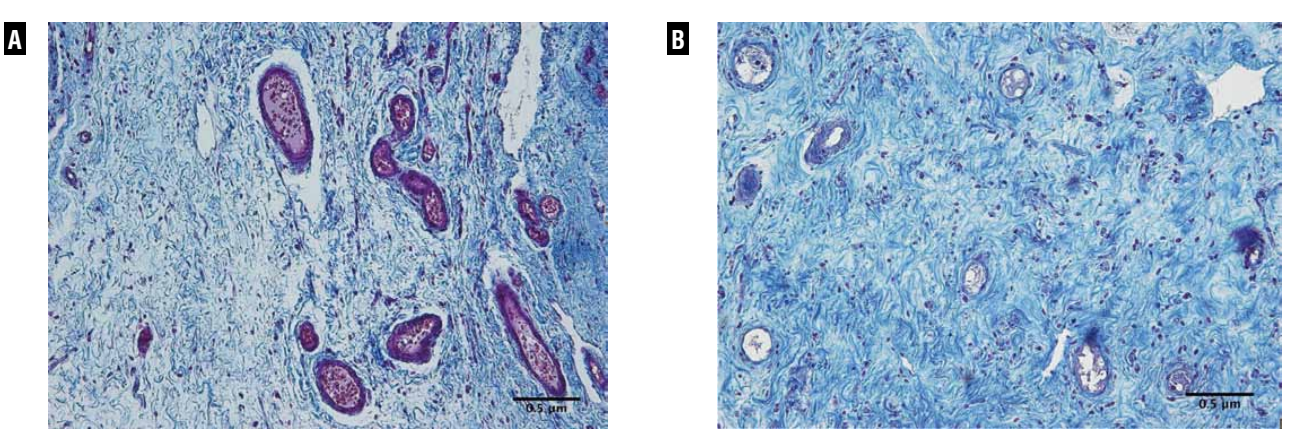


Figure 3 - Testicular appendix vessels. A) Testicular appendix of 2 years-old patient with cryptorchidism. Masson's trichrome X200. B) Testicular appendix of 1 year-old patient in the control group. Masson's trichrome X200. Note the marked increase of vessels in the appendix of the cryptorchidic patient.



chidism (mean = 4.93%) in comparison with the control group (mean = 9.032%). Figure-4 contains photomicrographs comparing the smooth muscle arrangement in the cryptorchidism and the control group appendices.

In the qualitative analysis, type III collagen was observed in both groups, although type III collagen was prevalent in the testicular appendices of patients with cryptorchidism and type I was prevalent in the patients of the control group. Figure-5 shows examples of the two groups stained with Picro Sirius Red with polarization. The photomicrographs presented a large difference in colors between the groups. This difference can suggest changes in the collagen fiber organization.

DISCUSSION

Knowledge of the presence, form and location of testicular and epididymal appendices is important in clinical practice because of the possibility of torsion of these structures, their association with

Figure 4 – Distribution of smooth muscle cells of the testicular appendix. A) Testicular appendix of 3-year-old patient with cryptorchidism. Imunohistochemical staining for alfha-actin X200. B) Testicular appendix of 11-year-old patient of the control group. Imunohistochemical staining for alfha-actin X200. Note the marked increase of SMCs in the appendix of the control patient when compared to the cryptorchidic patient.

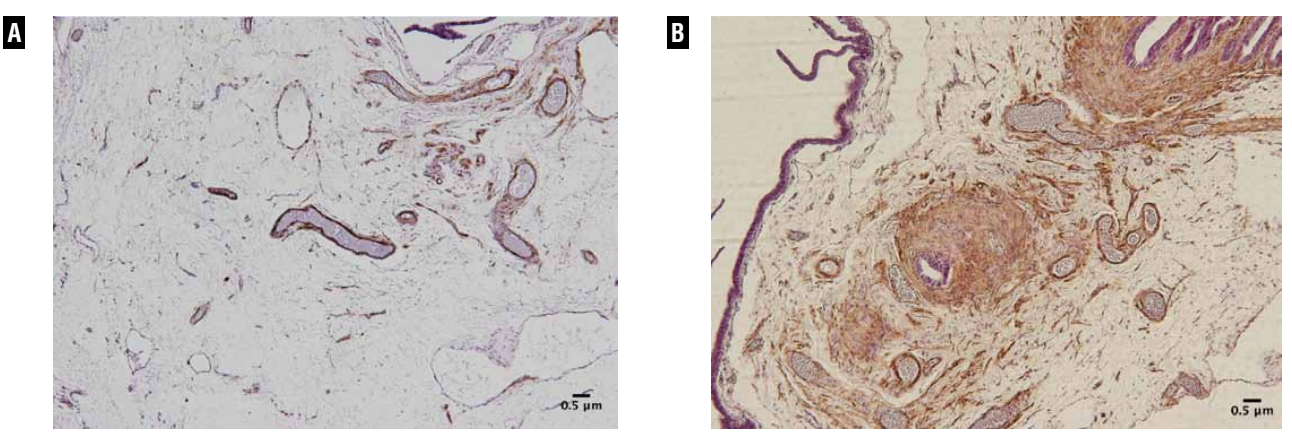
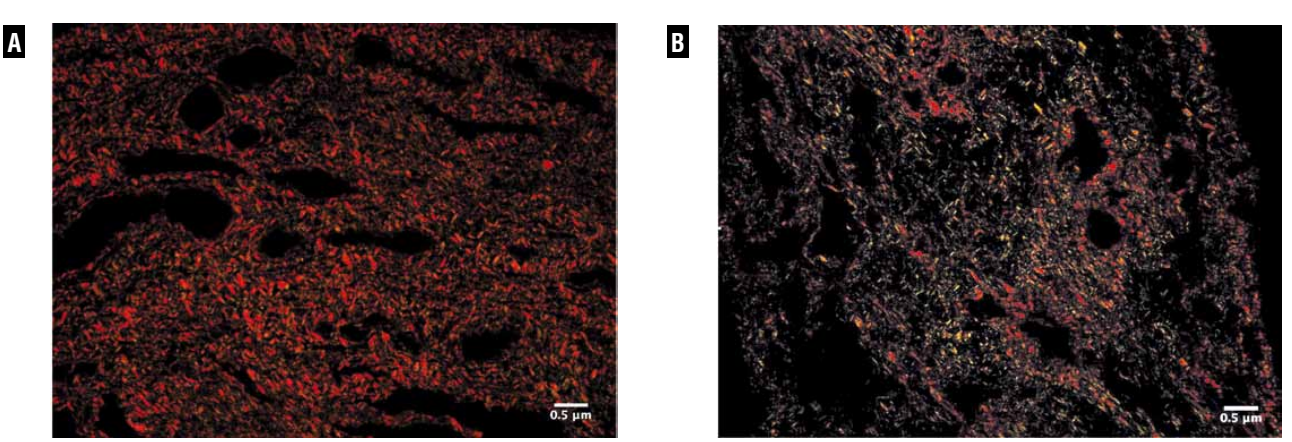


Figure 5 – Qualitative collagen distribution in testicular appendices. A) Testicular appendix of 3-year-old patient with cryptorchidism showing prevalence of yellowish color. Picrosirius-polarization method, X400. B) Testicular appendix of 11-years- old patient of the control group showing prevalence of greenish color. Picrosirius- polarization method, X400.



anatomical anomalies and the higher incidence of tumors (3,15). Cryptorchidism is one of the most common congenital anomalies among males, with a rate between 2 and 5% of full-term births, a rate that can reach 30% in premature babies (16-18). There are few studies in the literature on the incidence and analysis of the structure of testicular appendices in patients with cryptorchidism and their relation with epididymal anomalies and patency of the vaginal process (11).

Previous studies have shown that testicular appendices occurs significantly less often in patients with cryptorchidism. This can indicate a possible role of testicular appendices in the testicular migration process (19). In the present study, 61.1% of the testes with cryptorchidism presented testicular appendices, a much higher rate than the 24% reported by Jozsa (19). We did not find a significant difference in the number of appendices in the testis with cryptorchidism in relation to those of the control group, and also did not find a significant difference in the incidence of appendices in relation to the testicular position in the patients with cryptorchidism.

Cryptorchidism can be associated with various anatomical anomalies, but epididymal anomalies and patency of the vaginal process are among the most frequent (20-22). Epididymal anomalies are associated with cryptorchidism, with prevalence that ranges from 36 to 79% (20,21). In this study we found 27.7% of the testes with cryptorchidism also had epididymal anomalies and 56.9% had patency of the vaginal process. Of the 44 testes with appendices, 68.18% had patency of the vaginal process and 25% presented epididymal anomalies. There was no significant difference between the incidence of epididymal anomalies or patency of the vaginal process in the patients with cryptorchidism with and without appendices.

Previous studies have shown that the testicular appendices of patients with cryptorchidism show significant alterations in the estrogen and androgen receptors (19,23). Patients with hydroceles also present alteration in the expression of androgen receptors and epithelial destruction in the testicular appendices (19). These alterations are argued to be associated with increased hydrostatic pressure in the scrotum of patients with hydroceles (19,23).

Reports of structural and ultrastructural changes in the testicular appendices of patients with cryptorchidism are rare (10,19). In this study we did not observe any alterations in the epithelium of the testicular and epididymal appendices between the patients with cryptorchidism and those in the control group, where the majority of cases were made up of patients with hydroceles. We found a large quantity of veins and connective tissue in both the testicular and epididymal appendices. These findings are in agreement with those of Sahni et al. (10) and can confirm the theory that the epithelial layer of the appendices along with the subepithelial capillaries and lymphatic vessels form a functional unit (8,19).

In our samples, we observed the presence of elastic fibers in the appendices, more so in those of patients with cryptorchidism. The increased synthesis of elastic fibers may be associated with excessive distension of an organ (24). We also observed a decrease in the quantity of smooth muscle fibers and predominance of type III collagen in the patients with cryptorchidism. We can speculate that the smooth muscle fiber reduction is the primary event in fibrotic tissue formation. This event is probably correlated to a hydrodistension process. It has been variously shown that a shift towards greenish color in the Picrosirius polarization method is associated with less organized and/or degraded collagen (25). This color change can also occur in earlier phases of the remodeling and repair of connective tissues, when the synthesis of type III collagen is enhanced. Thus, based on these findings, the results of the Picrosirius-polarization method suggest that collagen matrix at the testicular appendices in patients with cryptorchidism is disrupted or degraded, rather than fibrotic, which is consistent with higher hydrostatic pressure. These findings lead us to suggest that in testes without complete migration, testicular appendices may result from the higher intra- abdominal pressure to which these testes are exposed.

In conclusion, we did not find a difference in the incidence of testicular appendices in relation to the testicular position in the patients with cryptorchidism. There also was no increased incidence of anatomical anomalies associated with the testes containing appendices. The testicular appendices showed a significant structural alteration in the pa-

tients with cryptorchidism: although the epithelium was not changed, the testicular appendices of the patients with cryptorchidism had a larger quantity of elastic fibers and smaller quantity of smooth muscle cells and predominance of type III collagen, remodeling in patients with cryptorchidism.

ACKNOWLEDGEMENT

By grants from the National Council for Scientific and Technological Development (CNPQ - Brazil) and the Rio de Janeiro State Research Foundation (FAPERJ), RJ, Brazil

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Johansen TE: Anatomy of the testis and epididymis in cryptorchidism. Andrologia. 1987; 19: 565-9.
2. Nöske HD, Kraus SW, Altinkilic BM, Weidner W: Historical milestones regarding torsion of the scrotal organs. J Urol. 1998; 159: 13-6.
3. Rolnick D, Kawanoue S, Szanto P, Bush IM: Anatomical incidence of testicular appendages. J Urol. 1968; 100: 755-6.
4. Vermeulen CW, Hagerty CS: Torsion of the appendix testis (hydatid of Morgagni) report of two cases with a study of the microscopic anatomy. J Urol. 1945; 54: 459-65.
5. Krukowski ZH, Auld CD: Torsion of the appendix epididymis in a maldescended testis. Br J Urol. 1983; 55: 244-5.
6. Grosfeld JL, O'neil JA, Coran AG, Fonkalsrud EW: Undescended Testis, Torsion and Varicocele; In: Pediatric Surgery 6th (ed.), Chapter 75. Mosby. 2006; pp. 1193-214.
7. Józsa T, Csízy I, Kutasy B, Cserni T, Flaskó T: Decreased incidence of appendix testis in cryptorchidism with intraoperative survey. Urol Int. 2008; 80: 317-20.
8. Posinovec J: Is the unstalked hydatid a functioning organ?. Verh Anat Ges. 1969; 63: 751-9.
9. Jacob M, Barteczko K: Contribution to the origin and development of the appendices of the testis and epididymis in humans. Anat Embryol (Berl). 2005; 209: 287-302.
10. Sahni D, Jit I, Joshi K, Sanjeev: Incidence and structure of the appendices of the testis and epididymis. J Anat. 1996; 189: 341-8.
11. Favorito LA, Cavalcante AG, Babinski MA: Study on the incidence of testicular and epididymal appendages in patients with cryptorchidism. Int Braz J Urol. 2004; 30: 49-52.
12. Turek PJ, Ewalt DH, Snyder HM 3rd, Duckett JW: Normal epididymal anatomy in boys. J Urol. 1994; 151: 726-7.
13. Favorito LA, Sampaio FJ: Anatomical relationships between testis and epididymis during the fetal period in humans (10-36 weeks postconception). Eur Urol. 1998; 33: 121-3.
14. Mandarim-de-Lacerda CA, Fernandes-Santos C, Aguila MB: Image analysis and quantitative morphology. Methods Mol Biol. 2010; 611: 211-25.
15. Sundarasivarao D: The Müllerian vestiges and benign epithelial tumours of the epididymis. J Pathol Bacteriol. 1953; 66: 417-32.
16. Kogan S, Hadziselimovic F, Howards SS, Snyder III HM, Huff D: Pediatric andrology. In: Adult and pediatric urology, 3rd (ed.), St Louis. Mosby Year Book. 1996.
17. Gill B, Kogan S: Cryptorchidism. Current concepts. Pediatr Clin North Am. 1997; 44: 1211-27.
18. Cendron M, Huff DS, Keating MA, Snyder HM 3rd, Duckett JW: Anatomical, morphological and volumetric analysis: a review of 759 cases of testicular maldescent. J Urol. 1993; 149: 570-3.
19. Józsa T, Dienes B, Telek A, Hargitai Z, Pór A, Kiss C: Differential expression of androgen and estrogen receptor of appendix testis in patients with descended and undescended testes. Int J Urol. 2008; 15: 171-4.
20. Marshall FF: Anomalies associated with cryptorchidism. Urol Clin North Am. 1982; 9: 339-47.
21. Mollaeian M, Mehrabi V, Elahi B: Significance of epididymal and ductal anomalies associated with undescended testis: study in 652 cases. Urology. 1994; 43: 857-60.
22. Barthold JS, Redman JF: Association of epididymal anomalies with patent processus vaginalis in hernia, hydrocele and cryptorchidism. J Urol. 1996; 156: 2054-6.
23. Samnakay N, Cohen RJ, Orford J, King PA, Davies RJ: Androgen and oestrogen receptor status of the human appendix testis. Pediatr Surg Int. 2003; 19: 520-4.
24. Cortivo R, Pagano F, Passerini G, Abatangelo G, Castellani I: Elastin and collagen in the normal and obstructed urinary bladder. Br J Urol. 1981; 53: 134-7.
25. Ushiki T: Collagen fibers, reticular fibers and elastic fibers. A comprehensive understanding from a morphological viewpoint. Arch Histol Cytol. 2002; 65: 109-26.

Correspondence address:
Dr. Luciano Alves Favorito
Rua Professor Gabizo 104/201
Tijuca, Rio de Janeiro, RJ, 20271-062, Brazil
Fax number: + 55 21 3872-8802
E-mail: lufavorito@yahoo.com.br

ORIGINAL ARTICLE

Vol. 39 (2): 248-256, March - April, 2013
doi: 10.1590/S1677-5538.IBJU.2013.02.14

Analysis of ureteral length in adult cadavers

Hugo F. F. Novaes, Paula C. S. Leite, Rafaela A. Almeida, Ney C. B. Sorte, Ubirajara Barroso Júnior

Urology Department, Fundação para o Desenvolvimento da Ciência. Escola Bahiana de Medicina e Saúde Pública, Bahia, Brazil

ABSTRACT

Introduction: In some occasions, correlations between human structures can help planning surgical intra-abdominal interventions. The previous determination of ureteral length helps pre-operatory planning of surgeries, reduces costs of auxiliary exams, the correct choice of double-J catheter with low morbidity and fewer symptoms, and an adequate adhesion to treatment.

Objective: To evaluate ureteral length in adult cadavers and to analyze its correlation with anthropometric measures.

Materials and Methods: From April 2009 to January 2012 we determined ureteral length of adult cadavers submitted to necropsy and obtained the following measures: height, distance from shoulder to wrist, elbow-wrist, xiphoid appendix-umbilicus, umbilicus--pubis, xiphoid appendix-pubis and between iliac spines. We analyzed the correlations between ureteral length and those anthropometric measures.

Results: We dissected 115 ureters from 115 adult corpses from April 2009 to January 2012. Median ureteral length didn't vary between sexes or according to height. It was observed no correlation among ureteral length and all considered anthropometric measures in all analyzed subgroups and in general population. There were no significant differences between right and left ureteral measures.

Conclusions: There is no difference of ureteral length in relation to height or gender (male or female). There is no significant correlation among ureteral length and the considered anthropometric measures.

ARTICLE INFO

Key words:
Ureter; Body Height; Stents; Human Engineering; Cadaver

Int Braz J Urol. 2013; 39: 248-56

Submitted for publication:
August 22, 2012

Accepted after revision:
December 21, 2012

INTRODUCTION

The search of human body proportionality is ancient and has not ended. The first recorded observation of human body proportions was made by the roman architect Vitruvius, posteriorly disseminated by Leonardo da Vinci's masterpiece "The Vitruvian man" (1).

In some occasions, correlations between different human structures could help planning intra-abdominal surgeries. In the era of minimally invasive surgeries, the previous knowledge of ureteral length allows a correct planning of a reconstructive surgery or ureteral reimplantation, reducing costs, without the need of invasive subsidiary exams, such as cystoscopy or radiological profiles (for example, intravenous pyelography or computerized tomography). The correct choice of the length of a double-J catheter reduces symptoms and morbidity (2-7) and one of the determinants is the catheter length (3,8,9). A very long catheter can cause irritative symptoms, while a short one allows migration (3,8,10,11).

Some authors searched for correlations between ureteral length and anthropomorphic measures (2,3,10,12) and some have found poor

correlations with no clinical use (2,12), while others haven't found any (3,10). Such studies were conducted using image or endoscopic methods.

There are no Brazilian data about ureteral length or studies about correlations between ureteral and anthropometric measures using cadavers. So we decided to dissect ureters of human cadavers submitted to necropsies at the medical legal institute, and determined their lengths and correlations with some anthropometric measures. According to our knowledge, this is the first Brazilian study that correlates ureteral length with anthropometric data and the first in medical literature to determine these measures in cadavers (the measurement is more accurate than other methods being used so far).

MATERIALS AND METHODS

This is a transverse study that evaluates the length of ureters (point of interest) and other anthropometric measures (predictors) in one particular moment. Data were collected from April 2009 to January 2012 and were obtained from the dissection of cadaveric ureters of patients with more than 16 years old, submitted to necropsy at the Medical Legal Institute Nina Rodrigues, in Salvador, Bahia.

The necropsied cadavers (with violent or natural cause of death) were submitted to retroperitoneal dissection (Figure-1), separating and removing the kidney and corresponding ureter with its bladder insertion (Figure-2). Renal pelvis

Figure 1 - Retroperitoneum.

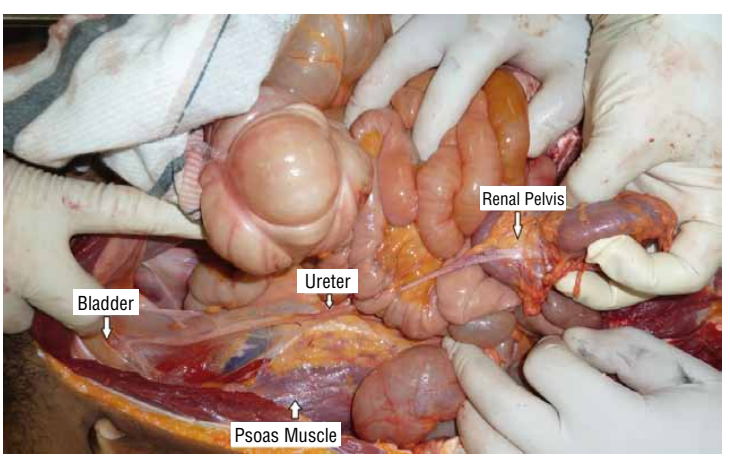
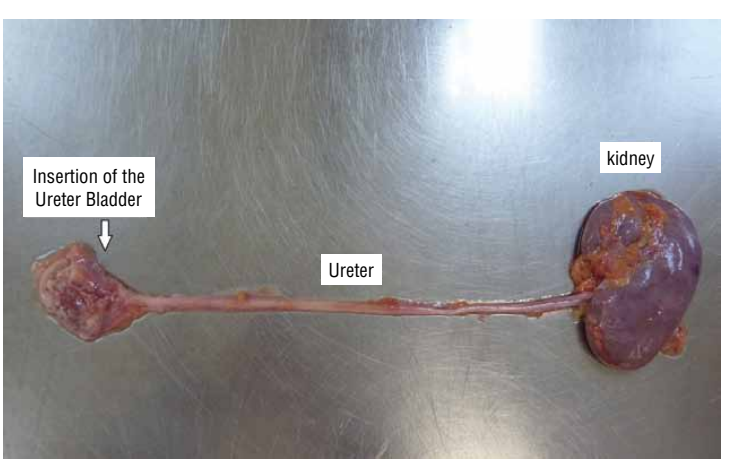


Figure 2 - Kidney, ureter and bladder cuff.



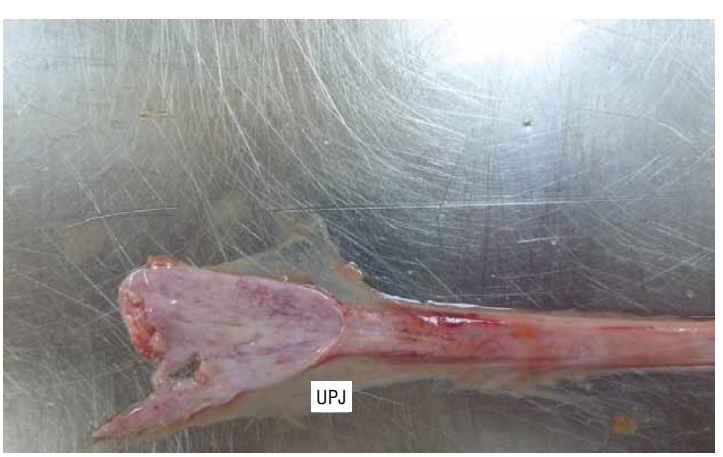
was detached from the renal parenchyma and the whole structure (renal pelvis, ureter and bladder insertion) was rectified at the bench (Figure-3). The ureter was delimited by the ureteral-pielic junction (UPJ) and ureteral-bladder junction (UBV). UPJ was identified at the point of proximal enlargement of the ureter and the UBV at the point of distal enlargement. In case of doubt, the extremities were opened longitudinally to help identify these points (Figure-4). The dissection was done bilaterally and at the bench the structure was rectified in order to measure the ureteral length using a millimetric scale with 5 mm gradation.

The following measures were also obtained: distance from shoulder to wrist (acromial process-head of the ulna), elbow-wrist (olecranon-head of the ulna), xiphoid appendix-umbilicus, umbilicus-pubis, xiphoid appendix-pubis and between iliac spines. The cadavers were randomly chosen, and those with primary or secondary ureteral diseases leading to anatomic distortion (megaureter, neoplasms, adhesions, duplicity), ureteral trauma or previous ureteral surgery (confirmed or suspected) or any evident ureteral disease were excluded.

Calculation of the sample was made using the software BioEstat 5.0, based on the data of Jeon et al. (4), that described a correlation between height and ureteral length with a correlation coefficient r = 0.369. With a statistical power of

Figure 3 - Ureter (red arrows indicate UPJ at left and UBJ at right).

Figure 4 - UPJ after longitudinal incision.



90% with a type error I of 5%, it was verified the need of a sample of 83 ureters.

For statistical analysis, the sample was categorized according to gender (male and female) and height (subgroup H1: cadavers with less than 165 cm height, subgroup H2: height from 166 to 175 cm; and subgroup H3: height equal or superior to 176 cm). Student t test was used for the analysis of the ureteral length according to gender. For analysis of ureteral length according to height subgroups, it was used analysis of variance (ANOVA).

Pearson correlation was used to analyze the correlation between ureteral length and the anthropometric measures. Multiple linear regression was used to evaluate the possibility of obtaining a predictor equation of ureteral length. Backward strategy was used to determine the predictive variables of the final model. In order to correct the possible effects of extreme values, it was obtained correlation and regression analysis of all values of ureteral lengths and to all values that, in a normal standardized scale, had z scores of -1.96 to 1.96.

P values lower than 5% ($p < 0.05$) were considered significant. All analysis were made using SPSS software version 14.0.

This study was approved by the Medical Ethical Committee of our institution and by the scientific board of the medical legal institute.

RESULTS

The ureters of 115 adult cadavers were dissected, with ages equal or superior to 16 years old, from April 2009 to January 2012. Twenty two were female and 93 male (1 female: 4.22 male). Median female age (standard deviation) was 51.48 (± 13.63) and 38.95 (± 19.0) male ($p = 0.002$). Median (standard deviation) height was 159.5 (± 6.36) for females and 171.59 (± 7.42) for males ($p \leq 0.001$) (Table-1).

Table 1 - Different aspects of the studied population.

	Female Median (sd)	Male Median (sd)	P
Age	51.48 (± 13.63)	38.95 (± 18.99)	< 0.001
Height	159.50 (± 6.36)	171.59 (± 7.42)	< 0.001
SW distance	52.68 (± 2.76)	57.57 (± 4.21)	< 0.001
SE distance	28.47 (± 2.04)	31.07 (± 3.28)	< 0.001
EW distance	24.20 (± 1.68)	26.49 (± 1.76)	< 0.001
XU distance	19.40 (± 3.09)	20.02 (± 3.45)	0.546
UP distance	16.63 (± 2.28)	17.05 (± 2.39)	0.635
XP distance	36.04 (± 3.45)	36.38 (± 5.01)	0.618
IS-IS	25.45 (± 2.97)	23.30 (± 2.62)	0.004
Ureteral length	26.73 (± 4.84)	25.05 (± 3.27)	0.135

SW: Shoulder-wrist; SE: Shoulder-elbow; **EW:** Elbow-wrist; XU: xiphoid appendix-umbilicus; **UP:** umbilicus-pubis; **XP:** xiphoid appendix-pubis; **IS-IS:** between antero-superior iliac spines.

Medial ureteral length (standard deviation) was 25.36 (± 3.67) cm. When we analyzed ureteral length according to gender, there was no significant differences (p = 0.135), although female ureters presented a median length (sd) of 26.73 (± 4.85) and male ureters 25.05 (± 3.26) cm (Table-1).

When we analyzed the height subgroups, there were also no statistical differences of ureteral length: medial length (sd) of subgroups H1, H2 and H3 were respectively 25.09 (± 4.88) cm, 25.51 (± 2.99) cm and 25.46 (± 3.07) cm (p = 0.860).

Among 115 analyzed cadavers, there were only 7 with different contralateral measures of ureters, varying from 1.5 to 6.5 cm. In these cases, left ureter was longer than the contralateral in three corpses. No pathologies were identified in these 7 cadavers.

There were no correlations between ureteral length and anthropometric measures in general population (Table-2). When the sample was categorized according to gender, there was also no correlation (Table-2). There were low correlations in some analysis of the population categorized according to height. Among the 34 cadavers of subgroup H1, ureteral length correlated with the shoulder-elbow distance, with a Pearson index of -0.368 (p = 0.038). In subgroup H2, there was also a low correlation between height and ureteral length (Pearson index -0.337 and p = 0.013). In subgroup H3, it was identified a correlation between height and ureteral left length, with Pearson index of -0.292 and p = 0.042 (Table-3). And finally, when we categorized the population simultaneously according to gender and height, there were no significant correlations (Tables 4 and 5).

DISCUSSION

Median ureteral length was 14 to 37 cm (median 25.36 ± 3.67 cm) in accordance to literature (16 to 35 cm) (2-4,8,10). There was no statistical significant difference between men (25.05 ± 3.27 cm) and women (26.73 ± 4.84 cm) (p = 0.135), although median height of male population was bigger (171.59 ± 7.42 cm male and 159.50 ± 6.36 cm female) (p < 0.001). The cause of this finding is unknown, but probably due to the larger pelvis of women, with a longer ureteral path. There

Table 2 - Correlations between ureteral length and several anthropometric measures.

	General population Pearson (p value)	Male Pearson (p value)	Female Pearson (p value)
Height	-0.012 (0.900)	0.124 (0.244)	0.090 (0.689)
Shoulder-wrist distance	- 0.098 (0.310)	0.002 (0.982)	-0.164 (0.466)
Shoulder-elbow distance	-0.118 (0.221)	-0.029 (0.786)	-0.266 (0.231)
Elbow-wrist distance	-0.025 (0.794)	0.061 (0.575)	0.054 (0.812)
Xiphoid appendix-umbilicus distance	-0.079 (0.414)	-0.078 (0.470)	-0.049 (0.827)
Umbilicus-pubis distance	0.111 (0.249)	-0.084 (0.438)	-0.157 (0.484)
Xiphoid appendix-pubis distance	-0.113 (0.241)	-0.109 (0.317)	-0.149 (0.509)
Iliac spines distance	0.172 (0.073)	0.111 (0.303)	0.180 (0.423)

Table 3 - Correlations between ureteral length and other anthropometric measures in general population according to height.

	H1 Pearson (p value)	H2 Pearson (p value)	H3 Pearson (p value)
Height	-0.119 (0.496)	-0.337 (0.013)	0.329 (0.116)
Shoulder-wrist distance	-0.316 (0.073)	-0.058 (0.683)	0.127 (0.562)
Shoulder-elbow distance	-0.363 (0.038)	-0.060 (0.672)	0.117 (0.596)
Elbow-wrist distance	-0.137 (0.447)	-0.012 (0.931)	0.119 (0.588)
Xiphoid appendix-umbilicus distance	-0.096 (0.594)	-0.237 (0.090)	0.118 (0.593)
Umbilicus-pubis distance	-0.096 (0.595)	0.034 (0.811)	-0.429 (0.046)
Xiphoid appendix-pubis distance	-0.144 (0.424)	-0.127 (0.369)	0.053 (0.814)
Iliac spines distance	0.249 (0.163)	0.088 (0.537)	0.178 (0.418)

Obs: The indicated values in the table refer to Pearson coefficients and the significance of parametrical analysis (). **H1:** Cadavers with height equal or inferior to 165 cm; **H2:** cadavers with height from 166 to 175 cm; **H3:** cadavers with height equal or superior to 176 cm.

Table 4 - Correlations between ureteral length and other anthropometric measures in male population according to height.

	H1 Pearson (p value)	H2 Pearson (p value)	H3 Pearson (p value)
Height	-0.124 (0.625)	-0.322 (0.024)	0.329 (0.116)
Shoulder-wrist distance	-0.258 (0.335)	-0.041 (0.783)	0.127 (0.562)
Shoulder-elbow distance	-0.162 (0.548)	-0.059 (0.693)	0.117 (0.596)
Elbow-wrist distance	-0.334 (0.207)	-0.030 (0.840)	0.119 (0.588)
Xiphoid appendix-umbilicus distance	-0.295 (0.267)	-0.268 (0.069)	0.118 (0.593)
Umbilicus-pubis distance	-0.089 (0.744)	0.029 (0.847)	-0.429 (0.046)
Xiphoid appendix-pubis distance	-0.108 (0.690)	-0.154 (0.303)	0.053 (0.814)
Iliac spines distance	0.227 (0.398)	0.002 (0.987)	0.178 (0.418)

Obs: The indicated values in the table refer to Pearson coefficients and the significance of parametrical analysis (). **H1:** Cadavers with height equal or inferior to 165 cm; **H2:** cadavers with height from 166 to 175 cm; **H3:** cadavers with height equal or superior to 176 cm.

Table 5 - Correlations between ureteral length and other anthropometric measures in the female population according to height.

	H1 Pearson (p value)	H2 Pearson (p value)
Height	-0.017 (0.948)	-0.526 (0.362)
Shoulder-wrist distance	-0.273 (0.288)	0.103 (0.870)
Shoulder-elbow distance	-0.477 (0.053)	0.158 (0.800)
Elbow-wrist distance	-0.019 (0.942)	-0.395 (0.511)
Xiphoid appendix-umbilicus distance	-0.011 (0.966)	0.229 (0.710)
Umbilicus-pubis distance	-0.145 (0.578)	-0.158 (0.800)
Xiphoid appendix-pubis distance	-0.121 (0.643)	0.103 (0.870)
Iliac spines distance	0.112 (0.668)	0.289 (0.637)

Obs: The indicated values in the table refer to Pearson coefficients and the significance of parametrical analysis (). **H1:** Cadavers with height equal or inferior to 165 cm; **H2:** cadavers with height from 166 to 175 cm; **H3:** cadavers with height equal or superior to 176 cm.

was also no statistical difference between distances in both sexes (xiphoid appendix-pubis (p = 0.618), xiphoid appendix-umbilicus (p = 0.546) and umbilicus-pubis (p = 0.635) independently of the subgroups of height, suggesting that the abdominal length of ureter doesn't alter according to gender or subgroup of height, and therefore, the distance between the kidney and the bladder may also not vary.

There was also no statistical difference of ureteral length according to the subgroups of height. Median ureteral length in subgroup H1 (height equal or lower than 165 cm) was 25.09 ± 4.88 cm, in subgroup H2 (height from 166 to 175 cm) 25.51 ± 2.99 cm and in subgroup H3 (height equal or superior to 176 cm) was 25.46 ± 3.07 cm (p = 0.860). Accordingly, we observed that correlation between height and abdominal length (represented by the distances from xiphoid appendix-umbilicus, umbilicus-pubis, xiphoid appendix-pubis) is low (r = 0.282 with p = 0.003; r = 0.295 with p = 0.002; and r = 0.227 with p = 0.019, respectively), disqualifying the affirmative that higher people have longer ureters. So, the choice of a double-J catheter should not be based upon height and is recommended the use of a median length catheter to all population.

In only 7 of a total of 115 necropsied cadavers there were differences between length of right and left ureters. Hruby et al. (2) analyzed 100 patients with endoscopy and also didn't find any difference, while Paick et al. (3) found 1 cm difference between left and right ureters in a sample of 203 patients. The 5 mm scale used in our work could justify the lack of observance of small differences between contralateral units. Another difficulty to compare data with living beings is related to the way of obtaining the measures. In vivo, ureters can contract or dilate according to manipulation, and image exams may not reflect the correct measure of the ureteral length.

There was no significant correlation between ureteral length and the studied anthropometric measures, in general population or according to gender or height. Hruby et al. (2) in a similar study used endoscopy and radioscopy to measure ureteral length and found a weak correlation with height, xiphoid appendix-pubis distance and

elbow-wrist distance, and established an equation that predicts ureteral length in only 26% (2). Breau et al. (12) and Jeon et al. (4) also reported weak correlations between height and ureteral length. Shah et al. (10) and Pilcher et al. (8) found no correlations between height and ureteral length. In view of these facts, this study reaffirms previous works regarding the lack of correlation between different segments of the human body and the ureteral length, making impossible the proposal of a predictive formula. The absence of correlation between height and ureteral length would also explain the absence of difference of ureteral length according to different heights.

Among all limitations of this study, we point out that the sample was a convenient one, that represented the black population with low income of Salvador (except for one white cadaver, all others were black or brown). Brazil has a vast territorial extension, with a very heterogeneous population with African, European and Asiatic descendents. And these differences are more evident in different regions of the country, with different median heights. Low income populations are more prone to deficits of physical development, with lower heights. However, since it was not found any correlation between height and all other anthropometric measures, probably this bias did not interfere the results.

CONCLUSIONS

There are no significant statistical differences in median ureteral length according to different genders or heights; median length is 25.36 (± 3.67) cm.

There is not a significant correlation between ureteral length and all evaluated segments of human body, making impossible the prediction of the length of the ureter accordingly.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Schott GD: The extent of man from Vitruvius to Marfan. Lancet. 1992; 340: 1518-20.
2. Hruby GW, Ames CD, Yan Y, Monga M, Landman J: Correlation of ureteric length with anthropometric variables of surface body habitus. BJU Int. 2007; 99: 1119-22.
3. Paick SH, Park HK, Byun SS, Oh SJ, Kim HH: Direct ureteric length measurement from intravenous pyelography: does height represent ureteric length? Urol Res. 2005; 33: 199-202.
4. Jeon SS, Choi YS, Hong JH: Determination of ideal stent length for endourologic surgery. J Endourol. 2007; 21: 906-10.
5. Ho CH, Huang KH, Chen SC, Pu YS, Liu SP, Yu HJ: Choosing the ideal length of a double-pigtail ureteral stent according to body height: study based on a Chinese population. Urol Int. 2009; 83: 70-4.
6. Lee BK, Paick SH, Park HK, Kim HG, Lho YS: Is a 22 cm Ureteric Stent Appropriate for Korean Patients Smaller than 175 cm in Height? Korean J Urol. 2010; 51: 642-6.
7. Kawahara T, Ito H, Terao H, Yoshida M, Ogawa T, Uemura H, et al.: Choosing an appropriate length of loop type ureteral stent using direct ureteral length measurement. Urol Int. 2012; 88: 48-53.
8. Pilcher JM, Patel U: Choosing the correct length of ureteric stent: a formula based on the patient's height compared with direct ureteric measurement. Clin Radiol. 2002; 57: 59-62.
9. Ho CH, Chen SC, Chung SD, Lee YJ, Chen J, Yu HJ, et al.: Determining the appropriate length of a double-pigtail ureteral stent by both stent configurations and related symptoms. J Endourol. 2008; 22: 1427-31.
10. Shah J, Kulkarni RP: Height does not predict ureteric length. Clin Radiol. 2005; 60: 812-4.
11. Slaton JW, Kropp KA: Proximal ureteral stent migration: an avoidable complication? J Urol. 1996; 155: 58-61.
12. Breau RH, Norman RW: Optimal prevention and management of proximal ureteral stent migration and remigration. J Urol. 2001; 166: 890-3.

Correspondence address:
Dr. Hugo F. F. Novaes
Urology Department,
Fundação para o Desenvolvimento da Ciência
Escola Bahiana de Medicina e Saúde Pública, Bahia, Brazil
Rua Clara Nunes, 543 / 702, Pituba
Salvador, Bahia, 41810-425, Brazil
E-mail: hugonovaesbr@yahoo.com.br

EDITORIAL COMMENT

The fact that few studies on the anatomy ureteral in the years fifty and seventy to reflect the originality of this article highlighted (1,2). As for its clinical applicability in adult surgical matter of finding not occur significant difference in mean length ureteral between different age and height between males and females, and the average length of 25.36 (± 3.67) cm. As the author rightly emphasizes the difficulty of comparing the data in the literature relates to the way of obtaining measurements, since in vivo, the ureter can contract and shorten as their handling, and imaging studies may not reflect the exact measurement ureteral length as most existing studies can cite as published by Chew et al., 2007 (3).

Remember important study in our country's existing anatomy of the ureteropelvic junction by Sampaio published in 1996 with great applicability to endopielotomia technique (4).

REFERENCES

1. Benjamin JA, Tobin CE: Abnormalities of the kidneys, ureters, and perinephric fascia: anatomic and clinical study. J Urol. 1951; 65: 715-33.
2. Wexler NS, Roberts JA: A correlation of anatomic and physiologic parameters in the ureter. Invest Urol. 1974; 11: 286-7.
3. Chew BH, Knudsen BE, Nott L, Pautler SE, Razvi H, Amann J, et al.: Pilot study of ureteral movement in stented patients: first step in understanding dynamic ureteral anatomy to improve stent comfort. J Endourol. 2007; 21: 1069-75.
4. Sampaio FJ: The dilemma of the crossing vessel at the ureteropelvic junction: precise anatomic study. J Endourol. 1996; 10: 411-5.

Dr. Maurício Hachul
Urologist
Rua Vergueiro 1855 / 114, Vila Mariana
Sao Paulo, SP, 04101-000, Brazil
Telephone: + 55 11 5579-0008
E-mail: hachul.mauricio@uol.com.br

ORIGINAL ARTICLE

Vol. 39 (2): 257-267, March - April, 2013
doi: 10.1590/S1677-5538.IBJU.2013.02.15

Genes responsible for vaginal extracellular matrix metabolism are modulated by women`s reproductive cycle and menopause

Oksana Shynlova, Maria A. T. Bortolini, May Alarab

Samuel Lunenfeld Research Institute, Mount Sinai Hospital (OS, MATB, MA); Division of Urogynecology and Reconstructive Pelvic Surgery, Mount Sinai Hospital, University of Toronto (MATB, MA) and Department of Obstetrics & Gynecology, University of Toronto (OS, MA), Toronto, Canada

ABSTRACT

Objectives: To analyze the expression of genes involved in extracellular matrix (ECM) biogenesis and remodeling in vaginal tissue of women with clinically normal pelvic floor support (defined as controls) according to the phase of menstrual cycle and postmenopausal women with and without pelvic organ prolapse (POP).

Materials and Methods: This study examined the expression of matrix metalloproteinases (MMPs), their tissue inhibitors (TIMPs), and the Lysyl oxidase (LOX) family genes in the anterior vaginal wall of Caucasian women by real-time RT-PCR. Initially, mRNA expression was assessed in premenopausal controls in the secretory (group 1, n = 10) vs. proliferative (group 2, n = 8) phase of menstrual cycle. In addition, we compared premenopausal controls in the proliferative phase (group 2) vs. postmenopausal controls (group 3, n = 5). Finally, we analyzed postmenopausal controls (group 3) vs. postmenopausal women with advanced POP (group 4, n = 13).

Results: According to the phase of menstrual cycle, MMP1 was significantly reduced ($p = 0.003$), whereas the expression of TIMP1 and LOXL4 was significantly up-regulated during proliferative phase (both $p < 0.01$) when compared to the secretory phase in premenopausal control women. Regarding menopausal status/ageing, all MMPs were down-regulated, while TIMP3, TIMP4 and LOXL2 were significantly up-regulated in postmenopausal control women when compared to premenopausal controls ($p = 0.005$, $p = 0.01$ and $p < 0.001$, correspondingly). TIMP4 and LOXL2 mRNA levels were significantly decreased in postmenopausal POP patients compared to asymptomatic postmenopausal controls ($p < 0.01$ for both).

Conclusions: Our results indicate that ovarian cycle and age-related changes influence the expression of genes encoding proteins responsible for ECM metabolism in human vagina. Moreover, POP is associated with alteration in vaginal ECM components after menopause.

ARTICLE INFO

Key words:
Tissue Inhibitor of Metalloproteinases; Menopause; Vagina; Metabolism; Genes

Int Braz J Urol. 2013; 39: 257-67

Submitted for publication:
May 23, 2012

Accepted after revision:
January 10, 2013

INTRODUCTION

Pelvic organ prolapse (POP) results from the failure of the pelvic floor support (1). Similar to stress urinary incontinence, POP is a serious health problem for women of all ages (2). Currently, POP affects almost half of all women older than 50 years and it is estimated that 11.1% of them will have prolapse or incontinence surgery by age of 80 (3).

Epidemiological studies have shown that race, parity, ageing and ovarian hormones depri-

vation after menopause are some of the risk factors for POP (4,5). Findings of estrogen receptors (ERs) in the structures that support the pelvic organs, including the vagina, corroborate for the ovarian hormones modulation of the pelvic floor (6,7). Molecular studies provide addition support for the association between ovarian hormones and POP. Serum estradiol levels and ER are significantly lower ($p < 0.01$) in the uterine ligaments of women who had POP than in controls without POP before menopause (8). Also, decreased levels of estrogen and progesterone receptors have been described in postmenopausal POP women compared to asymptomatic ones ($p < 0.0001$) (9).

Collagens and elastin are the two major extracellular matrix (ECM) components of the pelvic floor connective tissues, providing resistance to stretching and other tensile forces, and elasticity and resilience to the tissues, respectively. Cross-linking of tropoelastin and procollagen to form mature functional collagen and elastin fibers is performed by one or more members of the Lysyl oxidase (LOX) family of enzymes (10). On the other hand, collagen is degraded by a family of Metalloproteinases (MMPs) which are regulated by theirs tissue inhibitors (TIMPs) (11). There is a general agreement that pelvic floor tissues of patients with POP present decrease in the total collagen content, with higher rate of immature collagen more susceptible to rupture as compared with women without prolapse (12), and that deficiency of ECM components underlies POP. However, studies that have examined connective tissue content of prolapsed vaginal wall, collagen/elastin metabolism and remodelling (13,14) often produce discrepant data possibly due to the population heterogeneity regarding age, race and hormonal status.

In our previous studies enrolling a population controlled by hormonal status and ethnicity, we detected a significant reduction of LOX, LOX like 1 (LOXL1) and LOXL3 gene and protein levels in vaginal samples of Caucasian premenopausal patients with POP as compared to asymptomatic premenopausal women (15). In addition, the expression and the activity of MMPs were increased whereas the expression of all TIMPs was significantly decreased in samples of vaginal tissue taken from premenopausal women with POP compared to controls (16). It is plausible that deficient or defective ECM metabolism enzymes represent key factors responsible for the development of POP.

Hence, we hypothesize that physiological ovarian hormones variation modulates the expression of specific groups of enzymes responsible for the formation and degradation of collagen and/or elastin fibers in the human vagina. Based on our previous findings indicating that vaginal tissues from Caucasian premenopausal POP women show altered ECM metabolism (15,16), and that menopause-related ovarian hormones deprivation influences the genesis of POP in women (17), we also believe that similar or different changes can be found in POP women after the menopause. Therefore, the three objectives of the present study were to identify the expression pattern of MMPs, TIMPs and LOXs family genes (Table-1) in the anterior vaginal tissue of Caucasian (1) premenopausal women without POP according to the menstrual cycle phase (proliferative vs secretory) ,(2) to investigate the effect of age--related menopause changes on their expression by comparing premenopausal vs. postmenopausal women without POP. (3) In addition we examined the expression of MMPs, TIMPs and LOXs in the vaginal biopsies of postmenopausal women with and without severe POP.

MATERIALS AND METHODS

Patient's selection and Tissue Collection

Institutional Review Board of Mount Sinai Hospital, University of Toronto approved this study. We recruited Caucasian women undergoing vaginal hysterectomy for cervical prolapse equal or greater than stage III by POP-Q (18) classification as "patients", and women with POP-Q of stage 0 undergoing total abdominal hysterectomy for benign conditions other than POP as "controls" (Surgical indication in Table-2). We rationalized that stage 0 is the "gold standard" for normal pelvic support. Women with history of urogenital malignancy, endometriosis, connective tissue disorders, emphysema, previous pelvic surgery, and on estrogen and/or progestogen or steroid therapy were excluded. The initial gynecological exam

Table 1 - Simplified description of the main role of the studied ECM proteins.

Target Genes	Protein Functions
LOX	ECM Biogenesis: Maturation of collagen and elastin
LOXL1	ECM Biogenesis: Maturation of collagen and elastin
LOXL2	ECM Biogenesis: Maturation of collagen and elastin
LOXL3	ECM Biogenesis: Maturation of collagen and elastin
LOXL4	ECM Biogenesis: Maturation of collagen and elastin
MMP1	ECM Remodelling: Degradation of collagen and elastin
MMP2	ECM Remodelling: Degradation of collagen and elastin
MMP9	ECM Remodelling: Degradation of collagen and elastin
MMP14	ECM Remodelling: Degradation of collagen and elastin
TIMP1	ECM Remodelling: Modulation of MMPs
TIMP2	ECM Remodelling: Modulation of MMPs
TIMP3	ECM Remodelling: Modulation of MMPs
TIMP4	ECM Remodelling: Modulation of MMPs

was performed by the Urogynecology staff (MA), and by the Gynecology team at Mount Sinai Hospital during regular activities. One of the authors (MB or MA) obtained written informed consent, confirmed the POP staging of all participants, and collected clinical data a week before the surgical procedure. Demographic data included: age, bone mass index (BMI), parity, family history for POP (positive if mother or sister affected by POP) and presence of SUI (diagnosed by physical exam with stress test and/or urodynamic evaluation). The patients were examined in the lying position with a referred full-bladder, and asked to perform the cought test and further Valsalva maneuver. The descensus of the vaginal compartments were measured at the maximum straining point using a cm scale ruler. Total vaginal length was measured at rest under POP reduction with a vaginal speculum. Afterward, straining examination in the standing position confirmed the full extent of the POP. The participants were divided in 4 groups according to the hormonal status and presence of POP. Group 1: premenopausal control women in the secretory phase of menstrual cycle (n = 10); Group 2: premenopausal control women in the proliferative phase of menstrual cycle (n = 8); Group 3: postmenopausal control women (n = 5); Group 4: postmenopausal women with severe POP (n = 13). We considered women in the postmenopausal phase if they reported that their menstrual periods had stopped for more than a year, and as premenopausal if they were having regular periods over the preceding twelve months. The phase of menstrual cycle and the endometrial atrophy was confirmed by endometrial histology report of uterine specimens. After removal of the

Table 2 - Patients` demographics.

Study groups	Group 1 Premenopausal secretory controls	Group 2 Premenopausal proliferative controls	Group 3 Postmenopausal controls	Group 4 Postmenopausal POP patients
n	10	8	5	13
Mean Age	46.2 (± 3)	43.2 (± 4)	57 (± 8)†	67.5 (± 7.1)*
Mean BMI	31 (± 6.2)	26.8 (± 6.5)	27.6 (± 8.3)	26.21 (± 5.7)
Mean Parity	1.6 (1.4)	1.72 (0.3)	1.6 (1.3)	3.56 (1.6)*
SUI (%)	0	0	0	54% *
Family History of POP (%)	0	11%	0	54% *
Stage of POP (n, %)	Stage 0 (10)	Stage 0 (8)	Stage 0 (5)	III C (8, 61.5%)* IV C (5, 38.5%)
Indication for Hysterectomy	Fibroids (8) Adenomyosis (2)	Fibroids (8)	Endometrial Hyperplasia (3) Benign Complex Ovarian Cyst (2)	POP (13)

†indicates statistical difference between Group 2 and 3.
*indicates statistical difference between Group 3 and 4.

uterus, full thickness tissue specimen (approximately 1 cm^2) was obtained by sharp dissection down to the avascular space of loose areolar tissue of the vagina using Metzenbaum scissors. The dissected structure corresponds to the adventitia layer that separates the vaginal from the bladder muscularis (19). As easily torn during dissection, adventitia was excluded from our analysis. To account for variations in stretch conditions and muscularis thickness throughout the vaginal length (20), the site of tissue collection was standardized at the anterior middle portion of the vaginal vault for all study groups. The authors (MB or MA), not blinded for the samples status, immediately received the tissue biopsies from the surgeon in the operative room and further performed the biochemical assays under direct supervision of the senior author (OS). Vaginal biopsy samples were washed in ice-cold PBS, flash-frozen in liquid nitrogen and stored at -80° C for RT-PCR analysis.

Real Time-Polymerase Chain Reaction (PCR) Analysis

Reverse transcription (RT): RNA was extracted from the frozen tissues using TRIZOL (Gibco, Burlington, ON), column purified using RNeasy Mini Kit (Qiagen, Mississauga, Canada) and treated with 2.5µL DNase I (2.73 Kunitz unit/µL, Qiagen). 2µg of RNA was reverse transcribed into cDNA in a total reaction volume of 100µL using the TaqMan Reverse Transcription Kit (Applied Biosystems, CA, USA). To assess for genomic DNA contamination in the RNA samples, a "RT (-) control" was used.

Real-time PCR protocol: The primer sequences were generated through Primer Express 2.1 (ABI), verified for specificity by BLAST analyses and designed to span from two adjacent exons ((15) Table-3). 20ng of cDNA from the previous step was subjected to Real-time PCR in a total reaction volume of 20µL containing SYBR Green Master Mix (BioRad) using Eppendorf realplex

Table 3 - Real-time PCR primer sequences of a panel of genes studied and housekeeping genes.

Target Genes	Primer Sequences	GenBank accession #
LOX	Forward 5' – AGGCCACAAAGCAAGTTTCTG – 3' Reverse 5' – AACAGCCAGGACTCAATCCCT – 3'	NM_002317
LOXL1	Forward 5' –-CTGTGACTTCGGCAACCTCAA – 3' Reverse 5' – TGCACGTCGGTTATGTCGAT – 3'	NM_005576
LOXL2	Forward 5' – TCGAGGTTGCAGAATCCGATT – 3' Reverse 5' – TTCCGTCTCTTCGCTGAAGGA – 3'	NM_002318
LOXL3	Forward 5' – CGGATGTGAAGCCAGGAAACT- 3' Reverse 5' – AGGCATCACCAATGTGGCA – 3'	NM_032603
LOXL4	Forward 5' – ACCGGCATGACATTGATTGC – 3' Reverse 5' – CATCATACTTGCAGCGGCACT – 3'	NM_032211
MMP1	Forward 5' – TACGAATTTGCCGACAGAGATG– 3' Reverse 5'–GCCAAAGGAGCTGTAGATGTCC– 3'	NM_002421
MMP2	Forward 5' – GAATACCATCGAGACCATGCG– 3' Reverse 5'–CGAGCAAAGGCATCATCCA– 3'	NM_004530
MMP9	Forward 5' – CCTCGAACTTTGACAGCGACA– 3' Reverse 5'–AATGATCTAAGCCCAGCGCGT– 3'	NM_004994
MMP14	Forward 5' – TGCCATGCAGAAGTTTTACGG– 3' Reverse 5'– CCTTCGAACATTGGCCTTGAT– 3'	NM_004995
TIMP1	Forward 5' – TTCTGGCATCCTGTTGTTGCT– 3' Reverse 5'–CCTGATGACGAGGTCGGAATT– 3'	NM_003254
TIMP2	Forward 5' – GCGTTTTGCAATGCAGATGTAG– 3' Reverse 5'– TCTCAGGCCCTTTGAACATCTT– 3'	NM_003255
TIMP3	Forward 5' – CTGCTGACAGGTCGCGTCTA– 3' Reverse 5'–GCTGGTCCCACCTCTCCAC– 3'	NM_000362
TIMP4	Forward 5' – TCTGAACTGTGGCTGCCAAAT Reverse 5'–AGCTTTCGTTCCAACAGCCAG– 3'	NM_003256
ACTB	Forward 5'– ACCTTCAACACCCCAGCCATGTACG - 3' Reverse 5' –CTGATCCACATCTGCTGGAAGGTGG – 3'	NM_001101
SDHA	Forward 5' –TGGGAACAAGAGGGCATCTG– 3' Reverse 5' – CCACCACTGCATCAAATTCATG – 3'	NM_004168
YWHAZ	Forward 5'–ACTTTTGGTACATTGTGGCTTCAA – 3' Reverse 5' –CCGCCAGGACAAACCAGTAT – 3'	NM_003406
TBP	Forward 5' – TGCACAGGAGCCAAGAGTGAA – 3 Reverse 5' – CACATCACAGCTCCCCACCA – 3'	NM_003194

Mastercycler (Eppendorf, Hamburg, Germany). After PCR, a dissociation curve was constructed by increasing temperature from 65°C to 95°C to verify the specificity of PCR products. In addition, a no-template control (H_2O control) was analyzed for possible contamination in the master-mix. A cycle threshold (Ct) mean value was recorded for each sample. PCR reactions were set up in triplicates and the mean of the 3 Cts was calculated. Values obtained for each gene were normalized to the geometric mean of 3 housekeeping genes: TBP, SDHA, YWHAZ and/or ACTB (21). A comparative Ct method (ΔΔCT method) (22) was applied to the raw Ct values to find a relative gene expression. mRNA levels for premenopausal proliferative phase women were expressed relative to secretory phase levels, mRNA levels for postmenopausal control patients were expressed as fold changes relative to the premenopausal control levels or to POP patients expression levels. Validation experiments were performed to ensure that the PCR efficiencies between the target genes and the housekeeping genes were approximately equal.

Statistical analysis

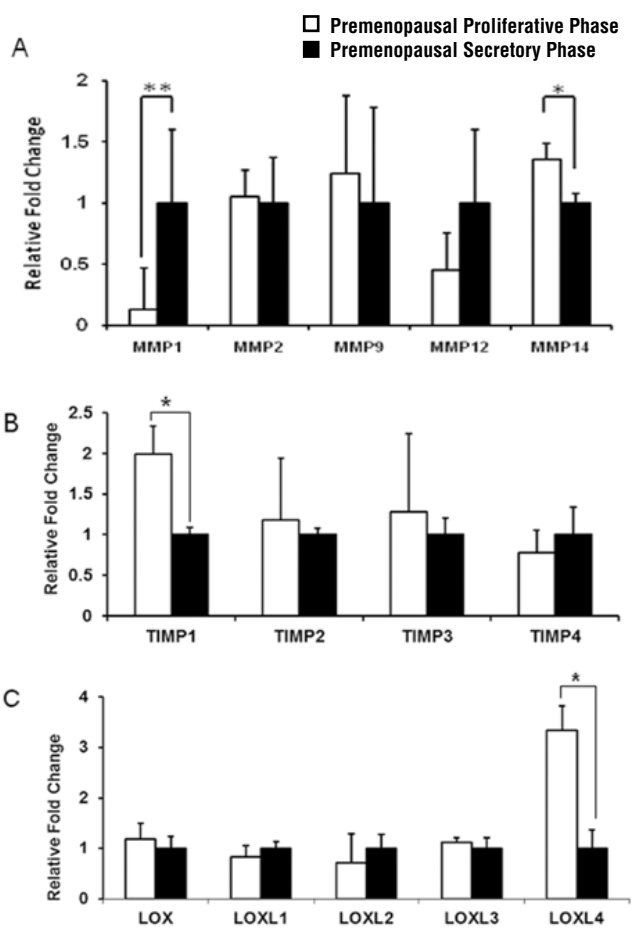
Unpaired comparison of the MMPs, TIMPs and LOX family gene expression in the study groups was performed using Wilcoxon rank-sum test (Prism version 4.02). Fisher's exact test was used to compare differences in the family history of POP and the incidence of SUI between the groups. The level of significance was set at $P < 0.05$. Results were reported as mean ± SEM.

RESULTS

Demographic data of four study groups population are summarized in Table-2. The groups were matched for race and BMI, however postmenopausal controls (Group 3) were older than premenopausal controls (Group 2) (57 vs. 43.2 years, respectively); and postmenopausal POP patients (Group 4) were significantly older than the controls (Group 3) (67.5 vs. 57 years, respectively) ($p < 0.05$ for both). Mean parity, family history of POP and incidence of SUI were significantly higher in patients when compared to controls in postmenopausal phases (Groups 3 and 4) ($P < 0.05$). In POP group, the majority of the women had stage III cervical prolapse.

To examine the effect of endocrine environment on ECM turnover in women with normal pelvic floor support, we first compared the expression of MMPs, TIMPs and LOXs (Table-1) between premenopausal groups (Groups 1 vs. 2, Figure-1). MMPs (MMP1, -2, -9, -12, -14), TIMPs (TIMP1, -2, -3, -4) and LOX (LOX, LOXL1, -2,-3,-4) family transcripts were expressed in all tissue samples.

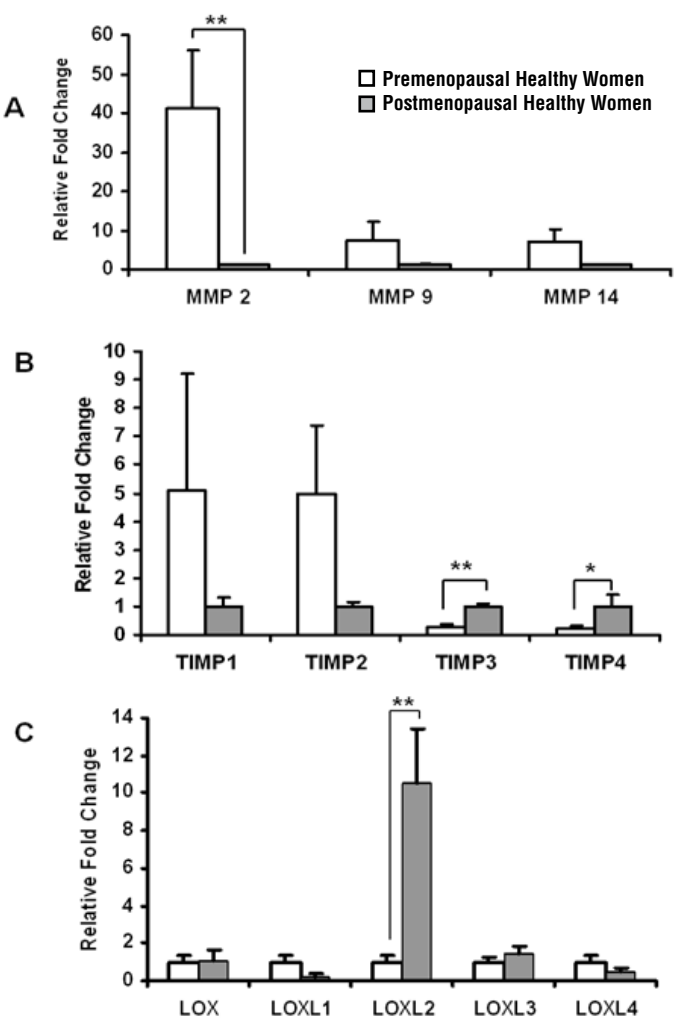
Figure 1 - ECM remodeling gene expression in vaginal tissue of premenopausal women. Real-time quantitative RT-PCR analyses were performed on total RNA to compare the level of expression of (A) MMPs, (B) TIMP1-4 and (C) LOX, LOXL1-4 in vaginal wall biopsy samples from premenopausal asymptomatic women in the proliferative phase (empty bars, n = 8) and the secretory phase of the menstrual cycle (black bars, n = 10). The results represent the mean ± S.E.M. A significant difference is indicated by * (p < 0.05).



MMP1 transcript levels were significantly decreased ($p < 0.05$), whereas MMP14 mRNA was significantly up-regulated ($p = 0.04$) (Figure-1A), TIMP1 mRNA was significantly higher ($p < 0.01$) (Figure-1B), and LOXL4 mRNA levels were significantly increased ($p < 0.01$) (Figure-1C) in vaginal samples collected from control women in the proliferative phase of their menstrual cycle as compared to the secretory phase.

We also examined the expression of the same genes in control premenopausal women compared to control postmenopausal women to explore the influence of advancing age and physiological ovarian hormones deprivation (Groups 2 vs. 3, Figure-2). The expression levels of all genes were reduced after menopause; moreover MMP1 and MMP12 mRNA were undetectable (Figure-2A). TIMP3 and TIMP4 were significantly elevated in the postmenopausal compared to the premenopausal women ($p = 0.005$ and 0.01, respectively) (Figure-2B). Interestingly, LOXL2 expression was 10 times higher in vaginal samples of postmenopausal compared to premenopausal control women (Groups 2 vs. 3) (Figure-2C).

Figure 2 - Expression of genes responsible for ECM metabolism in vaginal tissue of premenopausal and postmenopausal women. Real-time quantitative RT-PCR analysis of (A) MMP-2,-9,-14, (B) TIMP1-4 and (C) LOX, LOXL1-4 mRNA expression in vaginal samples from asymptomatic premenopausal (empty bars, n = 8) and postmenopausal women (thatched bars n = 5). The results represent the mean ± SEM. A significant difference is indicated by * (P < 0.05).



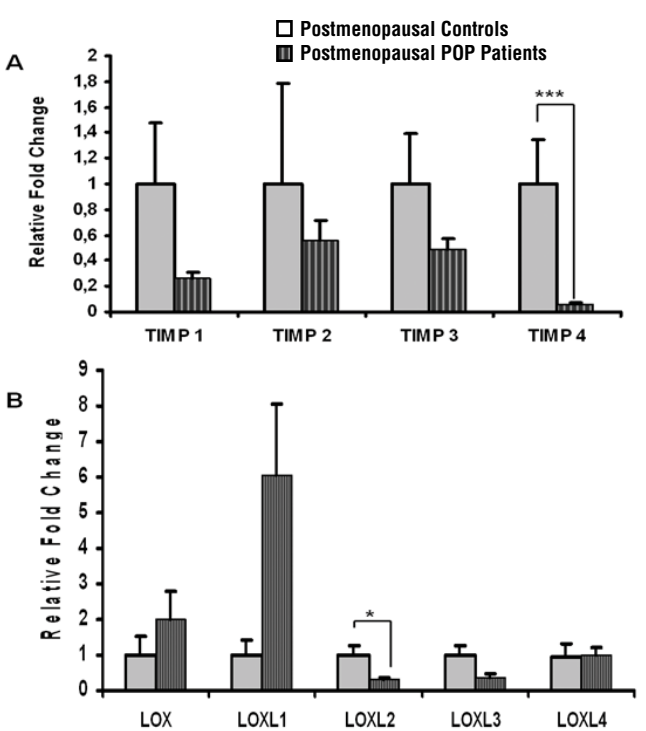
Our next goal was to compare the expression of MMPs, TIMPs and LOXs in postmenopausal POP patients versus postmenopausal asymptomatic controls (Group 3 vs. 4) (Figure-3). No MMPs expression was found in postmenopausal POP patients using the experimental conditions described in the Methods part of the manuscript. The quality of mRNA was confirmed by three different housekeeping genes. We also noticed that mRNA levels for all members of TIMP family were reduced in postmenopausal POP patients as compared to the asymptomatic postmenopausal controls, however only significantly for TIMP4 gene ($p = 0.002$) (Figure-3A). LOXL2 mRNA level was significantly reduced in postmenopausal patients with prolapse compared to postmenopausal control patients ($p = 0.004$). No significant difference between the two groups was observed for other LOX family genes (Groups 3 vs. 4) (Figure-3B).

DISCUSSION

POP is a disorder of multifactorial etiology (4,5). In our research, we tried to overcome some of the epidemiological biases often observed in POP studies by including a strictly homogeneous set of Caucasian women with particular hormonal status confirmed histologically.

Few data have shown that overall collagen metabolism is activated in the proliferative phase, while elastin metabolism changes are more pronounced in the secretory phase of the menstrual cycle (23,24). It is well described that reproductive organs are influenced by ovarian hormones during the menstrual cycle, pregnancy and menopause. The reproductive tract underwent cyclic remodelling in women's life span, with constant turnover of elastic and collagen fibres (25,26). In this study we have shown differential

Figure 3 - Analysis of TIMPs and LOX family gene expression in vaginal tissue of postmenopausal patients with severe POP and postmenopausal asymptomatic controls. Real-time quantitative RT-PCR analysis of (A) TIMP1-4 and (B) LOX, LOXL1-4 mRNA expression in vaginal samples from asymptomatic control patients (thatched bars, n = 5) and POP patients (vertical striped bars, n = 13). The results represent the mean ± SEM. A significant difference is indicated by * ($P < 0.05$).



expression of ECM remodelling genes MMP1 and TIPM1 from vaginal tissue samples of asymptomatic premenopausal women in the proliferative (estrogen dominated) phase compared to the secretory (estrogen/progestogen regulated) phase of the menstrual cycle. As TIMPs proteins are the major physiological inhibitors of MMPs, our data suggest that estrogen and progestogen may control vaginal collagen metabolism, and the estrogen environment is protective against collagen degradation via MMP1/TIMP1 homeostasis.

Our results are consistent with Chen and colleagues who showed an estrogen-mediated increase in TIMP1 expression in cultured fibroblasts from human vagina (27), as well as increased overall collagen metabolism during the proliferative phase of the menstrual cycle (18). Differently from MMP1 regulation, MMP14 gene expression was significantly increased in the proliferative versus the secretory phase of the menstrual cycle, suggesting that MMP14 expression might be regulated by estrogen. We also detected a significant increase in LOXL4 mRNA expression during the estrogen-dominant proliferative phase compared to the secretory phase. Although the information regarding hormonal modulation of human LOXL4 is scarce in the literature, in mouse vagina however the timing of LOXL4 elevation coincides with an increase in estrogen and a decrease of progesterone plasma concentration, supporting our results and pointing to the positive effect of estrogen on the expression of this gene (28). Taking together, we have concluded that genes responsible for vaginal ECM metabolism are modulated by the hormonal fluctuation during the menstrual cycle.

Ovarian hormones deficiency and ageing are also known to influence the quality of pelvic floor tissue. In accordance to our original hypothesis that physiological ovarian hormones variation can regulate ECM degrading proteins, we found that the expression of all MMPs studied was dramatically down-regulated in women after menopause. The transcripts for MMP1 and MMP12 were below the detection level of Real-time PCR instrument. It was also the reason we were not able to compare the expression of all proteinases in the postmenopausal POP group. As premenopausal women were significantly younger than postmenopausal women we speculated that advancing age has a major impact on the matrix-degrading enzymes. Surprisingly, we discovered that LOXL2 enzyme was significantly up-regulated in postmenopausal compared to premenopausal women without POP. Earlier report has confirmed an association of LOXL2 to Werner syndrome, which is characterized by premature aging (29), suggesting a potential connection between ageing and LOXL2 expression.

Positive family history of POP and the incidence of concomitant SUI were significantly higher in POP patients compared to controls, suggesting a possible familial inheritance of genes involved in the pathophysiology of this condition. Similar to what we previously found in premenopausal POP patients (16), we have shown that all TIMPs were decreased in vaginal biopsies of postmenopausal POP patients compared to controls indicating that the mechanism of their regulation is independent of menopausal status. Chen et al. detected a decrease of TIMP1, but not of TIMP2 and TIMP3 expression in vaginal tissue of pre- and postmenopausal patients with SUI and POP (14). Liang et al. however found the decrease in TIMP2 mRNA in uterosacral ligament but not in the cervical tissue of uterine prolapse women compared to controls (30). Our gene expression results reinforce other studies with regards to the reduction of TIMP1 in women with POP compared to asymptomatic controls (29).

We have previously described that the expression of LOX, LOXL1 and LOXL3 genes and proteins is diminished in premenopausal POP patients compared to asymptomatic controls (15). Here, we analyzed LOXs family genes and their possible association with POP in postmenopausal women. Our results showed that LOXL2 gene was significantly down-regulated in the vagina of postmenopausal patients with severe POP as compared to postmenopausal controls, corroborating with the idea that the ECM biogenesis is affected in women with POP. This finding suggests that advancing age and ovarian hormones deprivation modulate vaginal ECM components of women affected by POP as they were much older than postmenopausal women with normal floor sup-

port. It is probable that the molecular mechanisms underlying POP in women after menopause are different from the ones observed in premenopausal women. Importantly LOX enzymes and their substrate elastin expression also diminish with age (31); this correlation may reflect an increased incidence of pelvic floor diseases in the elderly population. However, the cause/effect relationship between the interval from menopause onset and development of defects in the ECM components in older POP patients is not elucidated. Future studies are needed to clarify those issues.

However, our study has limitations. The difficulty of obtaining adequate tissue sample according to our strict selection criteria (especially for the postmenopausal control group) has restricted the number of participants enrolled. The size of the tissue specimen also limited us to use only one methodology in the study, namely gene expression but not the protein expression analysis, which complicates the understanding of the basic biologic conditions of POP. We also acknowledge that the postmenopausal study groups were not matched for parity, a known factor in POP development.

CONCLUSIONS

In this study we have confirmed that vaginal tissue of Caucasian women with normal pelvic floor support before and after menopause shows different levels of ECM turnover and stability under physiological hormonal variances. In addition, we have shown that age and menopause influence the expression of genes involved in the ECM biogenesis and remodeling in vaginal tissues of older women with POP. We speculate that molecular mechanisms involving vaginal ECM deficiency in POP differ between women before and after menopause. The alteration in the expression of genes coding for MMPs, TIMPs and LOXs enzymes suggests that their protein expression and activities in pre- and postmenopausal tissues should be targets for future investigations. A better understanding of complex molecular mechanisms underlying POP before and after menopause will expand the diagnostic and clinical treatment capability. We encourage researches to acknowledge the hormonal status of the study candidates in relation to pelvic floor connective tissue disorders.

ACKNOWLEDGEMENT

The authors gratefully thank Nadiya Oleksiv for her assistance in the RT-PCR analyses. We also thank Dr Stephen Lye and Dr Harold Drutz for stimulating discussions.

SOURCE OF FUNDING

University of Toronto, Faculty of Medicine Dean's Fund- New Staff Grant and Research Fund, Department of Obstetrics and Gynecology, Mount Sinai Hospital, Toronto.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. DeLancey JO: Anatomy and biomechanics of genital prolapse. Clin Obstet Gynecol. 1993; 36: 897-909.
2. Subak LL, Waetjen LE, van den Eeden S, Thom DH, Vittinghoff E, Brown JS: Cost of pelvic organ prolapse surgery in the United States. Obstet Gynecol. 2001; 98: 646-51.
3. Olsen AL, Smith VJ, Bergstrom JO, Colling JC, Clark AL: Epidemiology of surgically managed pelvic organ prolapse and urinary incontinence. Obstet Gynecol. 1997; 89: 501-6.
4. Bump RC, Norton PA: Epidemiology and natural history of pelvic floor dysfunction. Obstet Gynecol Clin North Am. 1998; 25: 723-46.
5. Moalli PA, Jones Ivy S, Meyn LA, Zyczynski HM: Risk factors associated with pelvic floor disorders in women undergoing surgical repair. Obstet Gynecol. 2003; 101: 869-74.
6. Copas P, Bukovsky A, Asbury B, Elder RF, Caudle MR: Estrogen, progesterone, and androgen receptor expression in levator ani muscle and fascia. J Womens Health Gend Based Med. 2001; 10: 785-95.
7. Skala CE, Petry IB, Albrich SB, Puhl A, Naumann G, Koelbl H: The effect of hormonal status on the expression of estrogen and progesterone receptor in vaginal wall and periurethral tissue in urogynecological patients. Eur J Obstet Gynecol Reprod Biol. 2010; 153: 99-103.

8. Lang JH, Zhu L, Sun ZJ, Chen J: Estrogen levels and estrogen receptors in patients with stress urinary incontinence and pelvic organ prolapse. Int J Gynaecol Obstet. 2003; 80: 35-9.
9. Bai SW, Chung DJ, Yoon JM, Shin JS, Kim SK, Park KH: Roles of estrogen receptor, progesterone receptor, p53 and p21 in pathogenesis of pelvic organ prolapse. Int Urogynecol J Pelvic Floor Dysfunct. 2005; 16: 492-6.
10. Kagan HM, Li W: Lysyl oxidase: properties, specificity, and biological roles inside and outside of the cell. J Cell Biochem. 2003; 88: 660-72.
11. Shapiro SD: Matrix metalloproteinase degradation of extracellular matrix: biological consequences. Curr Opin Cell Biol. 1998; 10: 602-8.
12. Jackson SR, Avery NC, Tarlton JF, Eckford SD, Abrams P, Bailey AJ: Changes in metabolism of collagen in genitourinary prolapse. Lancet. 1996; 347: 1658-61.
13. Moalli PA, Shand SH, Zyczynski HM, Gordy SC, Meyn LA: Remodeling of vaginal connective tissue in patients with prolapse. Obstet Gynecol. 2005; 106: 953-63.
14. Chen BH, Wen Y, Li H, Polan ML: Collagen metabolism and turnover in women with stress urinary incontinence and pelvic prolapse. Int Urogynecol J Pelvic Floor Dysfunct. 2002; 13: 80-7; discussion 87.
15. Alarab M, Bortolini MA, Drutz H, Lye S, Shynlova O: LOX family enzymes expression in vaginal tissue of premenopausal women with severe pelvic organ prolapse. Int Urogynecol J. 2010; 21: 1397-404.
16. Alarab M, Shynlova O, Drutz H, Lye S: Extracellular Matrix remodeling is increased in Vaginal Tissue of Premenopausal Patients with Pelvic organ Prolapse. Reproductive Sci. 2008; 15 (Suppl 1): abstract #311A
17. Tinelli A, Malvasi A, Rahimi S, Negro R, Vergara D, Martignago R, et al.: Age-related pelvic floor modifications and prolapse risk factors in postmenopausal women. Menopause. 2010; 17: 204-12.
18. Bump RC, Mattiasson A, Bø K, Brubaker LP, DeLancey JO, Klarskov P, et al.: The standardization of terminology of female pelvic organ prolapse and pelvic floor dysfunction. Am J Obstet Gynecol. 1996; 175: 10-7.
19. Ricci JV, Lisa JR, et al.: The relationship of the vagina to adjacent organs in reconstructive surgery; a histologic study. Am J Surg. 1947; 74: 387-410.
20. Boreham MK, Wai CY, Miller RT, Schaffer JI, Word RA: Morphometric analysis of smooth muscle in the anterior vaginal wall of women with pelvic organ prolapse. Am J Obstet Gynecol. 2002; 187: 56-63.
21. Meller M, Vadachkoria S, Luthy DA, Williams MA: Evaluation of housekeeping genes in placental comparative expression studies. Placenta. 2005; 26: 601-7.
22. Schmittgen TD, Livak KJ: Analyzing real-time PCR data by the comparative C(T) method. Nat Protoc. 2008; 3: 1101-8.
23. Chen B, Wen Y, Zhang Z, Guo Y, Warrington JA, Polan M: Microarray analysis of differentially expressed genes in vaginal tissues from women with stress urinary incontinence compared with asymptomatic women. Hum Reprod. 2006; 21: 22-9.
24. Chen B, Wen Y, Zhang Z, Wang H, Warrington JA, Polan ML: Menstrual phase-dependent gene expression differences in periurethral vaginal tissue from women with stress incontinence. Am J Obstet Gynecol. 2003; 189: 89-97.
25. Woessner JF, Brewer TH: Formation and breakdown of collagen and elastin in the human uterus during pregnancy and post-partum involution. Biochem J. 1963; 89: 75-82.
26. Vassilev V, Pretto CM, Cornet PB, Delvaux D, Eeckhout Y, Courtoy PJ, et al.: Response of matrix metalloproteinases and tissue inhibitors of metalloproteinases messenger ribonucleic acids to ovarian steroids in human endometrial explants mimics their gene- and phase-specific differential control in vivo. J Clin Endocrinol Metab. 2005; 90: 5848-57.
27. Chen B, Wen Y, Wang H, Polan ML: Differences in estrogen modulation of tissue inhibitor of matrix metalloproteinase-1 and matrix metalloproteinase-1 expression in cultured fibroblasts from continent and incontinent women. Am J Obstet Gynecol. 2003; 189: 59-65.
28. Mogami H, Keller P, Acevedo JF, Word RA: Differential regulation of Lysyl oxidases in uterine and cervical tissues during pregnancy, parturition, and the puerperium. Reproductive Sciences. 2010; 17(Suppl 3): 76A, abstract # 34.
29. Murano S, Thweatt R, Shmookler Reis RJ, Jones RA, Moerman EJ, Goldstein S: Diverse gene sequences are overexpressed in werner syndrome fibroblasts undergoing premature replicative senescence. Mol Cell Biol. 1991; 11: 3905-14.
30. Chen BH, Wen Y, Li H, Polan ML: Collagen metabolism and turnover in women with stress urinary incontinence and pelvic prolapse. Int Urogynecol J Pelvic Floor Dysfunct. 2002; 13: 80-7; discussion 87.
31. Pascual G, Mendieta C, Mecham RP, Sommer P, Bellón JM, Buján J: Down-regulation of lysyl oxydase-like in aging and venous insufficiency. Histol Histopathol. 2008; 23: 179-86.

Correspondence address:
Dr. Oksana Shynlova,
Samuel Lunenfeld Research Institute at
Mount Sinai Hospital,
25 Orde st, Suite 6-1019
Toronto, Ontario, Canada. M5G1X5
Fax: 416-586-5993
E-mail: shynlova@lunenfeld.ca

ORIGINAL ARTICLE

Vol. 39 (2): 268-275, March - April, 2013
doi: 10.1590/S1677-5538.IBJU.2013.02.16

Effect of acute administration of sildenafil to rats with detrusor overactivity induced by chronic deficiency of nitric oxide

Ricardo Reges, Carlos D´Ancona, Fabíola Mônica, Edson Antunes

Division of Urology (RR, CDA) and Department of Pharmacology (FM, EA), Universidade Estadual de Campinas - UNICAMP, Sao Paulo; Division of Urology, Universidade Federal do Ceara - UFC (RR), Ceara, Brazil

ABSTRACT

Purpose: Recently, the effect of phosphodiesterase inhibitors (PDE5i) in the lower urinary tract symptoms (LUTS) associated to benign prostatic hyperplasia have been studied thoroughly. However, it remains unclear how the PDE5i improve LUTS. Therefore, the aim of the present study was to evaluate the potential of acute administration of the PDE5i sildenafil to improve detrusor overactivity (DO) induced by N^{ω}-nitro-$_{L}$-arginine methyl ester hydrochloride (L-NAME), an nitric oxide sinthase (NOS) inhibitor, in rats.

Materials and Methods: Twenty-seven MALE adult Wistar Rats were divided into the following groups: (1) control, (2) L-NAME, (3) sildenafil alone, and (4) L-NAME + sildenafil. The NOS blocker L-NAME (20 mg/rat/day) was given in the drinking water. Sildenafil (100µg/kg) was administrated intravenously (i.v.) acutely, diluted in cremophor, propylene glycol and water. All animals underwent to anesthetized cystometograms.

*Results: T*he chronic and systemic administration of L-NAME markedly increased the number of non voiding contractions (2.62 (± 0.89)), and frequency of micturition (1.97 (± 0.78)), as well increased volume threshold (2.83 mL (± 1.64)) compared with control group, the number of non voiding contractions (1.17 (± 0.75)), frequency of micturition (1.08 (± 0.65)) and volume threshold (1.16 mL (± 0.38)), $p < 0.001$, $p = 0.01$, and $p = 0.04$, respectively. Sildenafil infusion decreased the number of micturition cycles significantly from the baseline to end point (-0.93 (± 0.34)) in nitric oxide (NO) deficient animals compared with sildenafil infusion alone (control) in animals with normal NO level (0.13 (± 0.25)), $p = 0.03$.

Conclusion: Systemic reduction of nitric oxide causes detrusor overactivity and acute infusion of sildenafil reduces the number of micturition cycles in chronic NO-deficient rats.

ARTICLE INFO

Key words:
sildenafil nitrate [Supplementary Concept]; Nitric Oxide; Lower Urinary Tract Symptoms

Int Braz J Urol. 2013; 39: 268-75

Submitted for publication:
September 10, 2012

Accepted after revision:
February 20, 2013

INTRODUCTION

Initially, the rationale for the use of phosphodiesterase inhibitors (PDE5i) in the treatment of lower urinary tract symptoms (LUTS) was based on demographic data showing the frequent occurrence of both erectile dysfunction (ED) and LUTS in men as they age (1,2). Overall, these preliminary data provide experimental support for the clinical investigation of PDE5i in the treatment of benign prostatic enlargement (BPE)/ LUTS. After the first clinical study reported improvement of LUTS with sildenafil administration for men with DE and BPE in 2002 (2), it has been

observed a special interest in the role of PDE5i for the treatment of LUTS. Although nowadays there are several studies of high level of evidence supporting the use of PDE5i in LUTS, the mode of action of PDE5i is not yet fully understood.

Sildenafil, an oral PDE5i, is the most commonly prescribed medication for treating ED. By inhibiting the degradation of phosphodiesterase type-5, vascular smooth muscle relaxation is augmented, thus improving corpora cavernosa blood flow. Phosphodiesterase type-5 has been found throughout the urinary tract, including the corpus cavernosum, clitoris, tunica albuginea, bladder, urethra, prostate, ureter and vagina (3).

The nitric oxide synthase (NOS) inhibitor N^{ω}-nitro-$_L$-arginine methyl ester hydrochloride (L--NAME) causes significant increase in number of non voiding contractions (NVC) micturition cycles (MC) in rats. The increase in NVC and MC in experimental study has been associated with detrusor overactivity (DO) (4). In addition, the inhibition of arginases, which degrade L-arginine into L--ornithine, decreased neurogenic DO in chronic spinal cord-injured (SCI) rats (5). Recently, it was also showed that 4-week treatment with the NOS blocker L-NAME causes in vitro detrusor smooth muscle (DSM) supersensitivity to muscarinic agonists via increased levels of [H^3]-inositol-phophate, accompanied by reductions of β_3-adrenoreceptor mediated DSM relaxations (6).

Therefore, the aim of the present study was to evaluate the potential of acute administration of sildenafil to improve DO induced by an NOS inhibitor L-NAME in rats.

MATERIALS AND METHODS

Animals

The experimental protocols were approved by the Ethical Principles in Animal Research adopted by the Brazilian College for Animal Experimentation. Male Wistar rats (220-310 g) were used.

Experimental Groups

Animals were randomized into four experimental groups, as follows:

G1: rats (n=6): control group: the rats received drinking water ad libitum

G2: rats (n=6): received L-NAME (20mg/rat/day) in drinking water for 4 weeks

G3: rats (n=4): received sildenafil (100µg/Kg) (i.v.) in a single injection.

G4: rats (n=6): received L-NAME(20mg/rat/day) in drinking water for 4 weeks + sildenafil (100µg/Kg) (i.v.) in a single injection.

The volume of water drunk by each rat was approximately 50 mL/rat/day. Doses of L-NAME and sildenafil were chosen according to our previous studies (7).

Cystometry in Anesthetized Rats

Rats were anesthetized with an intraperitoneal injection of urethane (1.2g/kg), and the carotid artery cannulated for mean arterial blood pressure (MABP) monitoring. A 1-cm incision was made along the midline of the rat abdomen. The bladder was exposed and a butterfly needle (19G) was inserted into the bladder dome and connected to a pressure transducer and to an infusion pump. Before starting the cystometry, the bladder was emptied. Continuous cystometry (CMGs) was carried out by infusing saline into the rat bladder at a rate of 4 mL/hr. The following parameters were assessed: number of non-voiding contraction (NVCs), threshold pressure (TP) mmHg - at which micturition began, the peak pressure (PP) mmHg - during micturition, volume threshold (VT) mL - that was calculated by the time needed for the first micturition X 4 mL/60min., micturition cycle (MC) calculated as number of cycles per minute and basal vesical pressure (BP). NVCs were considered as spontaneous bladder contractions > 4 mmHg from the baseline pressure that did not result in a void. The urodynamic variables evaluated and the methodology used in the cystometry match those described in the literature (8).

Statistics

Data are expressed as mean ± SD of n experiments. Statistical significance of the differences was studied by analysis of variance (ANOVA) and posteriorly by Bonferroni method. $P < 0.05$ was accepted as significant. The software Gra-

phPad Prism® version 5.00 for Windows® (GraphPad Software, San Diego, California, USA, 2007) was used for the statistical analysis.

Drugs

N^{ω}-nitro-L-arginine methyl ester hydrochloride and urethane were obtained from Sigma Chem. Co. (St Louis, MO) and Sildenafil from Biolab (SP, Brazil).

RESULTS

The chronic and systemic administration of L-NAME (G2) resulted in a significant increase in total number of non voiding contractions (2.62 (± 0.89)) compared with control (G1) (1.17 (± 0.75)) ($p < 0.001$; Figure-1). Volume threshold was also significantly increased in L-NAME-treated rats (G2) (2.83 mL (± 1.64)) when compared with control (G1) (1.16 mL (± 0.38)) ($p = 0.01$; Figure-2). No significant difference was observed in the threshold pressure, peak pressure and basal bladder pressure comparing L-NAME group with control (Table-1).

The number per minute of micturition cycles significantly increased in L-NAME-treated groups (G2 and G4) when compared with control (G1) or sildenafil groups (G3) ($P = 0.04$; Figure-3). Sildenafil infusion to chronically L-NAME treated rats (G4) decreased the number of MC significantly from the baseline to end point (-0.93 (± 0.34)) compared with sildenafil infusion alone (G3) (0.13 (± 0.25)), $p = 0.03$ (Figure-4; Table-2).

The mean time for the response after sildenafil infusion was 21.58 (± 13.18) minutes.

L-NAME (G1) administration increased significantly the systemic blood pressure (BP) (200.3 (± 6.77)) mmHg compared with control (G2) (116.9 (± 3.35) mmHg) ($p < 0.05$). Comparing the baseline BP (before sildenafil) with endpoint BP (after sildenafil infusion) in the G4 group, it was not observed significant change in the arterial blood pressure (Figure-5).

DISCUSSION

In the present study, a chronic deficiency of NO was induced by L-NAME. The animals un-

Figure 1 - Number of non-void contractions (NVC) in NO-deficient animals (G2 and G4) compared with controls (G1 and G3). Analysis of variance (ANOVA) between groups ($p < 0.001$). G1 = Control, G2 = L-NAME, G3 = Sildenafil alone, and (G4 = L--NAME + Sildenafil.

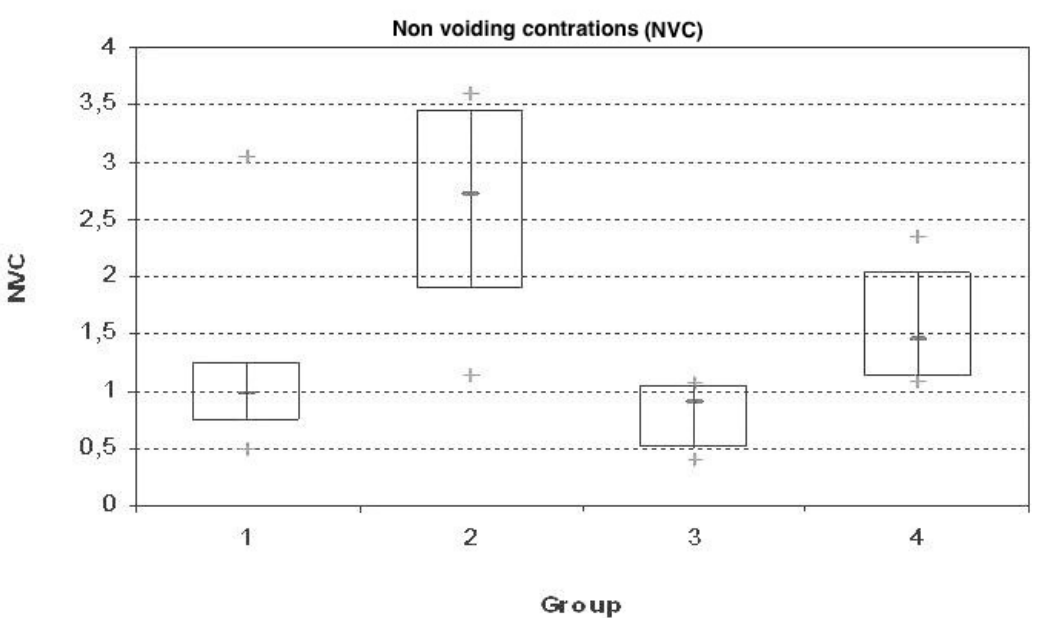


Figure 2 - Increase in volume threshold (VT) in NO-deficient animals (G2 and G4) compared with controls (G1 and G3). Analysis of variance (ANOVA) between groups (p = 0.01). G1 = Control, G2 = L-NAME, G3 = Sildenafil alone, and (G4 = L-NAME + Sildenafil.

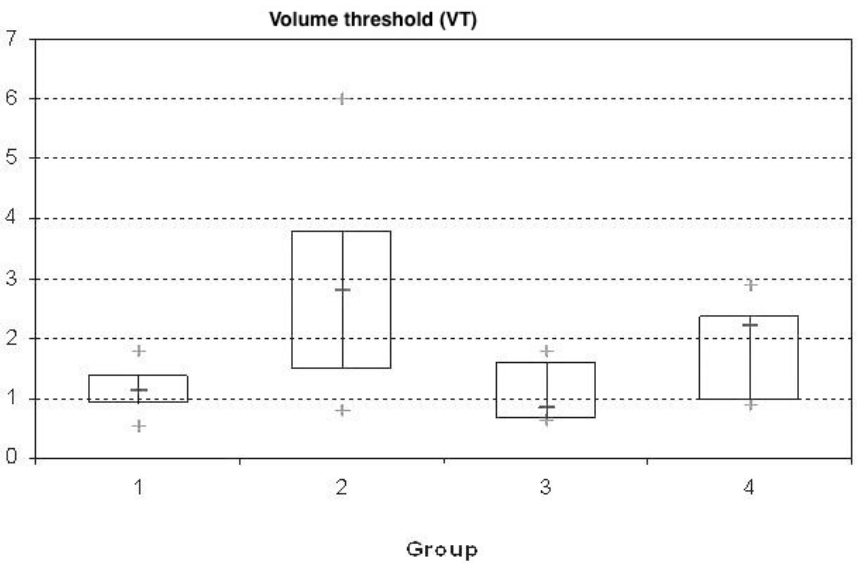


Table 1 - Administration of N$^{\omega}$-nitro-L-arginine methyl ester hydrochloride (L-NAME) to G1 and G4 rats resulted in increased number of non voiding contractions, volume threshold, and micturition cycles compared to the animals with normal level of nitric oxide at end point and before sildenafil infusion (G2 and G3, respectively).

Variables	Group 1 (n = 6) Mean / ± SD	Group 2 (n = 6) Mean / ± SD	Group 3 (n = 4) Mean / ± SD	Group 4 (n = 6) Mean / ± SD	P
NVC	1.17 / ± 0.75	2.62 / ± 0.89†	0.82 / ± 0.30	1.56 / ± 0.74‡	< 0.001
VT	1.16 / ± 0.38	2.83 / ± 1.64†	1.03 / ± 0.54	1.09 / ± 0.78‡	0.011
PT	19.14 / ± 3.02	19.13 / ± 2.90	21.63 / ± 4.50	28.16 / ± 11.39	0.06
PP	21.14 / ± 3.06	20.05 / ± 2.93	23.15 / ± 4.77	30.33 / ± 11.12	0.497
MC	1.08 / ± 0.65	1.97 / ± 0.78†	1.27 / ± 0.37	1.38 / ± 0.17‡	0.04
BP	11.69 / ± 6.77	13.73 / ± 3.35	16.50 / ± 3.11	21.00 / ± 14.08	0.21

G1 = Control, **G2 =** L-NAME, **G3 =** Sildenafil alone, and **G4 =** L-NAME+Sildenafil
NVC - non-voiding contraction, **TP -** threshold pressure, **PP -** peak pressure, **VT -** volume threshold, **MC -** micturition cycled and **BP** - basal pressure. **SD -** standard deviation, **ANOVA -** analysis of variance.
The symbols †(P < 0.05) and ‡(P < 0.05) denote statistically significant differences in relation to G1 and G3, respectively (Tukey's multiple comparison test).

Figure 3 - Increase in number of micturition cycle (MC) in NO-deficient animals (G2 and G4) compared with controls (G1 and G3). Analysis of variance (ANOVA) between groups ($p < 0.05$). G1 = Control, G2 = L-NAME, G3 = Sildenafil alone, and (G4 = L-NAME + Sildenafil.

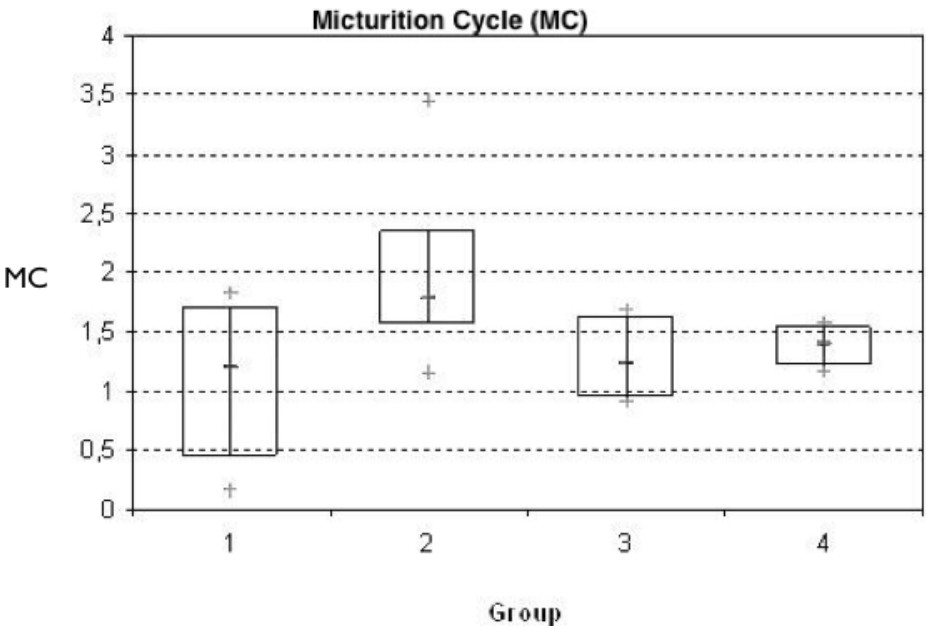
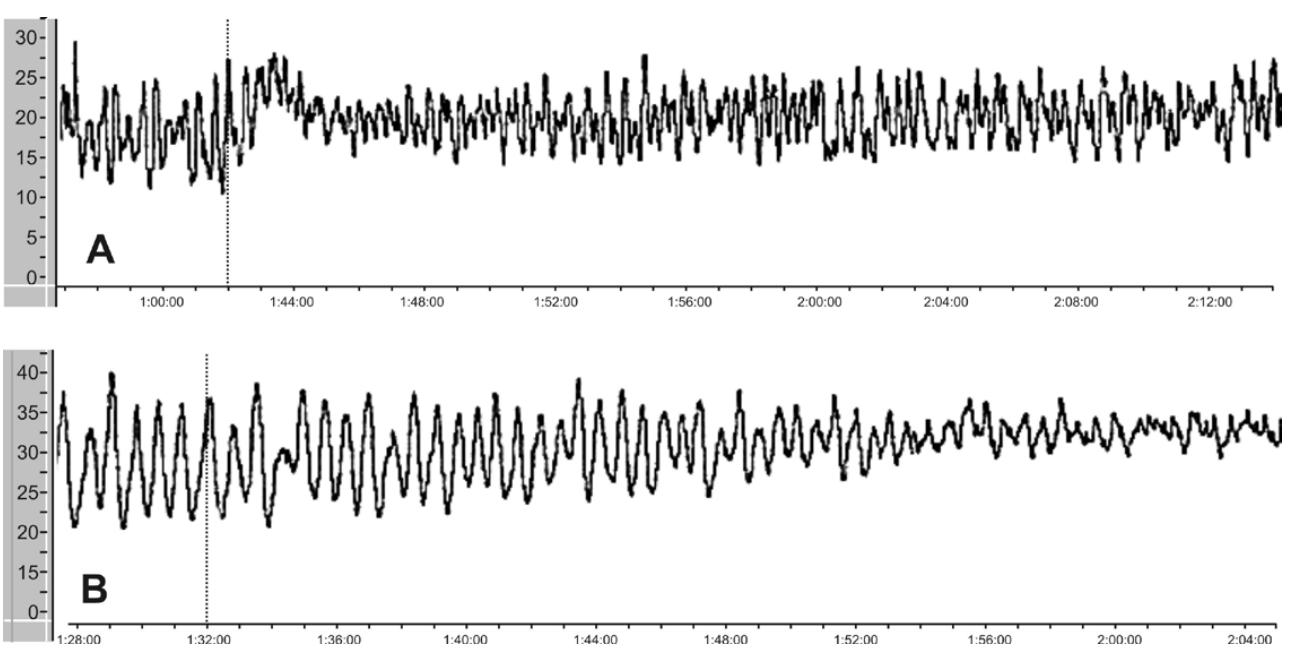


Figure 4 - Representative traces showing no change in the amplitude (mmHg) and number of micturition cycles (MC) after sildenafil infusion in a animal of G3 (A), and decrease in amplitude and number of MC after sildenafil infusion in a rat of G4 (B). The y-axis scale was stadardized to 5 mmHg and the x-axis for intervals of 4 minutes.



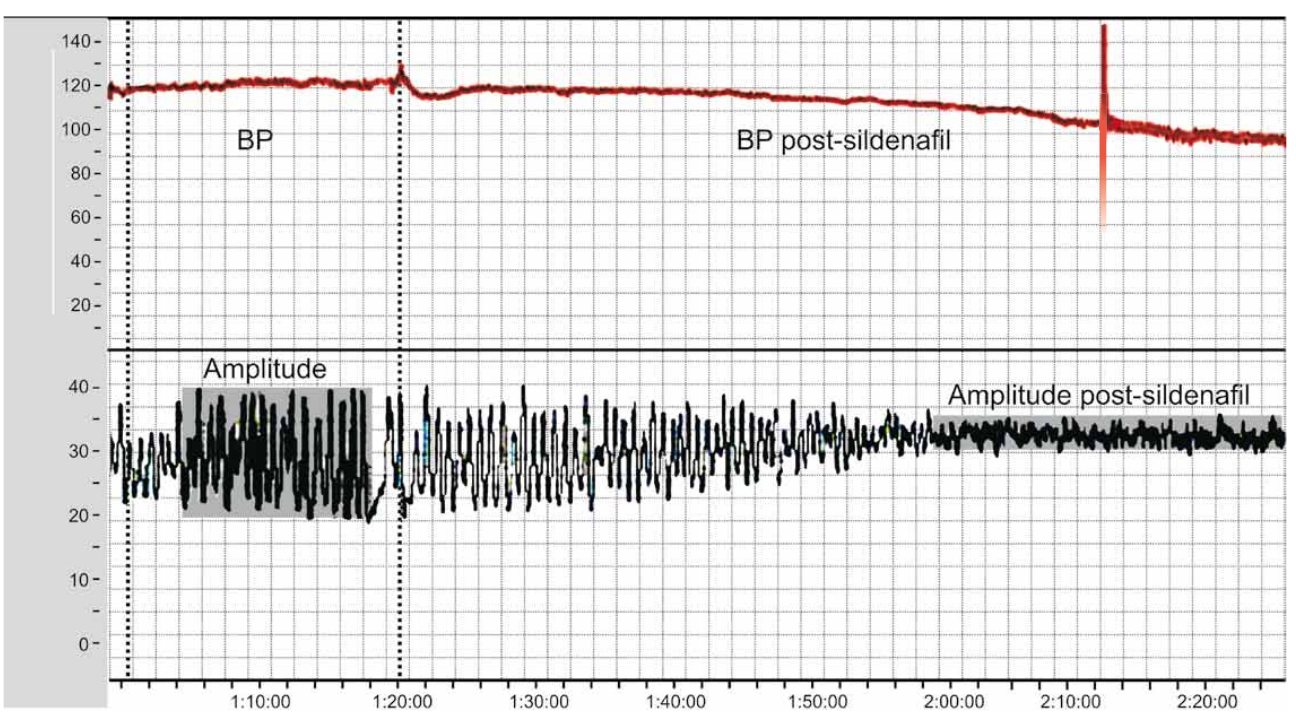
G3 = Single sildenafil injection
G4 = Chronic administration of L-NAME + single sindenafil injection

Table 2 - Change of number and amplitude of micturition cycles from baseline (before sildenafil) to end point (after sildenafil).

G3					G4				
Variable	N	Mean	SD	P	Variable	N	Mean	SD	P
DIF MC (mmHg)	4	0.13	0.25	0.875	DIF MC (mmHg)	6	-0.93	0.34	0.031
DIF AMPLIT (mmHg)	4	-0.50	1.71	0.999	DIF AMPLIT (mmHg)	6	-17.50	11.72	0.031

G3 = sildenafil and **G4 =** L-NAME + sildenafil
DIF MC = Difference (Delta) of micturition cycles
DIF AMPLIT = Difference (Delta) of amplitude
SD = Standard Deviation
P refer to comparison from baseline to end point for each group

Figure 5 - Representative traces showing no significative change in the BP (mmHg) after sildenafil in a NO-deficient animal (G4). It was also observed decrease in amplitude and number of MC after sildenafil infusion in the same animal.



BP = Blood pressure

derwent CMG and it was observed significant increase in NVC and VT in rats.

Therefore, a higher volume threshold becomes necessary to initiate micturition. There are two theories that could explain this observation: increased urethral resistance or detrusor impairment. However, as micturition of the L-NAME rats had detrusor pressure similar to control, it suggests an increased urethral resistance. This hypothesis is corroborated by in vitro pharmacological studies, which report that nitric oxide provides relaxation of the urethral smooth muscles (9,10). These results

suggest that NO has an inhibitory effect in muscular relaxation both in the bladder and in the urethra of animals with chronic deficiency of NO. As demonstrated in a previous experimental study, the systemic reduction of NO causes detrusor overactivity with a decrease in the functional relaxation of the urethra (4,6).

Chronic L-NAME treatment also increased the number of micturition cycles in comparison with control animals. In this study, acute administration of sildenafil during the voiding phase decreased amplitude and number of micturition cycles in the rats with chronic deficiency of NO; however, it did not alter these variables in the control group (G3), with normal NO level.

The exact mechanism through which phosphodiesterase inhibitors alleviate BPE/LUTS remains unclear (11). Also, the pathophysiological relationship between ED and LUTS is not clear yet, but there are several theories to explain it. The candidate mechanisms include pelvic atherosclerosis, autonomic hyperactivity, the calcium-independent Rho-kinase activation pathway and reduced NO levels. It is likely that there is an overlap between the roles of each of these candidate mechanisms, and an ultimate effect leading to smooth muscle relaxation in prostatic, bladder neck, or erectile tissues appears to be crucial. Probably the hypothesis of the reduction of NO is the best explanation.

Increased smooth muscle tension plays a central role in LUTS pathophysiology. The NO/cyclic guanosine monophosphate (cGMP) pathway is one of the major regulators of smooth muscle contractility. Nitric oxide can activate guanylate cyclase, the enzyme that produces cGMP. The accumulation of intracellular cGMP triggers a cascade, leading to decreased intracellular calcium level and subsequent relaxation of smooth muscle cells (SMCs). And the amount of cGMP results from the balance between production (NO) and degradation made by phosphodiesterase are enzymes that can hydrolyze and inactivate cyclic nucleotides. It is known that NO is involved in relaxation of the detrusor, bladder neck, urethra and prostate (6,12).

Clinical trials consistently demonstrated that PDE5i significantly improve IPSS, most studies failed to observe significant improvement in uroflowmetry parameters (13-15). A randomized, double-blind and placebo controlled clinical trial showed that tadalafil, a phosphodiesterase type 5 inhibitor, when administrated in association with tamsulosin improves significantly more the storage symptoms compared with isolated use of alfa-blocker (16). In consequence, the reduction demonstrated in the number per minute of micturition cycles after acute infusion of sildenafil in NO-deficient rats reinforces the hypothesis that PDE5 inhibitors may exert their effects probably via bladder.

This study has some limitations. It has been previously known that L-NAME is a non specific NOS inhibitor that when administered systemically could affect various levels of neuronal or endothelia. In consequence, it has the potential to alter physiology of lower urinary tract throughout these mechanisms. Additionally, we did not evaluate the pharmacological effects on molecular or morphological grounds of lower urinary tract.

CONCLUSIONS

Systemic reduction of nitric oxide causes detrusor overactivity and acute infusion of sildenafil reduces the number of micturition cycles in chronic NO-deficient rats.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Braun M, Wassmer G, Klotz T, Reifenrath B, Mathers M, Engelmann U: Epidemiology of erectile dysfunction: results of the 'Cologne Male Survey'. Int J Impot Res. 2000; 12: 305-11.
2. Blanker MH, Bohnen AM, Groeneveld FP, Bernsen RM, Prins A, Thomas S, et al.: Correlates for erectile and ejaculatory dysfunction in older Dutch men: a community-based study. J Am Geriatr Soc. 2001; 49: 436-42.
3. Uckert S, Hedlund P, Andersson KE, Truss MC, Jonas U, Stief CG: Update on phosphodiesterase (PDE) isoenzymes as pharmacologic targets in urology: present and future. Eur Urol. 2006; 50: 1194-207; discussion 1207.
4. Mónica FZ, Reges R, Cohen D, Silva FH, De Nucci G, D'Ancona CA, et al.: Long-term administration of BAY 41-2272 prevents bladder dysfunction in nitric oxide-deficient rats. Neurourol Urodyn. 2011; 30: 456-60.

5. Sasatomi K, Hiragata S, Miyazato M, Chancellor MB, Morris SM Jr, Yoshimura N: Nitric oxide-mediated suppression of detrusor overactivity by arginase inhibitor in rats with chronic spinal cord injury. Urology. 2008; 72: 696-700.
6. Mónica FZ, Bricola AA, Báu FR, Freitas LL, Teixeira SA, Muscará MN, et al.: Long-term nitric oxide deficiency causes muscarinic supersensitivity and reduces beta(3)-adrenoceptor-mediated relaxation, causing rat detrusor overactivity. Br J Pharmacol. 2008; 153: 1659-68.
7. Ribeiro MO, Antunes E, de Nucci G, Lovisolo SM, Zatz R: Chronic inhibition of nitric oxide synthesis. A new model of arterial hypertension. Hypertension. 1992; 20: 298-303.
8. Tsukimi Y, Mizuyachi K, Matsumoto H, Sato M, Ng B, Tajimi M: Mechanism of action by which aspirin alleviates detrusor hyperactivity in rats. J Pharmacol Sci. 2004; 95: 101-7.
9. Parlani M, Conte B, Manzini S: Nonadrenergic, noncholinergic inhibitory control of the rat external urethral sphincter: involvement of nitric oxide. J Pharmacol Exp Ther. 1993; 265: 713-9.
10. Andersson KE, Garcia Pascual A, Forman A, Tøttrup A: Non-adrenergic, non-cholinergic nerve-mediated relaxation of rabbit urethra is caused by nitric oxide. Acta Physiol Scand. 1991; 141: 133-4.
11. Dmochowski R, Roehrborn C, Klise S, Xu L, Kaminetsky J, Kraus S: Urodynamic effects of once daily tadalafil in men with lower urinary tract symptoms secondary to clinical benign prostatic hyperplasia: a randomized, placebo controlled 12-week clinical trial. J Urol. 2010; 183: 1092-7.
12. Uckert S, Sormes M, Kedia G, Scheller F, Knapp WH, Jonas U, et al.: Effects of phosphodiesterase inhibitors on tension induced by norepinephrine and accumulation of cyclic nucleotides in isolated human prostatic tissue. Urology. 2008; 71: 526-30.
13. McVary KT, Monnig W, Camps JL Jr, Young JM, Tseng LJ, van den Ende G: Sildenafil citrate improves erectile function and urinary symptoms in men with erectile dysfunction and lower urinary tract symptoms associated with benign prostatic hyperplasia: a randomized, double-blind trial. J Urol. 2007; 177: 1071-7.
14. McVary KT, Roehrborn CG, Kaminetsky JC, Auerbach SM, Wachs B, Young JM, et al.: Tadalafil relieves lower urinary tract symptoms secondary to benign prostatic hyperplasia. J Urol. 2007; 177: 1401-7.
15. Stief CG, Porst H, Neuser D, Beneke M, Ulbrich E: A randomised, placebo-controlled study to assess the efficacy of twice-daily vardenafil in the treatment of lower urinary tract symptoms secondary to benign prostatic hyperplasia. Eur Urol. 2008; 53: 1236-44.
16. Regadas RP, Reges R, Cerqueira JB, Sucupira DG, Josino IR, Nogueira EA, et al.: Urodynamic effects of the combination of tamsulosin and daily tadalafil in men with lower urinary tract symptoms secondary to benign prostatic hyperplasia: a randomized, placebo-controlled clinical trial. Int Urol Nephrol. 2013; 45: 39-43.

Correspondence address:
Dr. Ricardo Reges M. Oliveira
Rua Antonele Bezerra 280
Fortaleza, Ceara, 60160-070, Brazil
Fax: + 55 85 3262-3730
E-mail: consultoriodeurologia@gmail.com

ORIGINAL ARTICLE

Vol. 39 (2): 276-285, March - April, 2013
doi: 10.1590/S1677-5538.IBJU.2013.02.17

P2X7 receptor mediates activation of microglial cells in prostate of chemically irritated rats

Heng Zhang, Limei Liu, Zhong Yang, Jinhong Pan, Zhiwen Chen, Qiang Fang, Weibin Li, Longkun Li, Gengsheng Lu, Zhansong Zhou

Department of Urology (HZ, JP, ZC, QF, WL, LL, GL, ZZ); Department of Pathology (LL) and Department of Neurobiology (ZY), Southwest Hospital, Third Military Medical University, Chongqing 400038, China

ABSTRACT

Purpose: Evidence shows that adenosine triphosphate (ATP) is involved in the transmission of multiple chronic pain via P_2X_7 receptor. This study was to investigate the P_2X_7 and microglial cells in the chronic prostatitis pain.

Materials and Methods: Rats were divided into control group and chronic prostatitis group (n = 24 per group). A chronic prostatitis animal model was established by injecting complete Freund's adjuvant (CFA) to the prostate of rats, and the thermal withdrawal latency (TWL) was detected on days 0, 4, 12 and 24 (n = 6 at each time point in each group). Animals were sacrificed and the pathological examination of the prostate, detection of mRNA expression of P_2X_7 and ionized calcium binding adaptor molecule 1 (IBA-1) and measurement of content of tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) in the dorsal horn of L_5-S_2 spinal cord were performed on days 0, 4, 12 and 24. In addition, the content of TNF-α and IL-1β in the dorsal horn of L_5-S_2 spinal cord was measured after intrathecal injection of inhibitors of microglial cells and/or P_2X_7 for 5 days.

Results: The chronic prostatitis was confirmed by pathological examination. The expression of P_2X_7 and IBA-1 and the content of TNF-α and IL-1β in rats with chronic prostatitis were significantly higher than those in the control group. On day 4, the expressions of pro-inflammatory cytokines became to increase, reaching a maximal level on day 12 and started to reduce on day 24, but remained higher than that in the control group. Following suppression of microglial cells and P_2X_7 receptor, the secretion of TNF-α and IL-1β was markedly reduced.

Conclusion: In chronic prostatitis pain, the microglial cells and P_2X_7 receptor are activated resulting in the increased expression of TNF-α and IL-1β in the L_5-S_2 spinal cord, which might attribute to the maintenance and intensification of pain in chronic prostatitis.

ARTICLE INFO

Key words:
Prostatitis; Cells; Receptors, Purinergic P2X7; Tumor Necrosis Factor-alpha; Interleukin-1

Int Braz J Urol. 2013; 39: 276-85

Submitted for publication:
June 04, 2012

Accepted after revision:
January 17, 2013

INTRODUCTION

Chronic prostatitis, a common urological condition in young and middle-age men, is caused by multiple etiological factors. Pain is a major presentation of chronic prostatitis (1). Previous studies focused on the pathological changes in the prostate, while the pathways related to neurotransmission and the regulatory mechanisms of chronic prostatitis pain have not been studied. Recent studies have identified the chronic prostatitis pain as a visceral referred pain, which is usually accompanied by the dysfunction of pelvic floor muscles. The prostate is innervated largely by the pelvic nerves arising from the L_5-S_2 spinal cord (2,3).

It has also been shown that the transmission and regulation of pain are associated with not only the neurons but the microglia and astrocytes (4,5). Studies also demonstrated that astrocytes and microglias may secrete pro-inflammatory cytokines such as tumor necrosis factors (TNF), interleukin-1 (IL-1), nerve growth factor (NGF), and nitric oxide (NO), which may lead to the neuronal injury and chronic pain (6,7). Especially, the microglias are widely distributed in the central nervous system (CNS). The detrimental stimulation of CNS (such as trauma, ischemia and infection) may activate microglias. Under this condition, their morphology, the receptor expression on these cells and their function alter; these cells are ameboid; the markers for activation increase on these cells (8). There is evidence showing that the microglias in the posterior horn of spinal cord are significantly activated after damage to peripheral nerves (9). This suggests that the activation of microglias in the spinal cord is related to the occurrence and transmission of neuropathic pain. However, the role of microglias in chronic prostatitis pain is still poorly understood, and molecules activated after injury on these cells and the exact mechanisms are unclear.

There are a lot of P_2X_7 receptors of adenosine triphosphate (ATP). ATP is a type of pain-causing neurotransmitter, and its receptors can be classified as P_{2X} receptors and P2Y receptors. P_2X_7 receptor is a special subtype of purinergic receptor P_{2X} family and an ATP-gated non-selective cation channel. P_2X_7 receptor contains 595 amino acids and three P_2X_7 receptors form homologous polymers generally. P_2X_7 receptor is a dual functional receptor. Under pathological conditions, P_2X_7 receptor is involved in the transmission of pain. It was reported that microglias may be activated by the P_2X_7 receptor, which is up-regulated in various types of chronic pain (10). Chessell et al. found P_2X_7 knockout mice failed to present with hyperalgesia to heat and mechanical stimulation after nerve injury (11). However, whether the microglias and P_2X_7 receptor in the posterior horn of L_5-S_2 spinal cord are activated to regulate the chronic prostatitis pain remains unclear. Hence, the present study was to investigate the role of microglias and P_2X_7 receptor in the chronic prostatitis pain and the possible therapeutic strategies for chronic pelvic pain syndrome.

MATERIALS AND METHODS

Animals

The specific pathogen free (SPF) rats weighing 200 ± 25 g were purchased from the Experimental Animal Center of the Third Military Medical University and randomized into experiment group and control group. All rats were intraperitoneally anesthetized with 1% pentobarbital and then the prostate was exposed through a ventral midline incision (1 cm). For rats in the experiment group, injection with complete Freund's adjuvant (CFA; Sigma--Aldrich, Sigma) was done once at bilateral ventral lobes (10μL for each). For rats in the control group, 20μL of normal saline was injected (Nackley et al. (12), Butler et al. (13) and Zhou et al. (14)). Then, the wound was closed. Rats were sacrificed on days 0, 4, 12 and 24 d after injection (n = 6 at each time point in each group) and pathological examination and detection of the mRNA expression of P_2X_7 and ionized calcium binding adaptor molecule 1 (IBA-1) and the content of TNF-α and IL-1β in spinal cord were performed. All procedures were performed in accordance with the guidelines for animal care and use of National Institute of Health, and this study was approved by the Ethics Committee of our Hospital.

Prostatic Inflammation Model Identification

Rats were intraperitoneally anesthetized with 10% chloral hydrate (0.3 mL/100 g) on days 0, 4, 12 and 24 after CFA injection, and transcardially perfused with 200 mL of 0.9% saline and then with 0.01 mol/L phosphate buffer (about 300 mL) containing 4% paraformaldehyde at 48° C Subsequently, the left and right prostatic tissues were collected, fixed in 4% paraformaldehyde at 4° C overnight, embedded in paraffin, cut into sections, stained, and finally examined under a microscope (15).

Detection of heat pain threshold

The thermal withdrawal latency (TWL) of the rats was detected at 4 d, 12 d and 24 d after injection respectively. The rats were placed in a box (2 cm x12 cm x 22 cm) with a glass floor and allowed to accommodate to the environment for 30 min. Then,

a light spot (5 mm in diameter; 50 W, 12 V) was produced through a radiant heat stimulator and used to stimulate the paw. The time to paw withdraw was recorded as the TWL. The stimulation was done for no longer than 30 s and measurement was performed 5 times in each rat with an interval of 10 min. between two detections. The maximal or minimal TWL was removed, and the TWL in remaining 3 measurements was employed for the calculation of average (16).

Detection of mRNA expression of P_2X_7 and IBA-1 in L_5-S_2 posterior horn

Animals were sacrificed by decapitation. The posterior horn of L_5-S_2 spinal cord was carefully collected on ice under a microscope, and stored at -70° C for use. Total RNA was extracted from the L_5-S_2 spinal cord using the RNAgents Total RNA Isolation System (Promega, Madison, WI) according to the manufacturer's instructions. The concentration and purity of total RNA were determined by spectrophotometric analysis at A_{260} and A_{280} (1.8-2.0). The quality of RNA was determined by methanal agarose gel electrophoresis following ethidium bromide staining. Total RNA (2μg) was subjected to reverse transcription using the Reverse Transcription System (Jikang, Shanghai, China) with random primer oligo$(DT)_{18}$ (0.5μg). The reaction conditions were as follows: 70° C for 5 min, 37° C for 60 min. and 70° C for 10 min., and products were then stored at -70° C.

The resulting cDNA (20 ng) was used as templates for real-time fluorescence quantitative (FQ) PCR using a SYBR green PCR core reagent kit (Applied Biosystems, Foster City, CA) in DNA Engine OPTIONTm^2 (MJ RESEARCH, USA). The primers were designed using the Geneworks software package as follows: P_2X_7: 5'-GACAAACAAAGTCACCCGGAT-3' (forward) and 5'-CGCTCACCAAAGCAAAGCTAAT-3' (reverse); IBA-1: 5'-TTGATCTGA ATGGCAATGGA-3' (forward) and 5'-CCTCC AATTAGGGCAACTCA-3' (reverse). The PCR conditions were as follows: reverse transcription at 50° C for 30 min., Hot Start Taq (1.25 unit/sample) activation for 15 min at 95° C, 40 cycles of denaturation at 94° C for 15 s, annealing at 56° C for 30 s, and extension at 72° C for 30 s. The SYBR Green fluorescence was acquired by a final extension at 79° C. The melting curve analysis was performed after each reaction. GAPDH (5'-TTTAACTCTGGTAAAGTGGATATTGTTG-3' [forward] and 5'-ATTTCCATTGATGACAA GCTTCC-3') served as an internal control (Table-1). The expression of target genes could be calculated according to the amplification standard curve and regression equation of GAPDH automatically by the DNA Engine OPTIONTm2. The expression of target genes was normalized to that of GAPDH as the relative expression. Average was obtained from 6 animals in each group.

Contents of TNF-α and IL-1β in L_5-S_2 posterior horn

L_5-S_2 posterior horn was homogenized in 0.5 mL of ice-cold lysis buffer containing 50 mM Tris, 150 mM NaCl, 1% TritonX 100, 0.5% sodium deoxycholate, l mM phenylmethanesulfonyl fluoride (PMSF), 0.1% sodium dodecyl sulfate (SDS), 10 mM NaF and 1 mM vitriolu acid sodium. Homogenates were centrifuged at 1800 rpm for 10 min. and the supernatant was collected and stored at -70° C. The contents of IL-1β and TNF-α were detected using the commercially available ELISA kits according to the manufacturer's instructions. Briefly, 50μg) was subjected to reverse transcription using the L of biotinylated antibody was added to 100μg) was subjected to reverse transcription using the L of samples in an anti-rat TNF-α or IL-1β pre-coated plate (Santa Cruz Biotechnology, Inc., Santa Cruz) followed by incubation for 3 h at room temperature. After wa-

Table 1 - Primers and conditions for RT-PCR.

	Forward	Reverse
P_2X_7	5'-GACAAACAAAGTCACCCGGAT-3'	5'-CGCTCACCAAAGCAAAGCTAAT-3'
IBA-1	5'-TTGATCTGA ATGGCAATGGA-3'	5'-CCTCC AATTAGGGCAACTCA-3'
GAPDH	5'-TTTAACTCTGGTAAAGTGGATATTGTTG-3'	5'-ATTTCCATTGATGACAAGCTTCC-3'

shing three times, a prepared 100μg) was subjected to reverse transcription using the L of strepta vidin horseradish peroxidase solution and 3, 3′, 5, 5′-tetramethylbenzidin substrate solution were added followed by incubation. Absorbance was measured at 492 nm in a microplate reader (Salzburger Labortechnik, Salzburg, Austria). A standard curve was delineated to determine the content of TNF-α and IL-1β. The sensitivity of this assay is > 10 pg/mL. Average was obtained from 6 animals in each group.

Intrathecal injection of agonist or antagonist of P_2X_7 and microglial cells

The second part of the experiment involved intrathecal cannulation according to the procedures described by Yaksh and Rudy (17). In brief, rats were intraperitoneally anesthetized with pentobarbital, a cannula (PE-10 tubing) was inserted through the cisterna magna at 6 cm to the L_1 spinal cord via the spinal subarachnoid space. A recovery period of 7 d was allowed, and rats showing no motor impairment following surgery were used for further experiments. The prostatitis was induced as mentioned above. From day 7, intrathecal injection of drug was done for consecutive 5 days. Rats were divided into 5 groups (n = 6 per group) and treated as follows: 1) P_2X_7 receptor agonist: 2'-3'-O-(4-Benzoylbenzoyl)--adenosine 5'-triphosphate (BzATP; 100μmol/L); 2) P_2X_7 receptor antagonist: oxidized ATP (oATP; 100μmol/L); 3) inhibitor of microglial cells: minocycline (50μg); 4) P_2X_7 receptor agonist + inhibitor of microglial cells; 5) in the control group, injection was done with artificial cerebrospinal fluid (ACSF; pH5.5) of equal volume. Six rats were included in each group. Drugs were injected at a volume of 15μL and flushing was done with 5μL of ACSF. Injections were finished within 5 min. The content of TNF-α and IL-1β in the spinal cord was determined.

Statistical analysis

All data were expressed as means ± standard error (SEM) and statistical analysis was performed with SPSS version 13.0 for Windows. When F-test showed significance, means were compared with the LSD test of post hoc analysis (Dunnett's t test). Analysis of variance (ANOVA) was conducted for comparisons of data among different groups. A value of $P < 0.05$ was considered statistically significant.

RESULTS

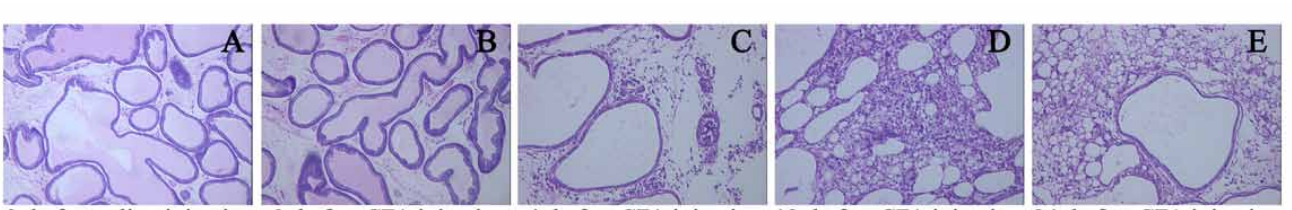
Pathological changes in prostate

Pathological examination showed thAT CFA treated prostates presented with degeneration, necrosis and exfoliation of mucosal cells in the prostate gland. Infiltration of large amounts of lymphocytes and monocytes was noted in the interstitium, and some lymphocytes aggregated in cluster. In the control group, the mucosal epithelial cells were regularly arranged, and the infiltration of leukocytes was not observed in the interstitium (Figure-1).

Detection of pain sensation

The TWL of CFA treated rats was 14.73 ± 0.93s, 12.15 ± 0.99s and 12.45 ± 1.19 respectively at 4 d, 12 d and 24 d after injection, respectively, which were significantly shorter than that in the

Figure 1 - Pathological changes of the prostate in different groups. In the control group (A) and inflammation 0 day group (B), the mucosal epithelium maintained an orderly organization, without leukocyte infiltration in interstitial tissues. In contrast, in the inflammation 4, 12, 24 days groups (C,D,E), degeneration, necrosis, and exfoliation of mucosal cells in the prostate gland were observed. Interstitial substance was infiltrated with large amounts of lymphocytes and monocytes.



control group ($P < 0.01$). This suggests that hyperalgia was induced following CFA injection at the prostate and the rat chronic prostatitis model was successfully established (Figure-2).

mRNA expression of P_2X_7 and IBA-1 in posterior horn

The mRNA expression of P_2X_7 and IBA-1 was significantly increased in the posterior horn of L_5-S_2 spinal cord in the experiment group on days 4, 12 and 24 as compared to the control group at the corresponding time points and to the experiment group at baseline ($P < 0.01$). The P_2X_7 expression reached a maximal level on day 12 ($P < 0.05$) (Figures 3A and 3B).

Content of TNF-α and IL-1β in L_5-S_2 posterior horn

The content of TNF-α and IL-1β in the posterior horn of L_5-S_2 spinal cord was significantly increased in the experiment group on days 4, 12 and 24, as compared to the control group at corresponding time points ($P < 0.01$) and to the experiment group at baseline ($P < 0.01$). The content of TNF-α and IL-1β reached a peak on day 12 ($P < 0.05$) (Figures 3C and 3D).

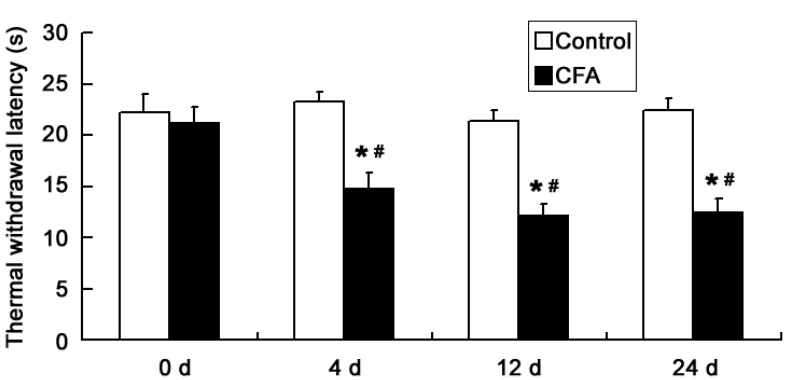
Content of TNF-α and IL-1β following inhibition of microglial cells and/or P_2X_7

In the experiment group, following injection of minocycline and oATP, the content of TNF-α and IL-1β was markedly reduced ($P < 0.01$). However, the P_2X_7 agonist (BzATP) could promote the secretion of TNF-α and IL-1β ($P < 0.01$ and < 0.05, respectively), and minocycline inhibited the bioeffects of BzATP ($P < 0.05$). In the control group, intrathecal injection of ASCF had no influence on the contents of TNF-α and IL-1β in the spinal cord (Figure-4).

DISCUSSION

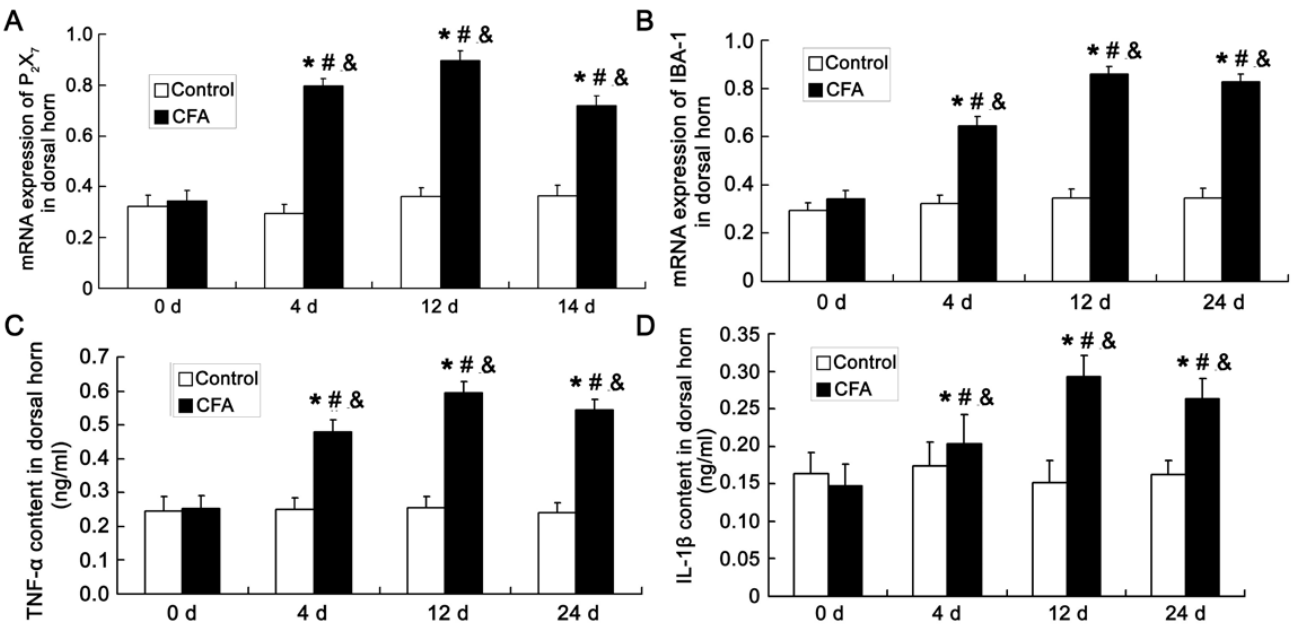
Chronic prostatitis is a common urological disease in young and middle-age men, and patients with chronic prostatitis account for 25-35% of inpatients at the urological clinic. Of all chronic prostatitis of different types, chronic nonbacterial prostatitis (IIIa) or chronic prostatitis/chronic pelvic pain syndrome (IIIb) is the most common and accounts for about 95% of chronic prostatitis (18,19). Generally, chronic prostatitis is characterized by refractory pelvic or perineal pain without evidence of urinary tract infection, which is usually accompanied by bladder and urethra dysfunction. Chronic prostatitis is a major reason for hospital visit. The etiology of chronic prostatitis and the pathogenesis of pain in chronic prostatitis are still poorly understood. In recent years, studies have shown that nanobacteria infection might be a major cause of chronic nonbacterial prostatitis (20). Currently, the antibiotic therapy achieves unfavorable efficacy for patients with chronic prostatitis, and effective strategies have not been developed for these patients to date. The diagnosis

Figure 2 - Thermal withdrawal latency (s) in two groups.



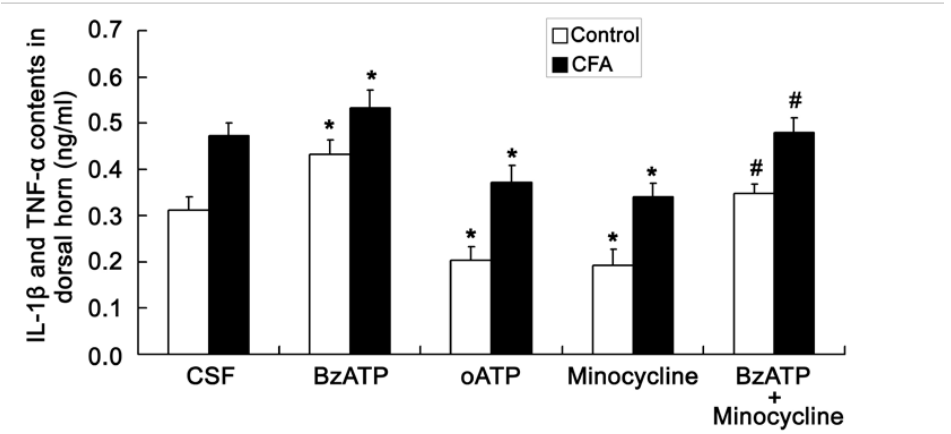
*P < 0.01 vs. control group; #P < 0.01 vs. 0 d.

Figure 3 - mRNA expression of P_2X_7 (A) and IBA-1 (B) in dorsal horn and contents of TNF-α (C) and IL-1β (D) (ng/mL) in dorsal horn at different time points in two groups.



*P < 0.01 vs. control group; #P <0.01 vs. 0 d; &P <0.05 vs. 4 d and 24 d.

Figure 4 - Contents of IL-1β and TNF-α in dorsal horn after injection of different agonist or antagonist (ng/mL).



*P < 0.05 vs. CSF; #P < 0.05 vs. BzATP.

and treatment of chronic prostatitis have been a challenge in urology. In addition, the long-lasting pain may result in physical and psychological disorders. Thus, the investigation of the etiology of chronic prostatitis and the pathogenesis of pain in chronic prostatitis are crucial for the accurate diagnosis and development of effective strategies for the treatment of chronic prostatitis.

Pain appears to be a most prominent manifestation of chronic prostatitis. However, the diagnosis and treatment of pain in chronic prostatitis are still challenging because of the complicated pathogenesis of chronic prostatitis pain (21). Patients with chronic prostatitis often experience pains not only at prostate, but at the sites adjacent to or tissues outside the prostate which are found to be also controlled by the L_5-S_2 spinal cord. Moreover, some patients feel pain even after prostatitis disappears. Hence, the pain in the chronic prostatitis is often characteristic of "extra-territorial" and "mirror" image pain. Increasing evidence demonstrates that there are abnormalities in the cell-mediated neurological regulation and the transmitters in the L_5-S_2 spinal cord in chronic prostatitis (22,23).

Accumulating studies have revealed that the pathological pain is due to not only neuronal dysfunction, but the activation of astrocytes and microglias (24), especially in the chronic exaggerated and continuing pain. Microglias and astrocytes are regarded as "immune cells" in the nervous system, and can secrete some pro-inflammatory cytokines such as IL-1β, TNF-α, NGF, NO, prostaglandin and bradykinin following activation, leading to the exaggeration and persistence of pain by acting on other glial cells and neurons (25,26). Therefore, microglial cells play important roles in the pathogenesis of pathological pain (27,28). In rats with sciatic inflammation, intrathecal injection of minocycline, an inhibitor of microglial cell activation, was found to inhibit the abnormal mechanical pain with low threshold (29).

In the pathogenesis of pathological pain, P_2X_7 plays an important role in the secretion of pro-inflamamtory cytokines mediated by microglial cell activation (27). P_2X_7 is an ATP receptor, a transmitter and modulator in the nervous system. P_2X_7 is a special subtype of purinergic receptor P_2X family. In rats with inflammatory pain, visceral pain and neuropathic pain, focal or intraperitoneal injection of antagonist of P_2X_7 (oATP or A-740003) was found to inhibit the mechanical hyperalgesia, allodynia and hypersensitivity (30,31). In the P_2X_7 receptor deficiency mice, the neuropathic hypersensitivity to mechanical or heat stimulation was absent following nerve injury (11). However, under physiological conditions, P_2X_7 receptor is not activated. Under the pathological conditions, P_2X_7 receptor is activated and involved in the pain transduction. Our findings also revealed that P_2X_7 receptor activation significantly increased the secretion of pro-inflammatory cytokines in animal inflammation model. Following activation, P_2X_7 receptor involves in the pain transduction, which is associated with the calcium related signal transduction (32,33).

In the present study, our results indicated that, in rats with chronic prostatitis pain, the expression of P_2X_7 and IBA-1 was elevated in the posterior horn of L_5-S_2 spinal cord, and the excretion of TNF-α and IL-1β was also up-regulated. However, after inhibition of P_2X_7 receptor and/or microglial cells, the secretion of TNF-α and IL-1β was dramatically reduced suggesting that P_2X_7 receptor mediates the microglial cell activation in rat with prostate prostatitis leading to the increased secretion of pro-inflammatory cytokines. It has been well established that TNF-α and IL-1β are responsive to inflammatory stimuli and cytotoxicity towards neurons, and they can induce chronic inflammation and pain (34). These findings suggest that there is neurogenic inflammation in the L_5-S_2 spinal cord as a result of microglial cell activation via the P_2X_7 receptor in rats with chronic prostatitis pain. These observations demonstrate that P_2X_7 mediated microglial cells activation in the L_5-S_2 spinal cord may take part in the regulation of chronic pain and might lead to the persistence and exaggeration of prostatitis pain. Hence, identification of new neurotransmission pathways and the mechanisms underlying the regulation of chronic prostatitis pain may be helpful to find novel therapeutic targets for chronic prostatitis pain. Currently, oral or intravenous non-steroidal anti-inflammatory drugs have been used to treat spinal cord inflam-

mation. However, the focal drug concentration is at a low level leading to unfavorable efficacy. In Traditional Chinese Medicine, the surface projection of L_5-S_2 spinal cord is also known as Shenshu point and acupuncture of Shenshu point has been used in the treatment of chronic prostatitis pain (35). To date, we have applied "water-needle therapy" for chronic prostatitis pain on the basis of our previous findings, in which the acupuncture of acupoint at L_5-S_2 spinal cord was performed followed by focal injection of B12, B1, hydrocortisone and Chinese herbs. This treatment achieves favorable efficacy, but is still in its infancy stage.

Of note, our findings can not explain the whole molecular mechanisms underlying the activation of microglial cells in chronic prostatitis because there are other receptors (such as P_2X_4, P_2Y_{12} and Toll like receptor) related to the neuropathic pain (36,37). Studies have shown that the P_2X_7 receptor is related to the P_2X_4 receptor in structure and function, and there is interaction between P_2X_7 and P_2X_4 in the microglial cell mediated pain (38). In addition, the activation of Toll-like receptor 4 in the dorsal horn and the release of IL-1β are dependent on the activation of P_2X_7 receptor, and inhibitors of P_2X_7 receptor (oxidized ATP, A-438079) may suppress the hyperalgesia to heat and mechanical stimulation following intrathecal injection of LPS (a agonist of Toll-like 4 receptor) (39). These findings demonstrate that the P_2X_7 receptor on the microglial cells can interact with the above molecules, which then aggregates the neuropathic pain However, the specific mechanisms of their interactions require further studies.

CONCLUSIONS

The chronic prostatitis is related to the activation of P_2X_7 and microglial cells and the high expression of TNF-α and IL-1β in the dorsal horn of L_5-S_2 spinal cord. Moreover, TNF-α and IL-1β expression in the L_5-S_2 spinal cord can be inhibited by inhibitors of P_2X_7 receptor and microglial cells. These findings indicate that chronic pelvic pain syndrome may cause secondary inflammation in the L_5-S_2 spinal cord by activating the microglial cells via P_2X_7 receptor, a phenomenon probably associated with the persistence and intensification of chronic prostatitis pain.

ABBREVIATIONS

ACSF = artificial cerebrospinal fluid
ANOVA = analysis of variance
ATP = adenosine triphosphate
BzATP = 2'-3'-O-(4-Benzoylbenzoyl)-adenosine 5'-triphosphate
CFA = complete Freund's adjuvant
CNS = central nervous system
IBA-1 = ionized calcium binding adaptor molecule 1
IL-1 = interleukin-1
NGF = nerve growth factor
NO = nitric oxide
oATP = oxidized ATP
PMSF = phenylmethanesulfonyl fluoride
SDS = sodium dodecyl sulfate
SEM = standard error
SPF = specific pathogen free
TNF-α = tumor necrosis factor-α
TWL = thermal withdrawal latency

ACKNOWLEDGMENT

This study was supported by the National Natural Science Foundation (NO.81100537).

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Luzzi GA: Chronic prostatitis and chronic pelvic pain in men: aetiology, diagnosis and management. J Eur Acad Dermatol Venereol. 2002; 16: 253-6.
2. Ishigooka M, Nakada T, Hashimoto T, Iijima Y, Yaguchi H: Spinal substance P immunoreactivity is enhanced by acute chemical stimulation of the rat prostate. Urology. 2002; 59: 139-4.
3. Chen Y, Song B, Jin XY, Xiong EQ, Zhang JH: Possible mechanism of referred pain in the perineum and pelvis associated with the prostate in rats. J Urol. 2005; 174: 2405-8.

4. Beggs S, Salter MW: Microglia-neuronal signalling in neuropathic pain hypersensitivity 2.0. Curr Opin Neurobiol. 2010; 20: 474-80.
5. Watkins LR, Milligan ED, Maier SF: Glial activation: a driving force for pathological pain. Trends Neurosci. 2001; 24: 450-5.
6. Madiai F, Hussain SR, Goettl VM, Burry RW, Stephens RL Jr, Hackshaw KV: Upregulation of FGF-2 in reactive spinal cord astrocytes following unilateral lumbar spinal nerve ligation. Exp Brain Res. 2003; 148: 366-76.
7. Kucher BM, Neary JT: Bi-functional effects of ATP/P2 receptor activation on tumor necrosis factor-alpha release in lipopolysaccharide-stimulated astrocytes. J Neurochem. 2005; 92: 525-35.
8. DeLeo JA, Tanga FY, Tawfik VL: Neuroimmune activation and neuroinflammation in chronic pain and opioid tolerance/hyperalgesia. Neuroscientist. 2004; 10: 40-52.
9. Beggs S, Salter MW: Stereological and somatotopic analysis of the spinal microglial response to peripheral nerve injury. Brain Behav Immun. 2007; 21: 624-33.
10. Suzuki T, Hide I, Ido K, Kohsaka S, Inoue K, Nakata Y: Production and release of neuroprotective tumor necrosis factor by P2X7 receptor-activated microglia. J Neurosci. 2004; 24: 1-7.
11. Chessell IP, Hatcher JP, Bountra C, Michel AD, Hughes JP, Green P, et al.: Disruption of the P2X7 purinoceptor gene abolishes chronic inflammatory and neuropathic pain. Pain. 2005; 114: 386-96.
12. Nackley AG, Suplita RL 2nd, Hohmann AG: A peripheral cannabinoid mechanism suppresses spinal fos protein expression and pain behavior in a rat model of inflammation. Neuroscience. 2003; 117: 659-70.
13. Butler SH, Godefroy F, Besson JM, Weil-Fugazza J: A limited arthritic model for chronic pain studies in the rat. Pain. 1992; 48: 73-81.
14. Zhou ZS, Song Bo, Lu GS: The research on the relationship between chronic prostatitis pain and spinal astrocytes activation. J Third Milit Med Univ 2005; 27: 1853-4.
15. Wu AN, Xiong EQ, Song B: Secretory alterations of urethral glands in complete Freund's adjuvant-induced prostatitis in rats. J Third Milit Med Univ 2009; 31: 2266-8.
16. Cheng H Yang JP, Zhang YB: Spinal microglia activation intrathecal minocycline inhibited by and attenuation on thermal hyperalgesia in CFA-induced inflammatory rats. Suzhou Univ J Med Sci 2007; 27: 14-6.
17. Yaksh TL, Rudy TA: Chronic catheterization of the spinal subarachnoid space. Physiol Behav. 1976; 17: 1031-6.
18. Millán-Rodríguez F, Palou J, Bujons-Tur A, Musquera-Felip M, Sevilla-Cecilia C, Serrallach-Orejas M, et al.: Acute bacterial prostatitis: two different sub-categories according to a previous manipulation of the lower urinary tract. World J Urol. 2006; 24: 45-50.
19. Zhou Z, Hong L, Shen X, Rao X, Jin X, Lu G, et al.: Detection of nanobacteria infection in type III prostatitis. Urology. 2008; 71: 1091-5. Erratum in: Urology. 2008 ;72: 723.
20. Shen X, Ming A, Li X, Zhou Z, Song B: Nanobacteria: a possible etiology for type III prostatitis. J Urol. 2010; 184: 364-9.
21. Baxter C, Bolus R, Mayer E: Choice and outcomes of alternative therapies in patients with interstitial cystitis (IC) and chronic pelvic pain (CPP). J Urol. 2010; 183: 580.
22. Zhang H, Liu L, Lu G, Chen Z, Fang Q, Yang Z, et al.: Chemical irritation of the prostate sensitizes P(2)X(3) receptor-mediated responses in rat dorsal root ganglion neurons. Neurourol Urodyn. 2011; 30: 612-8.
23. Baranowski AP: Chronic pelvic pain. Best Pract Res Clin Gastroenterol. 2009; 23: 593-610.
24. Watkins LR, Milligan ED, Maier SF: Glial activation: a driving force for pathological pain. Trends Neurosci. 2001; 24: 450-5.
25. Li HL, Qin LY, Wan Y: Astrocyte: a new star in pain research. Sheng Li Ke Xue Jin Zhan. 2003; 34: 45-8.
26. Aronica E, Gorter JA, Rozemuller AJ, Yankaya B, Troost D: Activation of metabotropic glutamate receptor 3 enhances interleukin (IL)-1beta-stimulated release of IL-6 in cultured human astrocytes. Neuroscience. 2005; 130: 927-33.
27. Mingam R, De Smedt V, Amédée T, Bluthé RM, Kelley KW, Dantzer R, et al.: In vitro and in vivo evidence for a role of the P2X7 receptor in the release of IL-1 beta in the murine brain. Brain Behav Immun. 2008; 22: 234-44.
28. Barger SW, Goodwin ME, Porter MM, Beggs ML: Glutamate release from activated microglia requires the oxidative burst and lipid peroxidation. J Neurochem. 2007; 101: 1205-13.
29. Ledeboer A, Sloane EM, Milligan ED, Frank MG, Mahony JH, Maier SF, et al.: Minocycline attenuates mechanical allodynia and proinflammatory cytokine expression in rat models of pain facilitation. Pain. 2005; 115: 71-83.
30. Fulgenzi A, Ticozzi P, Gabel CA, Dell'Antonio G, Quattrini A, Franzone JS, et al.: Periodate oxidized ATP (oATP) reduces hyperalgesia in mice: involvement of P2X7 receptors and implications for therapy. Int J Immunopathol Pharmacol. 2008; 21: 61-71.
31. Honore P, Donnelly-Roberts D, Namovic MT, Hsieh G, Zhu CZ, Mikusa JP, et al.: A-740003 [N-(1-{[(cyanoimino)(5-quinolinylamino) methyl]amino}-2,2-dimethylpropyl)-2-(3,4-dimethoxyphenyl)acetamide], a novel and selective P2X7 receptor antagonist, dose-dependently reduces neuropathic pain in the rat. J Pharmacol Exp Ther. 2006; 319: 1376-85.
32. Fumagalli M, Brambilla R, D'Ambrosi N, Volonté C, Matteoli M, Verderio C, et al.: Nucleotide-mediated calcium signaling in rat cortical astrocytes: Role of P2X and P2Y receptors. Glia. 2003; 43: 218-03.

33. Wang CM, Chang YY, Kuo JS, Sun SH: Activation of P2X(7) receptors induced [(3)H]GABA release from the RBA-2 type-2 astrocyte cell line through a Cl(-)/HCO(3)(-)-dependent mechanism. Glia. 2002; 37: 8-18.
34. Stalder AK, Carson MJ, Pagenstecher A, Asensio VC, Kincaid C, Benedict M, et al.: Late-onset chronic inflammatory encephalopathy in immune-competent and severe combined immune-deficient (SCID) mice with astrocyte-targeted expression of tumor necrosis factor. Am J Pathol. 1998; 153: 767-83.
35. Chen ZX: Observation on therapeutic effect of warm needle moxibustion on chronic non-bacterial prostatitis. Zhongguo Zhen Jiu. 2009; 29: 275-8.
36. Kim D, Kim MA, Cho IH, Kim MS, Lee S, Jo EK, et al.: A critical role of toll-like receptor 2 in nerve injury-induced spinal cord glial cell activation and pain hypersensitivity. J Biol Chem. 2007; 282: 14975-83.
37. Tozaki-Saitoh H, Tsuda M, Miyata H, Ueda K, Kohsaka S, Inoue K: P2Y12 receptors in spinal microglia are required for neuropathic pain after peripheral nerve injury. J Neurosci. 2008; 28: 4949-56.
38. Casas-Pruneda G, Reyes JP, Pérez-Flores G, Pérez-Cornejo P, Arreola J: Functional interactions between P_2X_4 and P2X7 receptors from mouse salivary epithelia. J Physiol. 2009; 587: 2887-901.
39. Clark AK, Staniland AA, Marchand F, Kaan TK, McMahon SB, Malcangio M: P2X7-dependent release of interleukin-1beta and nociception in the spinal cord following lipopolysaccharide. J Neurosci. 2010; 30: 573-82.

Correspondence address:
Dr. Zhansong Zhou
Department of Urology
Southwest Hospital, Third Military Medical University
30 Gaotanyanzheng
Chongqing 400038, China
Fax: + 86 23 6546-0268
E-mail: doczhang123@yeah.net

Vol. 39 (2): 286-287, March - April, 2013
doi: 10.1590/S1677-5538.IBJU.2013.02.18

Pseudoneoplastic mimics in an inactive bladder associated with ureteral strictures

Erich K Lang, Matthew Smith, Quan Nguyen

SUNY, Downstate Medical School, Brooklyn, NY, USA

This 54 year old, malnourished white male was seen to determine management of an inactive bladder and ureteric strictures of the remaining right renal moiety.

Following several lithotripsy and lithotomy procedures, which failed to control renal calculi, the left kidney was removed surgically 7 years ago. After several ESWOL and 2 percutaneous nephrolithotripsies, the patient developed a uretero-pelvic junction obstruction, which was corrected by dismembered pyeloplasty. A known stricture in the distal right ureter was not corrected at this time. Rather it was elected to place a percutaneous nephrostomy to protect renal function.

The patient was then lost to follow-up for 4 years. His percutaneous nephrostomy functioned satisfactorily, despite the fact that it never been exchanged or replaced.

At the time of this admission, his creatinine was 1.9; BUN 32, K 4.8, RBC 4.5 mil, WBC 6800, HCT 38, HB 13.8. Urine from the nephrostomy was clear, 1-2 RBC / hpf, culture was negative.

An antegrade pyelogram revealed an essentially normal collecting system, but a severe stricture in the distal ureter. Under fluoroscopic guidance a 0.014 7 cm platinum flexible-tip guide wire was passed across the stricture, followed by a 3 French catheter. Further injections demonstrated a 4 cm tight stricture, followed by another 0.5 cm tight napkin ring stricture (Figure-1). An exchange to a super-stiff Amplatz wire (0.038) was carried out and the strictures satisfactorily dilated with a 4 cm, 4 & 5 mm balloon (Figures 2 and 3).

A cystogram revealed innumerable defects in the bladder, reminiscent in appearance of malakoplakia, and distensible to only 120 mL (Figure-4).

Figure 1 - The collecting system of the right kidney is within normal limits. Parenchymal thickness is maintained. A tight stricture is shown in the distal ureter (arrow).

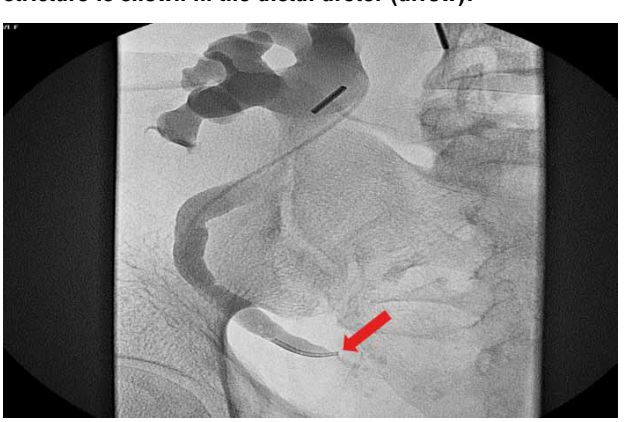
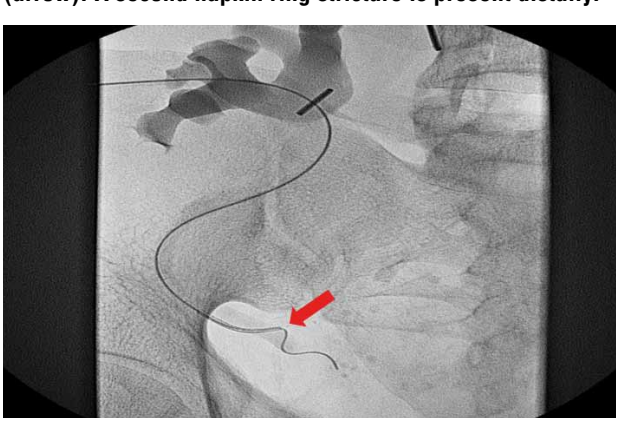


Figure 2 - Over an 0.038 Amplatz wire a 4 cm, 4 mm balloon catheter has been advanced across a tight 4 cm stricture (arrow). A second napkin ring stricture is present distally.



A washing of the bladder revealed debris, keratotic cells, but no evidence of malignancy, i.e. squamous metaplasia.

Despite the risk of squamous metaplasia in a bladder with malakoplakia, it was elected, to continue hydrodilation of the bladder to attain a normal volume while insuring drainage from the kidney via a double "J" stent (1,2). The option of cystectomy and replacement by ileal loop neo--bladder was dismissed because of the patient's history of ureteric strictures.

On 2 year follow-up the bladder had successfully increased volume to 550 ml, mucosal surfaces were normal in appearance, and the right ureter showed no evidence of restricturing after the "J" stent had been removed some 18 months earlier.

Figure 3 - Over an 0.038 Amplatz wire a 4 cm, 5 mm balloon catheter has been advanced across a second napkin ring stricture (arrow).

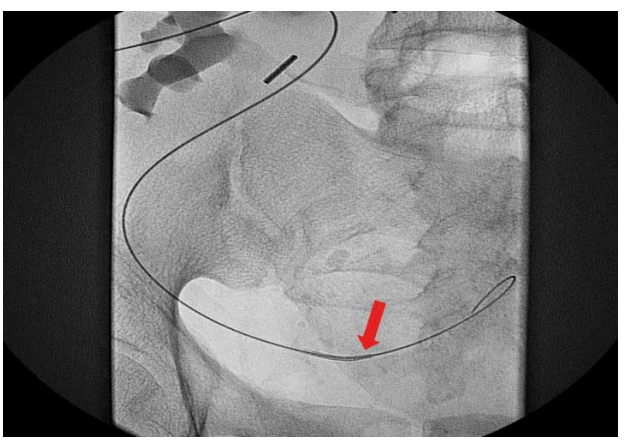
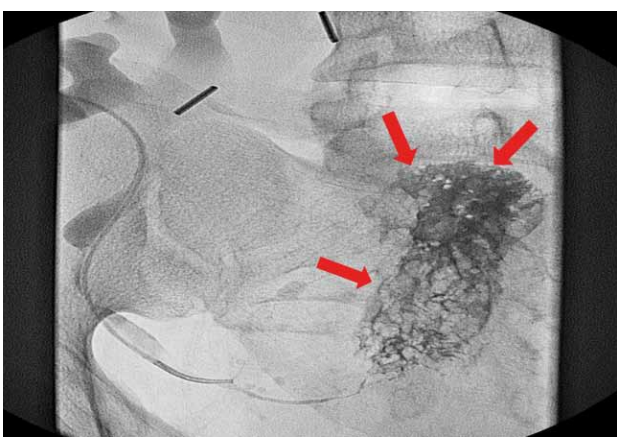


Figure 4 - A cystogram demonstrates a contracted bladder with innumerable defects. These are reminiscent of changes attributable to malakoplakia in this inactive bladder (arrows).



REFERENCES

1. Hameed O, Humphrey PA: Pseudoneoplastic mimics of prostate and bladder carcinomas. Arch Pathol Lab Med. 2010; 134: 427-43.

2. Senkul T, Yildirim S, Iseri C, Karademir K, Erden D, Baykal K: Histopathologic changes in the mucosa of ileal orthotopic neo-bladder--findings in 24 patients followed up for 5 years. Scand J Urol Nephrol. 2003; 37: 202-4.

ARTICLE INFO

Int Braz J Urol. 2013; 39: 286-7

Submitted for publication:
December 21, 2011

Accepted after revision:
May 04, 2012

Correspondence address:
Dr. Erich K. Lang
Departments of Urology and Radiology
SUNY, Downstate Health Science Center
455 Lenox Road
Brooklyn, NY, 11203, USA
E-mail: erich.lang@downstate.edu

RADIOLOGY PAGE

Vol. 39 (2): 288-290, March - April, 2013
doi: 10.1590/S1677-5538.IBJU.2013.02.19

Magnetic resonance imaging to detect vesico-symphyseal fistula following robotic prostatectomy

Ryan C. Hutchinson, David D. Thiel, Joseph M. Bestic

Department of Urology (RCH, DDT) and Department of Radiology (JMB) Mayo Clinic, Jacksonville, Florida, USA

ABSTRACT

Pubic complications following radical prostatectomy are rare. Osteitis pubis typically presents with symptoms related to irritation of the pubic rami including pain with ambulation and adduction of the leg. A 60-year-old male with prostatic adenocarcinoma underwent uneventful robotic assisted laparoscopic prostatectomy. The patient noted the onset of severe pubic pain exacerbated by ambulation approximately one month post-surgery. An abdominal/pelvic CT scan was negative for acute pathology. Due to continued discomfort, a multiplanar MRI of the pelvis was performed with and without intravenous contrast material (20 ml Omniscan). The MRI demonstrated irregularity of the bladder base and proximal urethra with a fistulous tract extending anteriorly in direct communication with the pubic symphysis joint space. Vague periarticular marrow edema-like signal and enhancement at the pubic symphysis was thought to represent osteitis pubis. The patient's symptoms resolved after one month of urethral catheter drainage, intravenous antibiotics, and anti-inflammatory therapy.

INTRODUCTION

Abnormal connections between the symphysis pubis and the lower urinary tract with associated inflammation of the pubis are rare occurrences. First described in 1924 by Beer (1) as a sequela of simple prostatectomy, osteitis pubis typically presents with symptoms related to irritation of the pubic rami, including pain with ambulation and adduction of the leg. It is classically described as a non-suppurative process; however bacterial abscesses have been cited in association (2). More recently, this finding has been associated with transurethral resection of the prostate with similar presentation, including fever, suprapubic pain, exostoses, and bilateral adductor abscesses (2). Conservative management with urinary drainage, antibiotic therapy, and anti-inflammatory medication is standard first-line therapy for most urinary fistulae, with surgical correction reserved for unresolved cases. MRI offers excellent imaging of soft tissue-based pathology and has been used to detect urinary fistulae with good results (3).

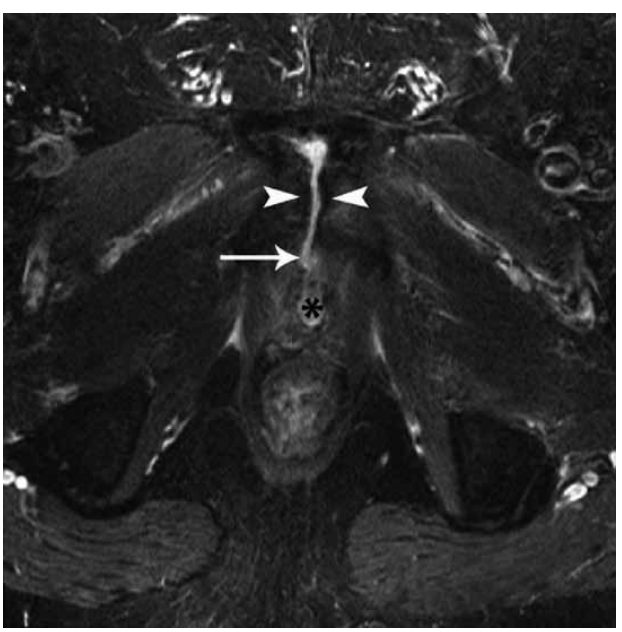
CASE REPORT

A 60-year-old male with prostatic adenocarcinoma underwent uneventful robotic assisted laparoscopic prostatectomy. The urethral catheter was found deflated and withdrawn from the glans on the evening of post-operative day number one. The catheter was replaced uneventfully, and he was discharged home the next day. Following standard catheter removal on post-operative day nine, he was readmitted with fever, rigors, and urinary cultures positive for quinolone-sensitive E.Coli. The patient defervesced with antibiotic therapy, which was continued for 14 days.

The patient noted the onset of severe pubic pain exacerbated by ambulation approximately one month post-surgery. An abdominal/pelvic CT scan was negative for acute pathology. Continued symptomatology prompted further evaluation with dedicated pelvic MRI (Figure-1). Multiplanar MRI imaging

of the pelvis performed with and without intravenous contrast material (20 ml Omniscan) demonstrated irregularity of the bladder base and proximal urethra with the fistulous tract extending anteriorly in direct communication with the pubic symphysis joint space. Vague periarticular marrow edema-like signal and enhancement at the pubic symphysis was thought to be infectious/inflammatory in etiology (Figure-2).

Figure 1 - Axial MRI.



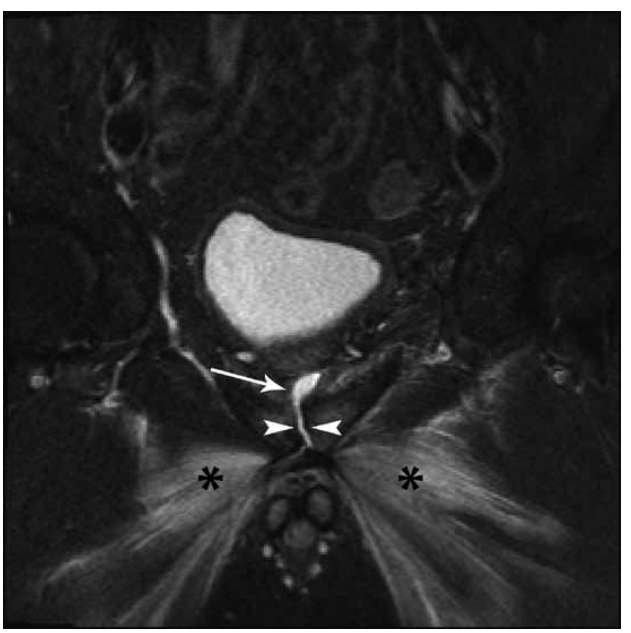
Axial image through the inferior pelvis demonstrates tract-like high signal intensity fistulous communication (white arrow) between the bladder base (black asterisk) and pubic symphysis (white arrowheads).

Similar inflammatory changes were also evident in the proximal adductor musculature bilaterally. The patient's symptoms resolved after one month of urethral catheter drainage, antibiotics, and anti-inflammatory therapy. A confirmatory CT cystogram before and after voiding demonstrated the absence of persistent fistula prior to catheter removal.

DISCUSSION

The role of MRI in the workup of a fistula involving the urinary tract is evolving. While limited by the same patient requirements as other MR exams, looking for urinary tract fistulae via MRI offers the advantage of being relatively less invasive than retrograde studies and offers excellent imaging of soft tissues, which can provide evidence for fistula even when the tract itself is not visualized. The ability to image inflammatory changes in soft tissues is especially helpful in a situation where external physical findings are not present or indeterminate. This is especially true in the case above where a symptomatic

Figure 2 - Coronal MRI.



Coronal image through the anterior pelvis demonstrates fluid signal within the pubic symphysis (white arrowheads) in continuity with fistulous tract (white arrow). Vague marrow signal abnormality involving the adjacent pubic bones with concomitant broad areas of edema-like signal tracking along the adductor musculature bilaterally (black asterisks). Signal abnormity in the pubic bones and adductor musculature is likely inflammatory in etiology.

patient had a negative CT scan of the pelvis while MRI demonstrated significant pathology. Continued progress in the field of MR imaging provides yet another useful imaging adjunct in the diagnosis and management of these challenging Urologic problems (3).

LIST OF ABBREVIATIONS

CT = computed tomography
MRI = magnetic resonance imaging
MR = magnetic resonance

REFERENCES

1. Beer E. Periostitis of the symphysis and descending rami of the pubis following suprapubic operations. Int J Med Surg 1924; 37: 224.
2. Kats E, Venema PL, Kropman RF: A rare complication after endoscopic resection of the prostate: osteitis pubis due to a prostate-symphysis fistula. J Urol. 1997; 157: 624.
3. Semelka RC, Hricak H, Kim B, Forstner R, Bis KG, Ascher SM, et al.: Pelvic fistulas: appearances on MR images. Abdom Imaging. 1997; 22: 91-5.

Correspondence address:
Dr. David D. Thiel
Mayo Clinic
Department of Urology
Davis Building 3E
4500 San Pablo Rd.
Jacksonville, FL 32224, USA
Fax: +1 904 953-2218
E-mail: thiel.david@mayo.edu

ARTICLE INFO

Int Braz J Urol. 2013; 39: 288-90

Submitted for publication:
January 05, 2013

Accepted after revision:
February 18, 2013

VIDEO SECTION

Vol. 39 (2): 291-292, March - April, 2013
doi: 10.1590/S1677-5538.IBJU.2013.02.20

Intravesical and transperitoneal laparoscopy in the management of tumor in the residual ureter

Anibal Wood Branco, Luciano Stunitz, Sandro Nichele, Paulo Henrique Douat, Murilo Luiz, Lucas Tha Nassif

Department of Urology Cruz Vermelha Hospital and Vita Batel Hospital, Curitiba, Brazil

The ocurrence of tumor in the residual ureter after an incomplete nephroureterectomy required by a tumor of renal collecting system is an uncommon but a well described situation. The recommended treatment in this situation is the radical excision of the remaining ureter, being the open technique the most used approach. The aim of this video is to demonstrate a new approach using intravesical and transperitoneal laparoscopy to remove the residual ureter following the oncological concepts. A 67 year-old male patient underwent an incomplete open right radical nephroureterectomy for a transitional cell carcinoma of the renal collecting system. After 16 months, the cystoscopy diagnostic revelead a recurrence of it in the residual ureter. An intravesical approach followed by a transperitoneal laparoscopy has removed the remaining ureter. Operative time was 110 minutes, blood loss 100 mL, the patient was discharged on the first postoperative day and the Folley cateter was removed on the seventh one. Pathological examination revelead low grade transitional cell carcinoma and free surgical margins, no recurrence was observed after six months. To our knowledge, this is the first treatment description of a tumor in the residual ureter with these techniques. This approach can be a minimal invasive alternative in this unusual situation.

ARTICLE INFO

Available at: www.brazjurol.com.br/videos/march_april_2013/Branco_291_292video.htm

Int Braz J Urol. 2013; 39 (Video #4): 291-2

Submitted for publication:
January 30, 2013

Accepted after revision:
March 01, 2013

Correspondence address:
Dr. Anibal Wood Branco
Av. República Argentina, 665 / 14a.
Curitiba, Parana, 80240-210, Brazil
E-mail: anibal@awbranco.com.br

EDITORIAL COMMENT

Dr. Branco et al. present a very nice case demonstrating and describing the laparoscopic excision of a retained distal ureter containing tumor 16 months following a right subtotal nephroureterectomy for upper tract urothelial carcinoma. This should be a very uncommon scenario as the standard of care is a complete nephroureterectomy to include the kidney, the entire ureter and a cuff of bladder. A significant percentage of patients experience disease recurrence in the ipsilateral distal ureter if it is allowed to remain in-situ. This case demonstrates a tumor recurrence that under most circumstances should be preventable. However, the authors provide us with a nice depiction of a minimally invasive surgical approach to manage this uncommon scenario.

Dr. Wade J. Sexton
Associate Member
Urologic Oncology Fellowship Director
Genitourinary Oncology Program
Moffitt Cancer Center
Tampa, Florida
E-mail: wade.sexton@moffitt.org

VIDEO SECTION

Vol. 39 (2): 293-294, March - April, 2013
doi: 10.1590/S1677-5538.IBJU.2013.02.21

Surgical Management of a Locally Advanced Symptomatic Recurrence of Penile Sarcoma Secondary to Prostate Brachytherapy

Tariq S. Hakky, Patrick Espiritu, Alejandro R. Rodriguez, Nicholas Gould, Philippe E. Spiess

Department of Genitourinary Oncology, Moffitt Cancer Center and Department of Urology, University of South Florida, Tampa, Florida, USA

ABSTRACT

Background: The surgical management of patients with symptomatic metastatic or locally advanced recurrences involving the penis remains poorly characterized. The aim of the present abstract and video is to detail our experience in the surgical management of a specific patient with a locally advanced symptomatic recurrence of penile sarcoma secondary to prostate cancer treated with primary brachytherapy.

Materials and Methods: A 70 year old male patient initially treated for localized prostate cancer with interstitial brachytherapy at an outside facility developed an unfortunate secondary malignancy consisting of a locally advanced penile sarcoma involving as well the prostate and base of the bladder. Despite our best efforts to control his pain, he developed a very symptomatic local recurrence with a secondary penile abscess and purulent periurethral drainage. At this time, it was felt a surgical resection consisting of a total penectomy, urethrectomy, cystoprostatectomy, and ileal conduit urinary diversion would be the best option for local cancer control in this particular patient.

Results: The patient underwent the surgical resection without any complications as illustrated in this surgical video, with a jejunal intestinal mass identified at the time of surgery which was resected with a primary bowel anastomosis performed. The patient was discharged from hospital uneventfully with his symptomatic local recurrence being successfully managed and the patient no longer requiring oral narcotics for pain control. The pathological report confirmed a locally advanced sarcoma involving the penile, prostate, and bladder which was resected with negative surgical margins and the jejunal mass was confirmed to represent a small bowel sarcoma metastatic site.

Conclusion: As highlighted in the present video, the treatment of a symptomatic sarcoma local recurrence contiguously involving the penis can be successfully managed provided the patient is informed of the potential morbidity and psychosocial implications imparted by performing a total penectomy and adjacent organ resection.

ARTICLE INFO

Available at: www.brazjurol.com.br/videos/march_april_2013/Hakky_293_294video.htm

Int Braz J Urol. 2013; 39 (Video #5): 293-4

Submitted for publication:
December 01, 2012

Accepted after revision:
January 30, 2013

Correspondence address:
Dr. Philippe E. Spiess,
Associate Professor
Department of Genitourinary Oncology
Moffitt Cancer Center
Tampa, FL, USA
E-mail: philippe.spiess@moffitt.org

EDITORIAL COMMENT

Radiation therapy for prostate cancer can be an effective treatment but does have its complications. In this very well done video the authors demonstrate their surgical technique for the management of a secondary penile sarcoma following brachytherapy for low-grade prostate cancer. A radical penectomy with vertical rectus myocutaneous flap in addition to radical cystoprostatectomy and ileal conduit was performed.

This was an impressive display of surgical skill for this rare but potentially devastating problem. I felt that the video was of very high quality and brings to light the difficult decisions which are needed in such a case. Although the video does suggest that the patient is free of recurrence clearly these highly aggressive tumor types are at high risk of recurrence with short to intermediate follow-up.

Dr. A. Karim Kader
Associate Professor,
Department of Urology
University of San Diego
San Diego, CA, USA
E-mail: jmthompson@ucsd.edu

VIDEO SECTION

Vol. 39 (2): 295-296, March - April, 2013
doi: 10.1590/S1677-5538.IBJU.2013.02.22

Initial Experience of Transurethral Resection with Pediatric Resectoscope for Incomplete Anterior Urethral Stricture

Won Seok Jang, Se Heon Jeong, Ho Song Yu, In Sang Hwang, Eu Chang Hwang, Sun-Ouck Kim, Kyung Jin Oh, Seung Il Jung, Dong Deuk Kwon, Kwangsung Park, Taek Won Kang

Department of Urology, Chonnam National University Medical School, Gwangju, Korea

ABSTRACT

Purpose: Endoscopic urethrotomy is an alternative method in treatment of urethral stricture. However, it have high recurrence rate because of the remained fibrotic tissue. Removal of the fibrotic tissue can maintain the patency of the urethral lumen after the procedure. We report the therapeutic efficacy of our initial experience using pediatric resectoscope for treating anterior urethral stricture in 16 cases.

Materials and Methods: From January 2009 to April 2011, transurethral resection with pediatric resectoscope was primarily performed on 16 patients with anterior urethral stricture. Retrograde urethrography, uroflowmetry, postvoid residual volume, IPSS score and QoL score were performed preoperatively. We used 11.5Fr pediatric resectoscope (Wolf) and monopolar electrosurgical generator. The stricture was incised under vision at the 12 o'clock location or the site of maximum scar tissue or narrowing in asymmetric strictures for working space. After incision, transurethral resection with pediatric resectoscope was performed to all scar tissues. Monopolar cutting current was set on 45 watt and coagulation current was set on 30 watt, fulgurate mode. Postoperatively, drainage of the bladder was performed for 7 days using an 18F latex catheter. Patients were followed up by IPSS score, QoL score, uroflowmetry and postvoid residual volume.

Results: Successful results without recurrence were achieved in 11 of 16patients. Postoperative urethral dilation had been performed average 2.4 times (0~6 times). When we classified the results by etiology, the number of successful results in strictures with a trauma, iatrogenic, or unknown cause was 5 (7/11), 3 (3/4) and 1 (1/1), respectively. In 5 patients who failed treatment, we repeated transurethral resection with pediatric resectoscope in 1 patient, and periodic urethral dilation in 4 patients. No operative complications occurred in any patients.

Conclusions: Transurethral resection with pediatric resectoscope is an effective therapeutic method for anterior urethral stricture. More long-term follow-up and large scale studies are needed to confirm the efficacy of this procedure.

ARTICLE INFO

Available at: http://www.brazjurol.com.br/videos/march_april_2013/Jang_295_296video.htm

Int Braz J Urol. 2013; 39 (Video #6): 295-6

Submitted for publication:
January 10, 2013

Accepted after revision:
February 27, 2013

Correspondence address:
Dr. Taek Won Kang
Department of Urology,
Chonnam National University Medical School 8,
Hak-dong, Dong-gu,
Gwangju #501-757, South Korea
Fax: + 82 62 227-1643
E-mail: sydad@hanmail.net

EDITORIAL COMMENT

The video by Jang et al. nicely demonstrates the technique of transurethral resection of an anterior urethral stricture. The small caliber of modern day pediatric equipment is useful in certain cases when transurethral resection is performed. The patient population consisted of sixteen patients treated primarily for stricture disease. The average stricture length was short 0.6 cm.

For patients with short strictures, excision with primary anastomosis remains the gold standard against which other techniques should be compared (1). It has proven to be a highly successful surgery and its results durable over time (2).

In this initial report, many patients required multiple dilations to maintain urethral patency. The main concern is that they will continue to require periodic dilations. The follow-up period is short (six months) and the failure rate at this short interval is 31% (5/16).

We look forward to longer term data as there may exist a subset of patients who can be treated effectively with durable results in this manner.

REFERENCES

1. Jordan GH, Schlossberg SM: Surgery of the penis and urethra. In: Wein AJ, et al, (ed.), Campbell-Walsh Urology. Vol 1. 9th ed. Philadelphia, Pa: WB Saunders Co; 2007; pp. 1023-97.

2. Mundy AR: Management of urethral strictures. Postgrad Med J. 2006; 82: 489-93.

Dr. Hubert Swana
Pediatric Urology
Nemours Children's Hospital Orlando
Orlando, FL, USA
E-mail: hswana@nemours.org

INTERNATIONAL
BRAZ J UROL

INFORMATION FOR AUTHORS

Manuscripts submitted for publication should be sent to:

Sidney Glina, M.D, PhD
Editor, International Braz J Urol

by e-mail with attached text files and figures to:
submission@brazjurol.com.br

Manuscripts must be written in current English or Portuguese. Non-native English speakers should ask a native specialist in medical English for checking the grammar and style. Either American or British English may be used but should be consistent throughout the manuscript.

A submission letter signed by all authors must accompany each manuscript. This letter must state that: a)- the paper or portion thereof have not been previously published and are not under consideration by another Journal, b)- that all authors have contributed to the information or material submitted for publication, and that all authors have read and approved the manuscript, c)- that the authors have no direct or indirect commercial financial incentive associated with publishing the manuscript, d)- that the source of extra-institutional funding, specially that provided by commercial companies, is indicated, e)- that the study had been reviewed and approved by a certified Ethical Board or Committee, f)- a non-plagiarism statement (I (We) declare that all material in this assignment is my (our) own work and does not involve plagiarism). After accepted for publication, the manuscript will become property of the **International Braz J Urol.**

Conflict of Interest – Any conflict of interest, mainly financial agreement with companies whose products are alluded to in the paper, must be clearly disclosed when submitting a manuscript for review. If accepted, a disclosure will be published in the final manuscript.

The requirements for authorship and the general rules for preparation of manuscripts submitted to the **International Braz J Urol** are in accordance with the Uniform Requirements for Manuscripts Submitted to Biomedical Journals (International Committee of Medical Journal Editors. Uniform Requirements for Manuscripts Submitted to Biomedical Journals. Ann Intern Med, 126: 36-47, 1997). An electronic version of the Uniform Requirements is available on various websites, including the International Committee of Medical Journal Editors web site: www.icmje.org.

In response to the concerns of the editors of scientific medical journals with ethics, quality and seriousness of published articles, a Committee on Publication Ethics (COPE) was established in 1997 and a guideline document was published. The International Braz J Urol signed, approved, and follows the COPE guidelines. The Editor strongly encourages the authors to carefully read these guidelines before submitting a manuscript **(www.publicationethics.org.uk/guidelines or www.brazjurol.com.br**, vol. 26 (1): 4-10, 2000).

Peer Review – All submissions are subject to editorial review. Typically, each manuscript is anonymously forwarded by the Editor to 4 Reviewers (at least 2). If the Editor receives conflicting or inconclusive revisions, the manuscript is always sent to 1 or 2 additional Reviewers before the Editor's decision. If considered necessary by the Editor or by the Reviewers, statistical procedures included in the manuscript will be analyzed by a statistician.

The **International Braz J Urol** contains six sections: **Original Article, Review Article, Surgical Technique, Challenging Clinical Case, Radiology Page** and **Video Section.** The articles should be written in Portuguese or English official orthography.

Abbreviations should be avoided, and when necessary must be specified when first

time mentioned. Unusual expressions may not be used. A list of abbreviations must be provided at the end of the manuscript.

Every manuscript submitted to publication should have a cover page containing the title, short title (up to 50 characters), authors and institution. Up to six key words should be provided. These words should be identical to the medical subject headings (MeSH) that appear in the Index Medicus of the National Library of Medicine **(http://www.nlm.nih.gov/mesh/meshhome.html)**. One of the authors should be designated as correspondent and the complete correspondence address, telephone and fax numbers and E-mail should be provided.

If any financial support has been provided, the name of the institution should be mentioned.

Original Article: Original articles should contain a Cover Page, Abstract, Introduction, Materials and Methods, Results, Discussion, Conclusions, References, Tables and Legends, each section beginning in a separate page and numbered consecutively. Original articles should cover contemporary aspects of Urology or experimental studies on Basic Sciences applied to urology. The manuscript text should contain no more than 2500 words, excluding the Abstract. The number of authors is limited to five. References should contain no more than 30 citations, including the most important articles on the subject. Articles not related to the subject must be excluded.

Review Article: Review articles are accepted for publication upon Editorial Board's request in most of the cases. A Review Article is a critical and systematic analysis of the most recent published manuscripts dealing with a urological topic. A State of the Art article is the view and experience of a recognized expert in the topic. An abstract must be provided.

Surgical Technique: These manuscripts should present new surgical techniques or instruments and should contain Introduction, Surgical Technique, Comments and up to five References. An abstract must be provided. At least five cases performed with the technique must be included.

Challenging Clinical Case: These manuscripts should present relevant clinical or surgical situations which can bring or consolidate our understanding of genesis, natural history, pathophysiology and treatment of diseases. *Structure of the articles*

Abstract (maximum 200 words) and should contain

- **Main findings:** Report case(s) relevant aspects
- **Case(s) hypothesis:** Proposed premise substantiating case(s) description
- **Promising future implications:** Briefly delineates what might it add? Lines of research that could be addressed

Full text (maximum 2000 words):

- **Scenario:** Description of case(s) relevant preceding and existing aspects;
- **Case(s) hypothesis and rational:** precepts, clinical and basic reasoning supporting the case(s) hypothesis and the raised scenario. Why is it important and is being reported?
- **Discussion and future perspectives:** what might it add and how does it relate to the current literature. 'Take-home message' - lessons learnt;
- **Table and/or Figure limits:** 2 (plates aggregating multiple images are encouraged) each exceeding table or figure will decrease 250 words of the full text;
- **Number of references:** 10-15.

Radiology Page: Will be published upon the Section Editor decision.

Video Section: The material must be submitted in the appropriate local, in the Journal's site, where all instructions may be found (Video Section link) Letters to the Editor: The letter should be related to articles previously published in the Journal, should be useful for urological practice and must

not exceed 500 words. They will be published according to the Editorial Board evaluation.

ILLUSTRATIONS:

The illustrations should not be sent merged in the text. They should be sent separately, in the final of the manuscript.

1) The number of illustrations should not exceed 10 per manuscript.
2) Check that each figure is cited in the text.
3) The legends must be sent in a separate page.
4) The legends of histological illustrations should contain the histological technique and the final magnification.
5) The International Braz J Urol encourages color reproduction of illustrations wherever appropriate.
6) All histological illustrations should be supplied in color.

ELECTRONIC SUBMISSION:

1) Do not embed the figures in the text, but supply them as separate files.
2) For Submitting Photographs Electronically, please:
Supply photographs as TIFF (preferable) or JPG files. The TIFF of JPG should be saved at a resolution of 300 dpi (dots per inch) at final size. If scanned, the photographs should be scanned at 300 dpi, with 125mm width, saved as TIFF file and in grayscale, **not embed in Word or PowerPoint.**
3) For Submitting Line Artwork Electronically please note that:
Line drawings must be supplied as EPS files (give an EPS extension, e.g. Fig01.eps). Use black text over light to mid grey and white text over dark grey or black shades. Use lower case for all labeling, except for initial capitals for proper nouns and necessary mathematical notation. Centre each file on the page and save it at final size with the correct orientation. We recommend a minimum final width of 65 mm, but note that artwork may need to be resized and relabeled to fit the format of the Journal.

4) IMPORTANT - Avoid - Do Not

a) **DO NOT** embed the images in the text; save them as a separate file
b) **DO NOT** supply artwork as a native file. Most illustration packages now give the option to "save as" or export as EPS, TIFF or JPG.
c) **DO NOT** supply photographs in PowerPoint or Word. In general, the files supplied in these formats are at low resolution (less than 300 dpi) and unsuitable for publication.
d) **DO NOT** use line weights of less than 0.25 point to create line drawings, because they will nor appear when printed.

TABLES: The tables should be numbered with Arabic numerals. Each table should be typed on a single page, and a legend should be provided for each table. Number tables consecutively and cites each table in text in consecutive order.
REFERENCES: The References should be numbered following the sequence that they are mentioned in the text. The references should not be alphabetized. They must be identified in the text with Arabic numerals in parenthesis. Do not include unpublished material and personal communications in the reference list. If necessary, mention these in the body of the text. For abbreviations of journal names refer to the "List of Journals Indexed in Index Medicus" (http://www.nlm.nih.gov). The authors must present the references according to the following examples; the names of **all authors** must be included; when exist more than six authors, list the first six authors followed **by et al.** The **initial** and the **final** pages of the reference should be provided:

Papers published in periodicals:

▪ Paterson RF, Lifshitz DA, Kuo RL, Siqueira Jr TM, Lingeman JE: Shock wave lithotripsy monotherapy for renal calculi. Int Braz J Urol. 2002; 28:291-301.
▪ Holm NR, Horn T, Smedts F, Nordling J, de la Rossete J: Does ultrastructural morphology of human detrusor smooth muscle cell characterize acute urinary retention? J Urol. 2002; 167:1705-9.

Books:
▪ Sabiston DC: Textbook of Surgery. Philadelphia, WB Saunders. 1986; vol. 1, p. 25.

Chapters in Books:
▪ Penn I: Neoplasias in the Allograft Recipient. In: Milford EL (ed.), Renal Transplantation. New York, Churchill Livingstone. 1989; pp. 181-95.

The Int Braz J Urol has the right of reject inappropriate manuscripts (presentation, number of copies, subjects, etc.) as well as proposes modifications in the original text, according to the Referees' and Editorial Board opinion.

THE EDITORS SUGGEST THE AUTHORS TO OBSERVE THE FOLLOWING GUIDELINES WHEN SUBMITTING A MANUSCRIPT:

The **Ideal Manuscript** may not exceed 2500 words.

The **Title** must be motivating, trying to focus on the objectives and content of the manuscript.

Introduction must exclude unnecessary information. It should briefly describe the reasons and objective of the paper.

Materials and Methods should describe how the work has been done. It must contain sufficient information to make the study reproducible. The statistical methods have to be specified.

The **Results** should be presented using Tables and Figures whenever possible. Excessive Tables and Figures must be avoided. The tables should not be repeated on the text.

The **Discussion** must comment only the results of the study, considering the recent literature.

Conclusions must be strictly based on the study findings.

References should contain no more than 30 citations, including the most important articles on the subject. Articles not related to the subject must be excluded.

The Abstract must contain up to 250 words and must conform to the following style: Purpose, Materials and Methods, Results and Conclusions. Each section of the manuscript must be synthesized in short sentences, focusing on the most important aspects of the manuscript. **The authors must remember that the public firstly read only the Abstract, reading the article only when they find it interesting.**

NOTE:
Recent issues of the International Braz J Urol must be observed concerning the presentation form of the manuscript.

INTERNATIONAL
BRAZ J UROL

MANUSCRIPT CHECKLIST

The authors should observe the following checklist before submitting a manuscript to the **International Braz J Urol**

- The sequence of manuscript arrangement is according to the Information for Authors.
- The Article is restricted to about 2,500 words and 6 authors.
- Abbreviations were avoided and are defined when first used and are consistent throughout the text.
- Generic names are used for all drugs. Trade names are avoided.
- Normal laboratory values are provided in parenthesis when first used.
- The references were presented according to the examples provided in the Information for Authors. The references were numbered consecutively, following the sequence that they are mentioned in the text. They were identified in the text using Arabic numeral in parenthesis. The names of all authors were provided. When exist more than six authors, list the first sixauthors followed by et al. The initial and the final pages of the reference should be provided. The number of references must be accordingly to the informed in the Instructions for Authors, depending on the type of manuscript.
- The staining technique and the final magnification were provided for all histological illustrations. The histological illustrations are supplied in color.
- Legends were provided for all illustrations, tables, and charts. All tables and charts were in separate pages and referred to in the text. All illustrations and tables are cited in the text.
- An Abstract was provided for all type of articles. The length of the Abstract is about 250 words.
- A corresponding author with complete address, telephone, Fax, and E-mail are provided.
- A submission letter and a disclosure form, signed by all authors, are included.
- The authors should included written permission from publishers to reproduce or adapt a previously published illustrations or tables.
- **Conflict of Interest** – Any conflict of interest, mainly financial agreement with companies whose products are alluded to in the paper, is clearly disclosed in the manuscript.
- **Check that each figure is cited in the text. The illustrations are not merged in the text.**
- The photographs are supplied as TIFF or JPG files and saved at a resolution of 300 dpi (dots per inch) at final size.
- The photographs should be scanned at 300 dpi, with 125mm width, saved as TIFF file and in grayscale, not **embed in Word or PowerPoint.**
- A list of abbreviations is provided.