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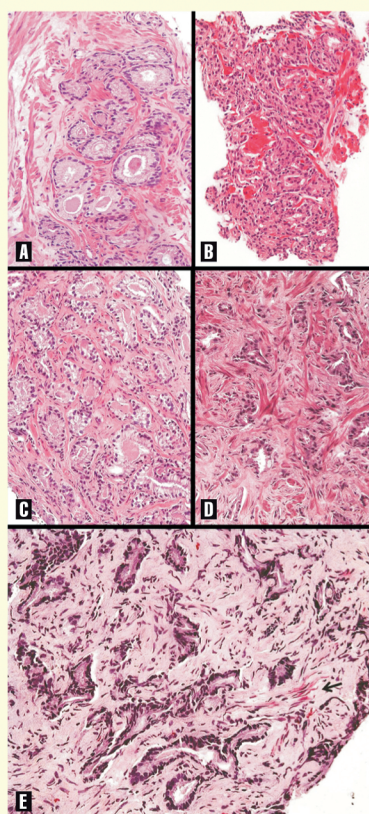


Figure 1 - (A) Grade 0 (absent reactive stroma): Gleason score 6 adenocarcinoma; (B) Grade 0 (absent reactive stroma): Gleason score 7 adenocarcinoma; (C) Grade 1 (slight reactive stroma); (D) Grade 2 (moderate reactive stroma); (E) Grade 3 (intense reactive stroma). Only some few residual smooth muscle fibers are seen (arrow) (hematoxylin-eosin, x165). (Page 322)



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Prostate Cancer: Prognosis and Recurrence

The May-June 2013 issue of the International Braz J Urol presents original contributions and editorials from many different countries such as Brazil, Korea, United Kingdom, Italy, Germany, Canada, Turkey, Colombia and USA. In this number we had several papers about prostate cancer and some of these papers will be highlights in our comments.

Doctor Novaes and colleagues from Federal University of Bahia and Federal University of São Paulo, Brazil, performed on page 305 a systematic review about single scrotal incision. The majority of cryptorchidic testes are in the superficial inguinal pouch of Denis Browne. The inguinal procedure requires two incisions. In this study the authors make a review about the scrotal incision to treat cryptorchidism and they conclude that Single scrotal incision orchiopexy proved to be an effective technique and is associated with low rates of complications.

Dr. Billis and colleagues from State University of Campinas (Unicamp), São Paulo, Brazil, performed on page 320 an elegant study about the adenocarcinoma on needle prostatic biopsies. The aim of this study is to establish any possible relation of reactive stroma grading on needle prostatic biopsies to biochemical recurrence and they conclude that increasing reactive stroma grade on biopsies is significantly associated with several clinicopathologic adverse findings, however, only grade 3 predicts time and risk to biochemical recurrence following radical prostatectomy on univariate but not on multivariate analysis.

Doctor Pontes-Junior and colleagues from University of São Paulo Medical School, Brazil, performed on page 335 a study about the correlation between Beta1 integrin expression and prognosis in clinically localized prostate cancer. The aim of this study was to evaluate the expression of $\beta 1$ integrin in



localized PC and to correlate the pattern of expression with recurrence after surgical treatment. The authors concluded that the loss of $\beta 1$ integrin immune expression was correlated with biochemical recurrence in patients treated with radical prostatectomy for localized prostate cancer.

Doctor Mazaris and colleagues from Lister Hospital in United Kingdom performed on page 364 a study about the transurethral resection and the risk of recurrence in superficial and invasive bladder cancer. The aim of this study was to determine which factors contributed to the absence of tumor in our series of radical cystectomy patients. The authors concluded that four factors were identified in our study to contribute towards a pT0 cystectomy result. Those included the absence of lymphovascular invasion, the completeness of transurethral resection, the experience of the surgeon and the use of a standardized technique for the transurethral resection. The time to cystectomy in this paper did not have a negative effect on pT0 final pathology result.

Doctor Sammon and colleagues in a multicentric study, performed on page 377 an interesting study about the Robot-assisted (RAPN) vs. Laparoscopic Partial Nephrectomy (LPN). The authors concluded that RAPN has supplanted LPN as the predominant minimally invasive surgical approach for renal masses. Perioperative outcomes after RAPN and LPN are comparable. Interpretation of these findings needs to take into account the lack of adjustment for case complexity and surgical expertise.

Doctor Karaman and Colleagues from Haydarpasa Numune Training and Research Hospital, Istanbul, Turkey performed on page 402 a interesting study about the Punishment Implemented by Families to Enuretic children. The authors studied more than 500 children and concluded that a quite high proportion of enuretic children were detected to be exposed to punishment methods. Even, some parents consider that these methods are a part of nocturnal enuresis treatment.

Doctor Sayeg and colleagues from Federal University of Sao Paulo, Brazil performed on page 414 a translational study in rabbits about the integration of collagen matrices into the urethra when implanted as onlay graft and they



concluded that Natural heterologous matrices implanted in the urethra as onlay graft were not incorporated into its walls but were able to fully restore the cell architecture of the organ, regardless of being seeded or not with autologous muscle cells.

LUCIANO A. FAVORITO, MD, PhD

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Single scrotal incision orchiopexy – a systematic review

Hugo Fabiano Fernandes Novaes, José Abraão Carneiro Neto, Antonio Macedo Jr, Ubirajara Barroso Júnior

Section of Pediatric Urology, Division of Urology Bahiana School of Medicine and Federal University of Bahia and Federal University of São Paulo

ABSTRACT

Objective: To conduct a systematic review on single scrotal incision orchiopexy.

Materials and Methods: A search was performed using Pubmed, through which 16 articles were selected out of a total of 133. The following conditions were considered exclusion criteria: other surgical methods such as an inguinal procedure or a laparoscopic approach, retractile testes, or patients with previous testicular or inguinal surgery.

Results: A total of 1558 orchiopexy surgeries initiated with a transscrotal incision were analyzed. Patients' ages ranged between 5 months and 21 years. Thirteen studies used high scrotal incisions, and low scrotal incisions were performed in the remainder of the studies. In 55 cases (3.53%), there was a need for inguinal incision. Recurrence was observed in 9 cases, testicular atrophy in 3, testicular hypotrophy in 2, and surgical site infections in 13 cases. High efficacy rates were observed, varying between 88% and 100%.

Conclusions: Single scrotal incision orchiopexy proved to be an effective technique and is associated with low rates of complications.

ARTICLE INFO

Key words:

Cryptorchidism; Orchiopexy; Scrotum; Surgical Procedures, Operative

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INTRODUCTION

Cryptorchidism is the most common pathology during childhood (1), affecting 2-4% of children at birth and decreasing to 1% in the first year of life (2,3). The majority of cryptorchidic testes are in the superficial inguinal pouch of Denis Browne, making inguinal exploration the traditional surgical correction procedure (4).

The inguinal procedure requires two incisions: one in the groin to release the testicle, and another scrotal incision to make a pocket to accommodate the testicle. In order to reduce the potential morbidity of this treatment, in 1989 Bianchi and Squire introduced a technique utilizing a single scrotal incision (5). It is suggested that this technique adds the advantages of a single incision, shorter operation time, ease

of dissection, accelerated healing, less pain, good maintenance of testicular position and an excellent cosmetic result (6).

Despite the good results of transscrotal orchiopexy found in the literature, its use is not widespread. The objective of this study is to perform a systematic review on the subject.

MATERIALS AND METHODS

Our Experience

We retrospectively evaluated 18 patients (22 testes) who underwent scrotal orchiopexy between August 2007 and August 2009. We reviewed the charts concerning laterality of the surgery, age of the patients, inguinal conduit persistence and whether the patients had undergone a previous surgery for undescended testis.

All patients were examined under anesthesia and the decision of whether or not to perform a transcrotal approach was based on the ability to push the testis to the level of external inguinal ring. The incision was horizontal at the lowest part of the hemiscrotum for unilateral cases (Figures 1a and 1b), and longitudinal at the scrotal raphe for bilateral undescended testes (Figure-2).

In the technique of scrotal orchiopexy we first manipulate the testis down toward the external inguinal ring. The testis is grasped with the thumb and the index finger. With the surgeon keeping traction on the testis the scrotal incision is performed and the testis and spermatic chord are freed from the cremasteric fascia (Figure-1a). When present, the inguinal conduit persistence is closed. Because the inguinal canal is short in children, the conduit can be closed at the level of the internal inguinal ring. The dissection of the inguinal conduit elongates enough the spermatic chord, making it possible for the testes to reach the scrotum easily. The testes are fixated in the scrotum by means of a Dartos pouch. The patients are discharged the same day of the surgery.

The patients were followed up regularly. They were directed to return to the office one week, one month and six months after surgery and then on a yearly basis.

Systematic review

A systematic review was performed through an electronic search on the Pubmed database using the following key words and combinations thereof: scrotal incision, cryptorchidism, orchiopexy and undescended testis. The search yielded 133 articles. Prospective and retrospective articles were selected which evaluated children with primary cryptorchidism who were treated surgically through single scrotal incision orchiopexy. Articles were excluded if they: evaluated secondary cryptorchidism from previous procedures or any other pathology; evaluated patients who underwent orchiopexy via another procedure (inguinal or laparoscopic). The effectiveness of the technique was evaluated, defined as the presence of the testis in the scrotum after the procedure, without subsequent hypotrophy or atrophy, and without the need for an inguinal incision.

Figure 1a - The testis mobilized and delivered through the incision.

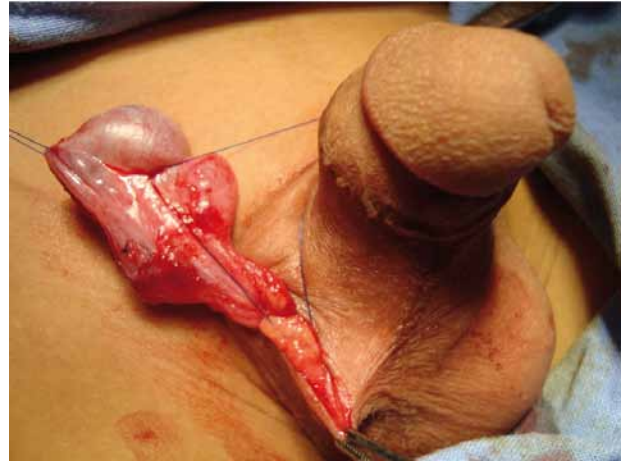


Figure 1b - Final appearance for unilateral undescended testis.



Figure 2 - Final appearance for bilateral undescended testes.



After evaluating the abstracts, 88 articles were excluded that dealt with other issues, 23 that used laparoscopy as a means of access, and 4 which were review articles; 18 articles remained to be reviewed. After verifying the references of the selected articles, we included two articles (7,8), giving a total of 20 articles. After analyzing the full articles, one study was excluded which studied the same group of patients as another study published later (9), one article which evaluated the technique in patients with retractile and reoperated testicles (10), another which only evaluated patients with secondary or relapsed cryptorchidism and another article which did not evaluate the surgical results of the orchiopexy separately from other inguinal procedures (8). In the end, 16 articles were selected for review. The present study was completed in March 2011.

RESULTS

Our results

Eighteen patients (22 testes) underwent scrotal orchiopexy. The surgery could be performed via scrotal approach in all cases and the testes could be brought down to the scrotum with no tension. No inguinal incision was necessary.

Persistence of inguinal conduit was found in 16 (72%) cases. In all, the conduit could be closed at the level of inguinal internal ring. There were no intraoperative or postoperative complications.

Two patients had unsuccessfully undergone previous inguinal surgery for undescended testis. The testes remained in the superficial inguinal pouch of Denis Browne and could be approached via scrotal approach with no complication.

In a mean follow up of 18 months (ranging from 3 to 37 months), no complication such as infection, testicular atrophy, hydrocele or hernia was observed.

Systematic review

Results are shown in Table-1. A total of 1558 orchiopexy surgeries initiated with a transscrotal incision were analyzed. The patients were examined in standing positions and un-

der anesthesia, excluding cases of retractile testis. The age range varied between 5 months and 21 years. All patients studied underwent single scrotal incision orchiopexy; 13 studies used high scrotal incisions, and low scrotal incisions were performed in the remainder. Eleven studies recorded the incidence of patent processus vaginalis, which was present in 324 of the 1090 cases evaluated (29.7%).

In 55 cases (3.53%) an inguinal incision was necessary. These patients had high testes (1,2,11-14) requiring retroperitoneal dissection (4,13,15) or the presence of the vaginal process or hernia (1,11,16).

Only one case presented intraoperative complications (injury to the vas deferens). Study 3 did not evaluate intraoperative complications. In one article, the authors reported no intraoperative or postoperative complications and 100% efficacy. In this study, the follow-up was only 3 months (17).

The post-operative follow-up was heterogeneous, ranging from 3 months to 5 years, with a lack of follow-up in some studies. Recurrence was observed in 9 cases (2-4,16,18), testicular atrophy in 3 (2,16), testicular hypotrophy in 2 patients (11) and surgical site infections in 13 (2,11,12,16,19-21). Hematomas occurred in 22 cases (2,3,12,15,16); in Study 2 alone 16 cases of hematoma were observed (15.09% of the patients in the study). Study 2 also showed the highest rates of testicular atrophy (2 cases) and recurrence (5 cases), out of a total of 106 orchiopexies.

Given the concept of efficacy that was pre-established (the post-operative result after single scrotal incision orchiopexy, with no subsequent atrophy, hypotrophy or any other complication that may result in orchiectomy), rates of success were found to be between 88 and 100%, as shown in Table-1.

DISCUSSION

Orchiopexy is a necessary procedure for the treatment of a common problem in the pediatric population. It is traditionally performed through an inguinal procedure, with a second incision made in the scrotum to set the testicle.

Table 1 - Review of the published literature about transscrotal orchidopexy.

Authors	Year	Design	No. of patients	No. of Orchiopexies	Incision	Patent Processus Vaginalis	Inguinal incision	Age	Intraoperative complications	Postoperative complications	Follow-up	Efficacy (%)	Reference
Lais A et al	1996	Prospective	50	50	High scrotal	13	3	5 months to 14 years (mean 5 years)	0	Hematoma: 3; Recurrence: 1; Hipotrophy: 2	3 to 5 years	88	15
Misra D et al	1997	Prospective	NE	67	High scrotal	0	9	1 to 12 years (mean: 6.8 years)	0	0	1 to 5 years	86.5	14
Jawad AJ	1997	Prospective	96	106	High scrotal	NE	14	14 months to 11 years (mean: 41 months)	0	Recurrence: 5; Atrophy: 2; Hematoma: 16; Infection: 2	8 to 36 months (mean: 16 months)	93.3	2
Caruso A et al	2000	Prospective	34	42	High scrotal	3	1	media: 03 years (mean: 14 months)	0	Recurrence: 1	6 months to 2 years (mean: 1 year)	96.6	4
Parsons JK et al	2003	Prospective	52	66	Low scrotal	13	0	Below 2 years-old: 16 patients; 2 to 6 years: 19 patients; 6 years: 17 patients	0	0	3 months	100	17
Russinko PJ et al	2003	Retrospective	78	85	High scrotal	58	1	4.5 years (0.5 a 24 years)	0	Recurrence: 2; Hematoma: 1; Atrophy: 1; Infection: 1	1 to 36 months (mean: 6 months)	95.2	16
Handa R et al	2006	Prospective	28	35	High scrotal	NE	0	10 months to 9 years	0	Infection: 1; Hernia: 1	2 to 6 months	100	19
Dayanc M et al	2007	Prospective	166	204	High scrotal	72	12	10 months to 12 years (mean 2.2 years; median 3.2 years)	NE	Hydrocele: 1; Hernia: 01;	16 to 68 months	92.7	1

Bassel YS et al	2007	Retrospective	103	121	High scrotal	75	0	6 months a 13 years (mean: 4.5 years)	0	Infection: 4	6 months to 1 year	100	21
Samuel DG et al	2008	Prospective	156	206	High scrotal	NE	1	1 a 21 years (mean: 4.26 years)	0	Infection: 2	2 years	99.95	11
Takahashi M et al	2009	Prospective	32	49	Low scrotal	14	0	11.5 a 114.0 months (mean: 39.3 months)	0	Recurrence: 1	12.1 to 68.8 months (mean: 39.1 months)	97.95%	18
Al-Mandil M et al	2008	Retrospective	56	63	Low scrotal	NE	0	4.6 years	0	Recurrence: 1; Hydrocele: 1; Hernias: 2; Infection: 1	6 to 42 months	98.41	20
Callearwaert PR et al	2009	Prospective	154	194	High scrotal	11	2	4 a 229 months (mean: 71 months)	0	Section of vas deferens: 1	3 to 22 months (mean: 10 months)	100	12
Gordon M et al	2010	Retrospective	118	122	Low scrotal	NE	3	10 months to 8 years	0	Recurrence: 12	6 months to 1 year	89.3	6
Cloutier J et al	2011	Retrospective	44	60	High scrotal	NE	1	53 (\pm 23) months	0	Recurrence: 1; Hematoma: 1	3 months to 2 years	99.3	3
Yucel S et al	2011	Retrospective	74	88	High scrotal	65	8	6 months to 11 years (mean: 4.9 years)	0	0	3 to 12 months (mean: 7.1 months)	90.9	13
Authors	2007 - 2009	Prospective	16 (more 2 patients was re-do)	20 (more 2 cases was re-do)	Low scrotal	16	0	3 to 108 months (mean 42.5 months)	0	0	3 to 17 months (mean: 18 months)	100	-

NE: not evaluated

After introduction of the single incision transcrotal technique by Bianchi and Squire in 1989, it became possible to treat these patients with high rates of effectiveness and lower postoperative morbidity.

After analyzing the selected articles, a high level of efficacy was observed in scrotal surgery, which was able to be performed in 85% of cases. Unfortunately, the location of the testis and the criteria for surgery are not well established in the studies, making it difficult to compare them in terms of efficacy.

The rate of relapse was small (1.43%), as was the rate of testicular atrophy/hypotrophy (0.3%). However, the follow-up times presented in the studies are short and many do not provide follow-ups. In 1995, Docimo et al. (22) conducted a systematic review, analyzing 8425 inguinal orchiopexies. Among these studies, the position of the testicles was identified in only 2491 of them. It was observed that the success rate of the surgery after 6 months of follow-up was 83.9% for the cases of intracanalicular testes and 92.3% for the cases with testes in the external inguinal ring. When the authors analyzed the literature that has been published since 1985, the success rates rose to 95.7% with intracanalicular testes and 100% with the testes located below the external inguinal ring. Finally, when analyzing a subgroup of patients who only underwent the inguinal procedure (1556 orchiopexies), the rate of success after 6 months was 86.4%. When analyzing the data after 1985 (677 orchiopexies), the success rate was 85.2%.

The incidence of patent processus vaginalis varied among the studies. In cryptorchidism, the processus vaginalis was patent in 20 to 73% of cases (13,17), and was lower in cases of retractile testes. Therefore, we can infer that the surgery was indicated for many gliding testis. The lack of surgical reports regarding the presence of this finding may interfere with the result.

The fact that only four hernias occurred postoperatively may reflect the lack of follow-up, but may also reflect the effectiveness of treating patent processus vaginalis through the scrotum. In children, the inguinal portion of the processus vaginalis is short (median 1 cm in children under 2 years and median 1.1 cm in children over 4 years) (23) and by using traction, it is possible to perform

the tubal ligation at practically the same level as the internal inguinal ring.

Only 11 articles report the location of the testis; in 8 of the studies the authors indicated scrotal orchiopexy for all the cases and in 8 studies the surgery was only indicated when it was possible to bring the testicles to the scrotum with the patient anesthetized (1,3,11,14,16,18,19,21). In 5 studies the rate of success was higher than 95% and two reported 100% of success. In our experience using this method, we were able to adequately position the testicles in the scrotum in all cases. In our department, low scrotal incision orchiopexy is indicated for cases where traction and relocation of the testis to the upper third of the scrotum is possible with the patient anesthetized. We have operated on 22 consecutive orchiopexies through low scrotal incisions (data not published) and found persistent processus vaginalis in 16 cases (72%). In all cases, it was possible to dissect and correct the patency of the processus vaginalis through the same incision. After a mean follow-up of 18 months, we did not find a single recurrence or any complication such as surgical site infection, hematomas, or atrophy.

This systematic review demonstrates the poor methodological quality of the articles which are available for the comparison of data. There was a significant lack of follow-up, some studies are retrospective, there is no randomization, and a great heterogeneity of techniques was used (high and low incisions); there was also a heterogeneity of cases selected (there are cases in which impalpable testes were treated by single scrotal incision). Given this heterogeneity, it is impossible to conduct a meta-analysis of the success rate.

Based on the evidence presented in the literature, we believe that in the cases in which the testicles are found in a low position, making it possible to move them to the scrotum, scrotal orchiopexy is the procedure of choice. However, for those testicles that are located in a higher position, comparative studies with inguinal orchiopexy should be performed.

CONCLUSIONS

Single scrotal incision orchiopexy is an effective technique associated with low complica-

tion rates. We believe that this technique is preferable in cases where the testicles are displaced up to the level of the external inguinal ring, even in reoperations and cases of previous inguinal surgery. Randomized, prospective, and multicenter studies are necessary to obtain better scientific evidence, specially for those testicles that are located in a higher position.

CONFLICT OF INTEREST

None declared.

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Correspondence address:

Dr. Ubirajara Barroso Jr.
Rua Sócrates Guanaes Gomes, 73 / 2004
Cidade Jardim
Salvador, Bahia, 40296-720, Brazil
E-mail: ubarroso@uol.com.br



High serum folate is associated with reduced biochemical recurrence after radical prostatectomy: Results from the SEARCH Database

Daniel M. Moreira, Lionel L. Bañez, Joseph C. Presti Jr, William J. Aronson, Martha K. Terris, Christopher J. Kane, Christopher L. Amling, Stephen J. Freedland

Division of Urologic Surgery, Department of Surgery, Duke Prostate Center (DMM, LLB, SJF), Department of Pathology (SJF), Duke University School of Medicine; Urology Section (DMM, LLB, SJF), Veterans Affairs Medical Center, Durham, NC; Department of Urology (JCPJr), Stanford University Medical Center and Urology Section, Department of Surgery (JCPJr), Veterans Affairs Medical Center, Palo Alto; Urology Section (JCPJr), Department of Surgery (WJA), Veterans Affairs Medical Center, Greater Los Angeles; Department of Urology (WJA), University of California at Los Angeles Medical Center, Los Angeles, California; Urology Section (MKT), Division of Surgery (MKT), Veterans Affairs Medical Centers and Division of Urologic Surgery (MKT), Department of Surgery (MTR), Medical College of Georgia, Augusta, Georgia; Division of Urology (CJK), Department of Surgery (CJK), University of California at San Diego Medical Center, San Diego, California and Division of Urology (CLA), Department of Surgery (CLA), Oregon Health & Science University, Portland, Oregon, USA

ABSTRACT

Introduction: To analyze the association between serum levels of folate and risk of biochemical recurrence after radical prostatectomy among men from the Shared Equal Access Regional Cancer Hospital (SEARCH) database.

Materials and Methods: Retrospective analysis of 135 subjects from the SEARCH database treated between 1991-2009 with available preoperative serum folate levels. Patients' characteristics at the time of the surgery were analyzed with ranksum and linear regression. Uni- and multivariable analyses of folate levels (log-transformed) and time to biochemical recurrence were performed with Cox proportional hazards.

Results: The median preoperative folate level was 11.6ng/mL (reference = 1.5-20.0ng/mL). Folate levels were significantly lower among African-American men than Caucasians ($P = 0.003$). In univariable analysis, higher folate levels were associated with more recent year of surgery ($P < 0.001$) and lower preoperative PSA ($P = 0.003$). In univariable analysis, there was a trend towards lower risk of biochemical recurrence among men with high folate levels ($HR = 0.61$, $95\%CI = 0.37-1.03$, $P = 0.064$). After adjustments for patients characteristics' and pre- and post-operative clinical and pathological findings, higher serum levels of folate were independently associated with lower risk for biochemical recurrence ($HR = 0.42$, $95\%CI = 0.20-0.89$, $P = 0.023$).

Conclusion: In a cohort of men undergoing radical prostatectomy at several VAs across the country, higher serum folate levels were associated with lower PSA and lower risk for biochemical failure. While the source of the folate in the serum in this study is unknown (i.e. diet vs. supplement), these findings, if confirmed, suggest a potential role of folic acid supplementation or increased consumption of folate rich foods to reduce the risk of recurrence.

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INTRODUCTION

Folate is a water-soluble B vitamin essential to innumerable bodily functions including nucleotide synthesis. Folate, the natural occurring form, can be found in green leafy vegetables such as spinach, broccoli and turnip greens. Folic acid is the synthetic form of folate used in vitamin supplements and in fortified foods. Since 1998, many countries, including the United States, have implemented mandatory folic acid fortification of flour and grain products to reduce the risk of neural-tube birth defects.

The human body needs folate to synthesize, repair and methylate DNA (1). Impairment to any of these functions may contribute to carcinogenesis. Indeed, folate deficiency has been implicated in the development of several tumors, including pancreatic, cervical and colon cancers (2). However, the role of folate in prostate cancer carcinogenesis is very controversial (3). There is evidence suggesting dietary folate may be protective against prostate cancer while folic acid supplementation may promote cancer (4). To date, no studies have examined the role of folate in prostate cancer recurrence after primary treatment. Therefore, we sought to analyze the association between serum folate levels and risk of biochemical recurrence after radical prostatectomy among men from the Shared Equal Access Regional Cancer Hospital (SEARCH) database.

MATERIALS AND METHODS

Study population

After obtaining Institutional Review Board approval from each institution, data from patients undergoing radical prostatectomy between 1991 and 2009 at 3 Veteran Affairs Medical Center (West Los Angeles and Palo Alto, CA and Durham, NC) were combined into the SEARCH database (5). The database includes information on patient age at surgery, race, height, weight, clinical stage, cancer grade on diagnostic biopsies, preoperative PSA, surgical specimen pathology (specimen weight, tumor grade, stage and surgical margin status) and follow-up PSA. Patients treated with preoperative hormonal therapy or radiotherapy were

excluded from the study. Of 1,596 patients in SEARCH from these 3 sites, 162 (10%) had preoperative folate levels available. We excluded 6 (4%) patients due to missing follow-up, 19 (12%) due to missing covariates and 2 (1%) due to very high folate levels ($> 60\text{ng/mL}$). This resulted in a study population of 135 subjects. Folate levels were determined by retrospective chart review. No banked sera were available to measure these levels on the other men. Only folate levels obtained within one year prior to surgery were considered. All patients were followed with serial PSA determinations and clinical visits at intervals according to attending physician discretion. Biochemical recurrence was defined as a single PSA above 0.2ng/mL , 2 concentrations at 0.2ng/mL or secondary treatment for an elevated PSA. Additional treatment after surgery was at the judgment of the patient and treating physician.

Statistical analysis

As folate levels were not normally distributed, folate was examined after logarithmic transformation. The association of folate levels with patients' characteristics at the time of the surgery and tumor features, such as race (Caucasian, African American, other), body-mass index (BMI, continuous, log-transformed), age at surgery (continuous), year of surgery (continuous), surgical center (1-3), preoperative PSA (continuous, log-transformed), surgical margin status (positive or negative), extracapsular extension (present or absent), seminal vesicle invasion (yes or no) and pathological Gleason score (2-6, 3+4, 4+3 and 8-10), were all analyzed using linear regression with folate being the outcome variable and the various patient characteristics being the predictor variable. Uni- and multivariable analyses of time to biochemical recurrence were performed with Cox proportional hazards. We adjusted our multivariable models for patient demographics, clinical and pathological findings (as described above). All statistical analyses were two-tailed and performed using Stata 10.1 (StataCorp, College Station, TX) and R 2.11.1 (R Foundation for Statistical Computing, Vienna, Austria). A $P < 0.05$ was considered to indicate statistical significance.

RESULTS

In the study population, the median (interquartile range [IQR]) of preoperative folate levels was 11.6ng/mL (7.3-18.5). The reference values for folate levels were 1.5-20.0ng/mL. The mean age was 62.4 years. There was a similar proportion of white (45%) and black men (49%; Table-1).

Preoperative folate levels were significantly higher among white men than black men ($P = 0.003$). In addition, higher folate levels were asso-

ciated with more recent year of surgery ($P < 0.001$) and lower preoperative PSA ($P = 0.003$). Given that year and PSA track together, we adjusted the results for year and found a trend towards lower PSA in patients with higher serum folate levels ($P = 0.069$). Age and BMI were unrelated to folate levels. Also, no significant associations were observed between folate levels and pathological features such as Gleason score, positive surgical margins, extracapsular extension or seminal vesicle invasion (Table-2).

The median follow-up was 36 months. During this time, 42 (31%) patients develop a biochemical recurrence. In univariable analysis, there was a trend towards lower risk of biochemical recurrence among men with high folate levels ($HR = 0.61$, $95\%CI = 0.37-1.03$, $P = 0.064$). After adjustments for patients characteristics' and pre- and post-operative clinical and pathological findings, higher serum levels of folate were independently associated with lower risk for biochemical recurrence ($HR = 0.42$, $95\%CI 0.20-0.89$, $P = 0.023$, Table-3).

Table 1 - Patient demographics and tumor characteristics.

Variable	Mean \pm SD or N (%)
Folate (ng/mL)	12.9 \pm 7.1
Age at surgery (years)	62.4 \pm 6.3
Race	
White	61 (45%)
Black	66 (49%)
Others	8 (6%)
BMI (kg/m ²)	27.0 \pm 4.5
Preoperative PSA (ng/mL)	8.5 \pm 6.1
Year of surgery (years)	2002 \pm 4.6
Pathological Gleason score	
2-6	48 (35%)
3+4	59 (44%)
4+3	17 (13%)
8-10	11 (8%)
Positive surgical margins	59 (44%)
Extracapsular extension	24 (18%)
Seminal vesicle invasion	14 (10%)

BMI: Body mass index, **PSA:** prostate-specific antigen, **SD:** Standard deviation.

DISCUSSION

Data concerning the association between folate and prostate cancer is limited, dispersed and controversial (6,7). Several studies examined the association between folate and prostate cancer risk with mixed results. For example, in a small case-control study by Hultdin et al., higher levels of folate were statistically significantly associated with increased prostate cancer risk ($OR = 1.60$; $P = 0.02$) (8). Results from a large randomized trial comparing folic acid supplementation versus placebo for reduction of colon adenoma development showed a secondary outcome of higher risk of prostate cancer in men receiving folic acid (4). Conversely, an Italian case-control study found lower folate intake (measure by questionnaire) was associated with higher risk of prostate cancer (9). Findings from the American Cancer Society Cancer Prevention Study II Nutrition Cohort demonstrated that neither dietary nor total folate intake were associated with incidence of prostate cancer (10). Moreover, higher folate serum levels were associated with a nonsignificant decrease risk of advanced prostate cancer. Data from the European Prospective Investigation into

Table 2 - Association of serum folate levels with patient and tumor characteristics.

Variable	Coefficient	P
Age at surgery (years)	0.00	0.857
Race		
White	ref	-
Black	-0.27	0.004
Others	-0.01	0.969
BMI (log[kg/m ²])	0.32	0.264
Preoperative PSA (log[ng/mL])	-0.17	0.007
Year of surgery (years)	0.04	< 0.001
Pathological Gleason score		
2-6	ref	-
3+4	-0.05	0.609
4+3	-0.14	0.369
8-10	0.16	0.376
Positive surgical margins	0.12	0.213
Extracapsular extension	0.03	0.784
Seminal vesicle invasion	-0.24	0.118

BMI: Body mass index, **PSA:** prostate-specific antigen.

Cancer and Nutrition Study, where 869 cases and 1,174 controls had serum folate level analyzed, showed no significant association between serum folate and prostate cancer risk (11). Additionally, in a U.K. population-based matched case-control study with nearly 3,000 subjects, serum folate levels were not associated with prostate cancer (12). In the same manuscript, they performed a meta-analysis of 7 studies and also found no correlation between serum folate and risk of prostate cancer. Thus, while the association between folate and prostate cancer risk is controversial and remains the subject of intense research, no studies

examined the association between folate and disease recurrence after primary treatment for localized prostate cancer. Therefore, in the present study we analyzed the association between serum folate levels and biochemical recurrence after radical prostatectomy.

In a cohort of men undergoing radical prostatectomy at several Veteran Affairs Medical Centers across the country, we found serum folate levels were associated with more recent year of surgery. This finding can be explained by the fortification of the US diet with folate since 1998 (13). We also observed that higher folate levels were associated with trends toward lower PSA levels even after adjusting for the more recent year of surgery. This result is in agreement with data from Stevens et al. who found a trend between higher dietary folate and lower risk of advanced prostate cancer at diagnosis (10). Finally, we found higher serum folate levels were independently associated with decreased risk of biochemical recurrence. As no prior study specifically examined this question, further validation of our findings is necessary. However, these findings are consistent with data from the American Cancer Society Cancer Prevention Study II Nutrition Cohort, which suggested that higher folate intake was associated with lower risk of advanced prostate cancer, though they did not look at outcomes after diagnosis in that study (10). In the U.K. population-based matched case-control study with nearly 3,000 subjects opposite results were found (12). Specifically, in that study, higher folate levels were associated with increased PSA velocity after diagnosis of prostate cancer suggesting folate was associated with faster prostate cancer progression. Again, disease progression such as recurrence after treatment, metastasis or death were not evaluated. Thus, the currently available evidence on folate and prostate cancer progression is limited and somewhat controversial. Studies looking at the association of folate and cancer progression including metastasis and disease-specific mortality are needed. Nevertheless, our results, if confirmed in larger studies, support the potential role of folic acid supplementation or increased dietary intake of folate rich foods in patients with prostate cancer undergoing radical prostatectomy to reduce the risk of recurrence.

Table 3 - Univariable and multivariable predictors of biochemical recurrence.

Variable	Univariable analysis			Multivariable analysis [†]		
	HR	95%CI	P	HR	95%CI	P
Folate (log[ng/mL])	0.61	0.37-1.03	0.064	0.42	0.20-0.89	0.023
Age at surgery (years)	1.04	0.99-1.09	0.132	1.10	1.02-1.76	0.011
Race						
White	ref.	-	-	ref.	-	-
Black	1.64	0.84-3.19	0.148	1.36	0.63-2.96	0.431
Others	0.74	0.16-3.40	0.695	2.88	0.52-15.99	0.228
BMI (log[kg/m ²])	1.47	0.25-8.55	0.667	11.32	0.95-134.89	0.055
Preoperative PSA (log[ng/mL])	2.06	1.30-3.27	0.002	1.57	0.82-3.01	0.172
Year of surgery (years)	1.03	0.95-1.11	0.445	1.17	1.06-1.29	0.003
Pathological Gleason score						
2-6	ref.	-	-	ref.	-	-
3+4	4.99	1.89-13.22	0.001	4.19	1.30-13.55	0.017
4+3	6.96	2.27-21.38	0.001	3.81	1.01-14.42	0.049
8-10	9.09	2.74-30.08	<0.001	5.87	1.37-24.99	0.017
Positive surgical margins	2.90	1.52-5.51	0.001	3.84	1.71-8.68	0.001
Extracapsular extension	2.85	1.48-5.47	0.002	2.06	0.83-5.10	0.117
Seminal vesicle invasion	2.97	1.40-6.28	0.019	3.30	1.15-9.45	0.026

[†]Adjusted for center

BMI: body mass index, **CI:** confidence interval, **PSA:** prostate-specific antigen.

It is important to highlight that dietary folate manipulation is still very controversial (6). From a biochemical standpoint, reduction of folate by antifolate drugs (e.g. methotrexate) can decrease cancer cell proliferation but it may induce DNA damage which, in turn, can lead to carcinogenesis (14,15). Alternatively, folic acid supplementation may prevent malignant cell transformation on the

one hand, but maintain the high cell proliferation rate that is characteristic in neoplastic cells on the other (16-18). From an epidemiological perspective, the largest randomized trial comparing folic acid supplementation versus placebo showed a higher risk of prostate cancer in men receiving folic acid supplementation, though this was a secondary outcome (4). The same results were not

seen in large observational cohort studies where folate was not associated with prostate cancer (10,19). The current findings of lower recurrence risk among men with high serum folate levels, if confirmed in subsequent studies, would support future randomized trials, which are ultimately needed to determine the true effects of a folic acid supplementation in patients with prostate cancer.

Our study was limited by being retrospective in nature. As such, we could not prospectively measure folate levels, but rather relied on tests that had been done for some clinical reason. As such, folate was not available for the majority of patients undergoing radical prostatectomy. Thus, our sample represents only the selected few for which folate levels were obtained for other reasons (e.g. anemia work up), which may limit the generalizability of our results. This also led to a limited sample size (N = 162) which reduced the statistical power of the study. Also, given folate intake as supplement or in the diet is associated with other micronutrients, the association of folate levels and cancer outcomes may be confounded by these nutrients. Moreover, folate deficiency is associated with certain diseases such as alcoholism, malabsorption and chronic liver disease which could potentially act as confounding factors. Finally, as we assessed serum folate levels, we were unable to determine the source of the folate - dietary or via supplementation. Thus, it is possible that men who had higher folate levels, whether obtained from a high-folate diet or via supplementation, were more likely to have other behaviors associated with a healthy lifestyle (e.g. more exercise, healthier diet) which, in turn, may correlate with lower risk of disease progression after surgery. Therefore, we were not able to determine the causality in the association between folate with PSA and biochemical recurrence in the current study.

The strengths of the present study include the use of serum folate levels which reflect the total body folate better than folate intake alone. In addition, the detailed demographic and pathological data allowed us to control for these potential confounders.

In conclusion, among men undergoing radical prostatectomy at several Veteran Affairs Medical Centers across the country, higher serum fo-

late levels were associated with lower preoperative PSA and lower risk for biochemical failure. While the source of the folate in the serum in this study is unknown (i.e. diet vs. supplement), these findings if confirmed in future prospective studies, suggest a potential role of folic acid supplementation or increased intake of folate rich foods to reduce the risk of recurrence. However, further studies are required to determine whether folate supplementation or diet modification to increase folate intake can reduce prostate cancer progression.

CONFLICT OF INTEREST

None declared.

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Correspondence address:

Dr. Daniel M. Moreira
Division of Urology,
Box 2626 DUMC, Duke University School of Medicine
Durham, NC, 27710, USA
FAX: + 1 919 668-7093
E-mail: daniel.moreira@duke.edu

EDITORIAL COMMENT

The authors present the correlation between serum folate levels and biochemical recurrence in a retrospective cohort of patients submitted to radical prostatectomy. The results suggest a potential beneficial effect of higher serum folate levels on reducing the risk of biochemical failure after surgery.

Folate, also named vitamin B9, is involved in synthesis, repair, and methylation of DNA. Folic acid is converted to folate in human body (1). Dietary factors, such as folate dietary intake, can trigger epigenetic mechanisms that could play a role in cancer development (1). Actually, a systematic review and meta-analysis of randomized controlled trials showed a significant, albeit border-

line, increase in incidence of overall cancer, and, especially, prostate cancer (1). On the other hand, the association between dietary or circulating levels of folate with prostate cancer risk was not demonstrated in other cohorts of patients (2,3).

Folate deficiency is implicated in developmental conditions such as neural tube defects in developing embryos (1). This is the reason why folic acid is added to foods in several countries, including Brazil. (RDC 344/02) Consequently, the potential role of folic acid supplementation in either promotion of or protection from the most incident male cancer, excluding skin cancer, is very important in terms of public health. Despite the limitations (a small, retrospective, and selected cohort of patients), the study represents a contribution to advance the knowledge of folate and prostate cancer relationship.

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Dr. Milton Berger
Universidade Federal do Rio Grande do Sul
E-mail: mberger.uro@uol.com.br



Adenocarcinoma on needle prostatic biopsies: Does reactive stroma predicts biochemical recurrence in patients following radical prostatectomy?

Athanase Billis, Luciana Meirelles, Leandro L.L. Freitas, Aline S. Polidoro, Hamilton A. Fernandes, Mariana M. Padilha, Luis A. Magna, Leonardo O. Reis, Ubirajara Ferreira

Department of Anatomic Pathology (AB, LM, LLLF, ASP, HAF, MMP); Medical Genetics/Biostatistics (LAM) and Urology (LOR, UF), School of Medical Sciences, State University of Campinas (Unicamp), Sao Paulo, Brazil

ABSTRACT

Objective: There is evidence that reactive stroma in different cancers may regulate tumor progression. The aim of this study is to establish any possible relation of reactive stroma grading on needle prostatic biopsies to biochemical recurrence.

Materials and Methods: The study group comprised 266 biopsies from consecutive patients submitted to radical prostatectomy. Reactive stroma was defined as stroma surrounding neoplastic tissue and graded as 0 (absent), 1 (slight), 2 (moderate), and 3 (intense) according to tumor stroma area relative to total tumor area.

Results: From the total of 266 needle prostatic biopsies, 143 (53.8%), 55 (20.7%), 54 (20.3%), and 14 (5.3%) showed grades 0, 1, 2, and 3, respectively. Increasing reactive stroma grade was significantly associated with clinical stage T2, higher preoperative PSA, higher biopsy and radical prostatectomy Gleason score, more extensive tumors in radical prostatectomy, and pathologic stage > T2. Only grade 3 was significantly associated with time and risk to biochemical recurrence. On multivariate analysis only preoperative PSA and 2 methods of biopsy tumor extent evaluation were independent predictors.

Conclusion: Increasing reactive stroma grade on biopsies is significantly associated with several clinicopathologic adverse findings, however, only grade 3 predicts time and risk to biochemical recurrence following radical prostatectomy on univariate but not on multivariate analysis. We have not been able to show that reactive stroma grade 3 on biopsies is an independent predictor of biochemical recurrence beyond that of preoperative PSA and other pathologic findings on biopsy.

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INTRODUCTION

Several human cancers may induce a stromal reaction (desmoplasia) as a component of carcinoma progression. This has been described in breast and colon carcinoma (1,2). In cancers with stromal reaction, it seems that the response is similar, if not identical, to wound repair response

(3). Prostate cancer may also be associated with wound repair type of reactive stroma composed of myofibroblasts and fibroblasts rather than normal prostate smooth muscle, which is displaced by the reactive stroma (4).

There is growing evidence that carcinogenesis is influenced and controlled by cellular

interactions derived from a complex relationship between stromal, epithelial and extracellular matrix components (4-15). The neoplastic stromal environment is different from the stroma of the normal tissue and is characterized by modified extracellular matrix composition, increased microvessel density, inflammatory cells and myofibroblasts (4,14,15).

A pioneer study by Ayala et al. (16) showed that the volume of reactive stroma in surgical specimens from patients submitted to radical prostatectomy was a significant predictor of biochemical recurrence. In a subsequent paper from the same group, Yanagisawa et al. (17) showed that intense reactive stroma on biopsies was an independent predictor of recurrence. The only other study dealing with the prognostic value of reactive stroma in prostate carcinoma is Tomas' et al. (18). Using histochemistry and immunohistochemistry in radical prostatectomies, the authors found that on multivariate analysis only vimentin expression in reactive stroma was a significant predictor of biochemical recurrence.

Our study aims to establish the possible association of prostate cancer reactive stroma on needle biopsies and biochemical progression following radical prostatectomy.

MATERIALS AND METHODS

This retrospective study comprised 266 needle prostatic biopsies from 266 consecutive patients submitted to retropubic radical prostatectomy. No patient in this series received radiotherapy or androgen manipulation before or after surgery. The biopsy of the prostate was performed with transrectal ultrasound guidance and a spring-loaded 18-gauge needle instrument. Prostate was imaged in gray-scale mode and hypervascularity on color and power Doppler. The mean number and range of the cores obtained was 9 cores and 2-20 cores, respectively; and the mean and range of the length in mm of all cores 53 mm and 3-150 mm, respectively. All biopsies were analyzed by the same senior uropathologist (AB).

Prostatic biopsies

The analysis was done on slides stained with hematoxylin and eosin. Reactive stroma was defined

as stroma surrounding the neoplastic tissue and that was not part of the normal preexisting host stroma. The scoring system was analyzed according to the stroma area relative to total tumor area and was based on the system proposed by Ayala et al. (16) and Yanagisawa et al. (17). Reactive stroma was graded as 0 (absent or up to 5% reactive stroma, Figures 1A and B), 1 (slight, 6% to 15% reactive stroma, Figure-1C), 2 (moderate, 16% to 50% reactive stroma, Figure-1D), and 3 (intense, > 50% reactive stroma, Figure-1E). In biopsies with cores showing different grades, the final grading was considered in the core with the most extensive carcinoma (index core). Reactive stroma is easily identified using hematoxylin and eosin stain and does not need special stains such as Masson's trichrome.

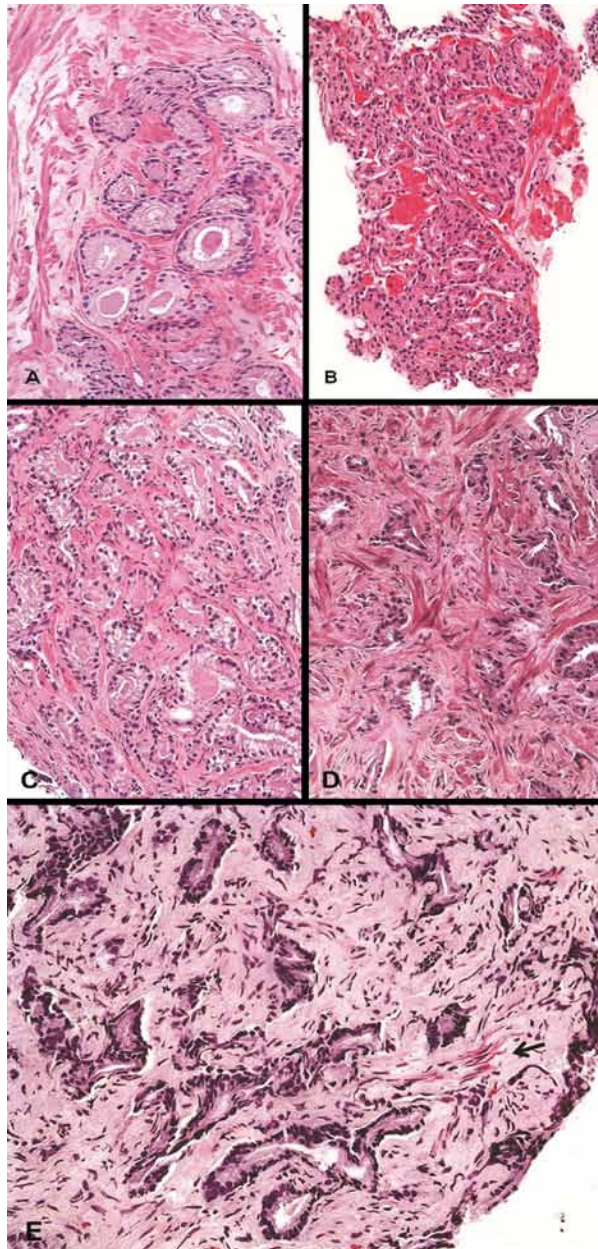
Grading of reactive stroma was related to several clinicopathological variables: age, clinical stage, preoperative PSA, biopsy tumor extent using several methods of evaluation, radical prostatectomy tumor extent, biopsy and radical prostatectomy Gleason score, pathologic stage, and surgical margin status. For tumor grading was used the revised Gleason system according to the consensus conference of the International Society of Urological Pathology (19).

Tumor extent on needle biopsy was evaluated as number of needle biopsy cores with carcinoma (NC), number of needle biopsy cores with carcinoma divided by the total number of cores (%NC), total length of cancer in mm in all cores (mmAC), and total length of carcinoma in all cores divided by the total length of the cores (%mmAC). Linear extent of carcinoma in mm was measured using a single micrometer eyepiece with a linear array. In cases of discontinuous foci 1mm apart, the tumor was considered as continuous and the measure included 1mm. In discontinuous foci more than 1mm apart, the final extent was the sum of the measures.

Surgical specimens

The surgical specimens were step-sectioned at 3 to 5mm intervals and totally embedded in paraffin. A mean of 32 paraffin blocks were processed and 6µm sections from each block were stained with hematoxylin and eosin. Each transversal section of the prostate was subdivided into 2 anterolateral and 2 posterolateral quadrants. The basal and the apical margins were amputated and

Figure 1 - (A) Grade 0 (absent reactive stroma): Gleason score 6 adenocarcinoma; (B) Grade 0 (absent reactive stroma): Gleason score 7 adenocarcinoma; (C) Grade 1 (slight reactive stroma); (D) Grade 2 (moderate reactive stroma); (E) Grade 3 (intense reactive stroma). Only some few residual smooth muscle fibers are seen (arrow) (hematoxylin-eosin, x165).



sectioned parasagittally in a direction perpendicular to the initial transverse incision (cone method) obtaining 8 sections from each margin. Positive surgical margin was defined as cancer cells in contact with

the inked specimen surface. Extraprostatic extension was diagnosed whenever cancer was seen in adipose tissue and, in case of desmoplastic response, whenever a protuberance corresponding to extension of tumor into periprostatic tissue was observed. Seminal vesicle invasion occurred whenever there was involvement of the muscular coat. Tumor extent at radical prostatectomy was evaluated by a semiquantitative point-count method previously described (20).

After radical prostatectomy, serum PSA was drawn every 3 months during the first year, every 6 months during the second year, and annually thereafter. No patient had radiotherapy or androgen manipulation before or after surgery. Total serum PSA was measured utilizing previous validated Immulite® PSA kit. Biochemical recurrence following surgery was considered as PSA ≥ 0.2 ng/mL according to recommendation of the American Urological Association (21). Patients without evidence of biochemical recurrence were censored at last follow-up. Institutional Committee of Ethics approved the study.

Statistical analysis

The data were analyzed using the Qui-square test for comparison of proportions, the Kruskal-Wallis test for comparison of means, the Kaplan-Meier product-limit analysis for the time to biochemical recurrence using the log-rank test for comparison between the groups, and a univariate and multivariate Cox stepwise logistic regression model to identify significant predictors of shorter time to biochemical recurrence. The P-values were two-sided at the significance level of < 0.05 . All statistical analyses were performed using the commercially available PASW Statistics (SPSS) 18.0.

RESULTS

Biopsies

Table-1 shows the clinicopathologic characteristics of 266 patients undergoing radical prostatectomy according to biopsy reactive stroma grade. From the total of 266 needle prostatic biopsies, 143 (53.8%), 55 (20.7%), 54 (20.3%), and 14 (5.3%) biopsies showed absent (grade 0), slight (grade 1), moderate (grade 2), and intense (grade 3) reactive stroma, respectively.

Table 1 - Clinicopathologic characteristics of 266 patients undergoing radical prostatectomy according to biopsy reactive stroma grade.

Characteristic	Grade 0	Grade 1	Grade 2	Grade 3	P value
No. of patients	143 (53.8%)	55 (20.7%)	54(20.3%)	14(5.3%)	
Mean (SD; range)					
Age, years	64 (6.6;46-76)	62 (6.8;45-73)	63 (7.2;43-73)	65 (4.3;57-72)	0.69
Preoperative PSA (ng/mL)	8.6 (5;0.6-29.7)	9.5 (4.3;2.6-22.1)	11 (7;0.9-29-7)	18.1 (14.7;3.43-50)	< 0.01
RP tumor extent (pos.points)	31.4 (30.9;1-192)	37.4 (30.4;1-147)	46.9 (45.3;5-222)	68.8 (71.2;20-225)	0.02
N (%)					
Clinical stage					
T1c	81 (58.7)	23 (44.2)	9 (19.6)	2 (16.7)	< 0.01
T2	57 (41.3)	29 (55.8)	37 (80.4)	10 (83.3)	< 0.01
RP Gleason score					
2-6	65 (45.5)	15 (27.3)	10 (19.2)	1 (7.1)	
7	73 (51.0)	35 (63.6)	40 (76.9)	11 (78.6)	
8-10	5 (3.5)	5 (9.1)	2 (3.8)	2 (14.3)	
Biopsy Gleason score					
2-6	103 (72.0)	30 (54.5)	32 (59.3)	7 (50.0)	0.02
7	36 (25.2)	21 (38.2)	19 (35.2)	4 (28.6)	
8-10	4 (2.8)	4 (7.3)	3 (5.6)	3 (21.4)	
Pathologic stage					
T2	110 (76.9)	42 (76.4)	35 (64.8)	5 (35.7)	0.01
> T2 (T3a/T3b)	33 (23.1)	13 (23.6)	19 (35.2)	9 (64.3)	
Surgical margin status					
Negative	86 (60.1)	29 (52.7)	28 (51.9)	5 (35.7)	0.27
Positive	57 (39.9)	26 (47.3)	26 (48.1)	9 (64.3)	

Increasing reactive stroma grade was significantly associated with clinical stage T2, higher preoperative PSA, higher biopsy and radical prostatectomy Gleason score, more extensive tumors in radical prostatectomy, and pathologic stage > T2. There was no significant association with age and positive surgical margin.

Radical prostatectomies

From the total of 266 men following radical prostatectomy, 92 (34.6%) patients had biochemical recurrence at a mean, median and range follow-up of 19, 8, and 3-111 months; 162 (60.9%) censored men remained at risk at a mean, median and range follow-up of 59, 57, and 3-141 months, respectively; and, 12 (4.5%) men had no serum PSA data.

At 5 years following radical prostatectomy, 69%, 59%, 57%, and 39% from a total of 254 patients with grade 0, 1, 2, and 3 biopsy reactive stroma, respectively, were free of biochemical recurrence; 12/266 (4.5%) men had no serum PSA data. Only patients with needle biopsies showing grade 3 reactive stroma were significantly associated with shorter time to biochemical recurrence following surgery (log-rank, $p < 0.01$) (Figure-2).

Figure 2 - Kaplan-Meier product-limit analysis comparing grades 0, 1, 2, and 3 reactive stroma on needle biopsy from 254 patients for time to biochemical recurrence following radical prostatectomy.

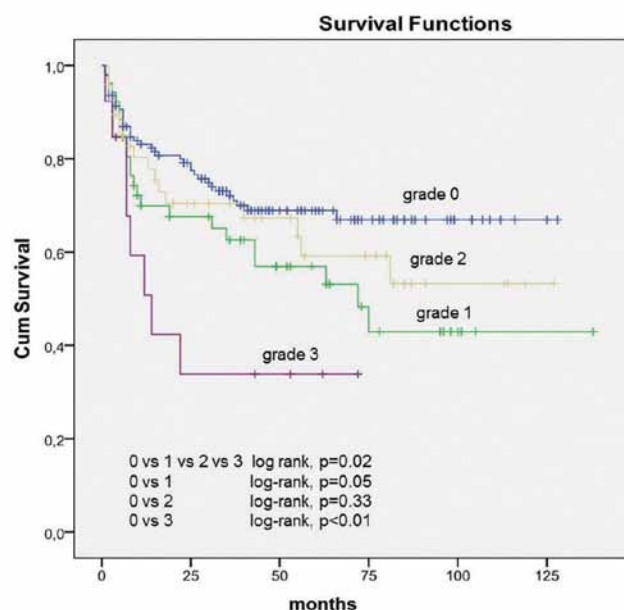


Table-2 shows the Cox proportional hazard analysis of several clinicopathologic factors predicting time to biochemical recurrence following radical prostatectomy. On univariate analysis, preoperative PSA, all methods of biopsy tumor extent evaluation, and only needle biopsies with grade 3 reactive stroma were significant predictors of time to biochemical recurrence. On multivariate analysis, only preoperative PSA and 2 methods of biopsy tumor extent evaluation were independent predictors using two models of analysis.

DISCUSSION

We used the system proposed by Ayala et al. (16) and Yanagisawa et al. (17) for definition and scoring of reactive stroma. Reactive stroma was evaluated as stroma surrounding the neoplastic tissue and not part of the normal preexisting host stroma. The scoring system was established according to the stroma area relative to total tumor area. In biopsies with cores showing different grades, the final grading was considered in the core with the most extensive carcinoma (index core). Reactive stroma is easily identified using hematoxylin and eosin stain and does not need special stains such as Masson's trichrome. No patient in this series received radiotherapy or androgen manipulation before or after surgery. This is important because both occurrences promote mesenchymal reaction that may interfere with the results.

All patients in our study had only one needle prostatic biopsy preceding surgery therefore there was no selection of biopsies. The mean number and range of the cores examined was 9 and 2-20, respectively and the mean and range of the length in mm of all cores 53 mm and 3-150 mm, respectively. Sampling of the biopsies is important for comparison of results.

According to intensity of reactive stroma, the frequency was 143/266 (53.8%), 55/266 (20.7%), 54 (20.3%), and 14/266 (5.3%) biopsies for grades 0, 1, 2, and 3, respectively. The frequency is higher in grade 0 and decreases to grade 3. On needle biopsies, Yanagisawa et al. (17) found a frequency of 1/224 (0.5%), 149/224 (66.5%), 59/224 (26.3%), and 15/224 (6.7%) biopsies for grades 0, 1, 2, and 3, respectively. In radical pros-

Table 2 - Cox proportional hazard analysis of several clinicopathologic factors predicting time to biochemical recurrence following radical prostatectomy.

Predictors	Hazard ratio (95% CI)	Wald test	P value
Univariate analysis			
Reactive stroma grade 1	1.655 (0.990-2.765)	3.696	0.06
Reactive stroma grade 2	1.320 (0.748-2.329)	0.915	0.34
Reactive stroma grade 3	2.863 (1.386-5.914)	8.071	< 0.01
Preoperative PSA	1.058 (1.036-1.080)	28.700	< 0.01
Biopsy Gleason score	1.293 (0.993-1.684)	3.645	0.06
Biopsy NC	1.126 (1.038-1.221)	8.139	< 0.01
Biopsy %NC	1.012 (1.005-1.019)	10.284	< 0.01
Biopsy mmAC	1.025 (1.007-1.043)	7.721	0.01
Biopsy %mmAC	1.017 (1.008-1.026)	13.306	< 0.01
Multivariate analysis			
Model 1			
Preoperative PSA	1.042 (1.012-1.073)	7.436	0.01
Biopsy %NC	1.011 (1.001-1.001)	4.288	0.04
Model 2			
Preoperative PSA	1.035 (1.003-1.068)	4.522	0.03
Biopsy %mmAC	1.016 (1.003-1.028)	6.216	0.01

tatectomies, Ayala et al. (16) found a frequency of 34/545 (6.2%), 161/545 (29.5%), 306/545 (56.1%) , and 44/545 (8%) surgical specimens for grades 0, 1, 2, and 3, respectively.

The higher frequency of grade 0 (53.8%) in our study contrasts with the frequency of 0.5% and 6.2% of grade 0 in Yanagisawa's et al. (17) and Ayala's et al. (16) studies. Considering that the method of evaluation of reactive stroma is the same, it is difficult to explain this striking difference in frequency. Absence of reactive stroma is easily discernible as we can see in Figures 1A and B. One possible reason may be the additional use of trichrome staining to visualize reactive stroma in those prior studies. There is also a role of inte-

robserver variability in interpretation of reactive stroma particularly when it is absent or lower grade. However, since distinction is more important for grade 3, which is relatively easier to identify, variation of interpretation in lower grades may not be significant.

Increasing reactive stroma grade was significantly associated with adverse clinicopathologic findings. Table-1 clearly shows the significant association with clinical stage T2, higher preoperative PSA, higher tumor extent in surgical specimen, higher Gleason score in biopsy and surgical specimen, and pathological stage >T2. There was no significant association with age and positive surgical margin. In Yanagisawa's et al. (17) study,

reactive stroma grade in biopsies was significantly associated with clinical and pathological stage, surgical margin stage, and Gleason score on biopsy. No correlation was found with preoperative PSA and age.

The Kaplan-Meier product-limit analysis showed that only patients with reactive stroma grade 3 had significantly shorter time to biochemical recurrence (Figure-1). In Cox univariate analysis reactive stroma grade 3 is a significant predictor of shorter time to biochemical recurrence (Table-2). Yanagisawa et al. (17) found that patients with reactive stroma grade 0 and 3 had significantly shorter time to biochemical recurrence. A comment on this finding is the fact that they included in the same group 15 patients with reactive stroma grade 3 and only one patient with grade 0 in their series. It is not plausible any influence of this single patient with reactive stroma grade 0 in the analysis.

On multivariate analysis we used two models including only variables significantly predictive of time to biochemical recurrence on univariate analysis (Table-2). In model 1, only preoperative PSA and number of needle biopsy cores with carcinoma divided by the total number of cores (%NC) were significant; in model 2, only preoperative PSA and the total length of carcinoma in mm in all cores divided by the total length of the cores (%mmAC). This result is at odds with Yanagisawa's et al. (17) study on biopsies. According to the authors reactive stroma grade 0 and 3 was an independent predictor of biochemical recurrence by Cox proportional hazard analysis. Their analyses were based on 205 patients with reactive stroma grade 1 and 2 vs. 16 patients with reactive stroma grade 0 and 3 (only one patient with grade 0).

In prostate cancer, the reactive stroma shows myofibroblasts that coexpress smooth-muscle marker (α -smooth-muscle actin) and mesenchymal marker (vimentin) and loss of late-stage smooth-muscle differentiation markers (desmin, calponin) (18). Based on these histological features, the only other study dealing with the prognostic value of reactive stroma in prostate carcinoma is Tomas' et al. (18). The authors quantified histochemically and immunohistochemically the stromal reaction

in radical prostatectomies. Patients with a higher vimentin or lower desmin expression had a shorter disease-free period and on multivariate analysis only vimentin expression was a significant predictor of biochemical recurrence. Further studies on needle prostatic biopsies may show utility of histochemistry and immunohistochemistry for the analysis of reactive stroma.

CONCLUSIONS

Increasing reactive stroma grade on needle prostatic biopsies is significantly associated with several clinicopathologic adverse findings. However, only grade 3 predicts time and risk to biochemical recurrence following radical prostatectomy on univariate analysis. On multivariate analysis we have not been able to show that reactive stroma grade 3 is an independent predictor of biochemical recurrence factoring in preoperative PSA and other pathologic findings on biopsy. An additional limitation of reactive stroma grade 3 as prognostic factor is the relatively rare occurrence of this finding (only 5.3% on needle biopsies).

ACKNOWLEDGMENTS

PSA = prostate specific antigen;
SD = standard deviation;
CI = confidence interval;
NC = number of needle biopsy cores with carcinoma;
%NC = percent of needle biopsy cores with carcinoma;
mmAC = total length of cancer in mm in all cores;
%mmAC = percent of total length of cancer in mm in all cores.

CONFLICT OF INTEREST

None declared.

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Correspondence address:

Dr. Athanase Billis
 Dep. of Anatomic Pathology
 School of Medical Sciences
 State University of Campinas (Unicamp)
 Rua Tessália Vieira de Camargo, 126
 Campinas, SP, 3083-887, Brazil
 Fax: + 55 19 3289-3897
 E-mail: athanase@fcm.unicamp.br



Association between Literacy, Compliance with Prostate Cancer Screening, and Cancer Aggressiveness: Results from a Brazilian Screening Study

Marcos Tobias-Machado, Gustavo F. Carvalhal, Celso H. Freitas Jr., Rodolfo B. dos Reis, Leonardo O. Reis, Lucas Nogueira, Roberto D. Machado, Wesley Magnabosco, René A. C. Vieira, Edmundo C. Mauad, André L. Carvalho, Eliney F. Faria, Cooperative Brazilian Uro-oncology Group (CBUG)

Department of Urology, Faculdade de Medicina do ABC (MTM), Department of Urology, Pontifícia Universidade Católica do Rio Grande do Sul (GFC), Barretos Cancer Hospital (CHFjr, RDM, WM, RACV, ECM, ALC, EFF), Department of Urology, Universidade de São Paulo, Ribeirão Preto (RBR), Department of Urology, Universidade de Campinas (LOR), Department of Urology, Universidade Federal de Minas Gerais (LN), MG, Brazil

ABSTRACT

Purpose: Little is known about the effects of literacy levels on prostate cancer screening. This study evaluates the association between literacy, compliance with screening, and biopsy findings in a large Brazilian screening study.

Materials and Methods: We analyzed 17,571 men screened for PCa with digital rectal examination (DRE) and total and free prostate-specific antigen (PSA) from January 2004 to December 2007. Of those, 17,558 men had information regarding literate status. Full urological evaluation in a specialized cancer center was recommended in the case of: a) suspicious DRE, b) PSA > 4.0 ng/mL, or c) PSA 2.5-3.9 ng/mL and free/total PSA (f/tPSA) ratio < 15%. Transrectal ultrasound guided prostate biopsy (14 cores) was performed upon confirmation of these findings after the patient's consent. Patients' compliance with screening recommendations and biopsy results were evaluated according to literacy levels.

Results: an abnormal PSA, a suspicious DRE, or both were present in 73.2%, 19.7%, and 7.1% of those men who underwent biopsy, respectively. PCa was diagnosed in 652 men (3.7%). Previous PSAs or DREs were less common among illiterate men ($p < 0.0001$). Additionally, illiterate men were less prone to attend to further evaluations due to an abnormal PSA or DRE ($p < 0.0001$). PSA levels > 10 mg/mL ($p = 0.03$), clinical stage > T2a ($p = 0.005$), and biopsy Gleason > 7 ($p = 0.02$) were more common among illiterate men. **Conclusions:** In a screened population, literacy levels were associated with prior PCa evaluations and with compliance with screening protocols. Illiterate men were at higher risk of being diagnosed with more advanced and aggressive PCa.

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Key words:

Prostatic Neoplasms; Health Literacy; Mass Screening; Diagnosis

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INTRODUCTION

Prostate cancer (PCa) is the most common non-cutaneous cancer and the second cause of

cancer mortality among men, regardless of geographic differences (1). In spite of recent evidence of the association between prostate cancer screening and reduced cancer specific mortality

rates (1,2) data on the effectiveness of screening measures are not uniform and there are important concerns with the magnitude of overdiagnosis (3,4). Therefore, several professional organizations recommend that physicians counsel their patients on the risks and benefits of screening, so that an informed decision about PCa testing can be made (5,6). Few studies discuss literacy levels as a variable that may affect screening efficacy (7,8). Men with low literacy levels may have less knowledge about PCa and may struggle with such complex decisions.

In Latin America, many underprivileged men do not have access to education or to the health care system (9). More specifically, in the Brazilian population, data concerning the relationship between literacy levels and PCa screening are lacking. It is also unknown whether literacy levels correlate with PCa aggressiveness or clinical stage at diagnosis in a screened population. Barretos Cancer Hospital (BCH) is a public institution located in the state of São Paulo, Brazil, which serves a large community of patients from many cities and states. As a tertiary healthcare center specialized in the treatment of cancer, it assists many men whose prostate cancers are suspected or diagnosed elsewhere and who are referred for diagnosis or treatment. Additionally, BCH conducts a study that utilizes five Mobile Cancer Prevention Units (MCPUs) offering opportunistic screening for some of the most common cancers (skin, breast, cervix and prostate) to patients in rural municipalities from six Brazilian states with poor access to specific health care, with the costs of screening, work up and treatment covered by the Brazilian public health system (9).

The purpose of this study was to verify whether illiterate Brazilian at our PCa screening study had higher risk of unfavorable disease, meaning higher PSA levels, more advanced clinical stage and higher Gleason score.

MATERIALS AND METHODS

From January 2004 to December 2007, 17,571 men aged ≥ 45 years voluntarily underwent prostate cancer screening at a mobile cancer prevention unit (MCPU) at least once. Of those, 17,558

men had information regarding literate status. This program included a total of 231 rural municipalities across six Brazilian states. Each MCPU had a general physician trained in performing prostate cancer screening (9). Men were evaluated by clinical history, digital rectal examination (DRE), and serum free and total prostate-specific antigen (PSA) levels. When men entered the MCPU they responded to our own general epidemiologic questionnaire applied by the physician, which included direct questions about urinary symptoms and literacy status. Regarding literacy two groups were defined: illiterate (men who had no formal education or were unable to read and write) and literate (men who had any degree of formal education or were able to read and write). Data regarding educational status were available for 17,558 men, who consisted of our final population. All PSA tests were performed with Hybritech® assays, and were analyzed by the same central laboratory.

Men with a suspicious DRE and/or serum PSA ≥ 4.0 ng/mL were recalled for further evaluation at BCH. From November 2004 onwards, men with serum PSA between 2.5 and 3.9 ng/mL, normal DRE and percentage of free/total PSA (%fPSA) $\leq 15\%$ were also recalled. Upon confirmation of screening data, a 14-core transrectal prostatic biopsy was performed at our center. All biopsies were evaluated at the same pathology laboratory. Cancers were staged according to the TNM system (American Joint Committee on Cancer Staging 2002) and the Gleason score was used for grade classification. The study protocol was reviewed and approved by the BCH ethics committee, and informed consent was obtained from all patients.

We compared variables about clinical data (PSA, clinical stage) and Gleason score in prostate biopsy between illiterate and literate men. Statistical analysis were performed using Epi info 6.02® software. Differences with $p < 0.05$ were considered statistically significant. This study was approved by Ethics Committee - number of protocol: 076/2007.

RESULTS

Mean age at the time of the first screening visit was 61.2 years (range 45 to 98 years), with more than 80% of men with ages between 50 and 74 ye-

ars. Regarding literacy status, of the 17,558 men analyzed, 3,403 (19.4%) were illiterate. Urinary symptoms were present in 1,697 men (9.7%). A total of 5,023 men (28.6%) had performed at least one DRE, and 5,108 men (29.1%) had performed at least one PSA prior to entering the study. Of the 17,558 men evaluated on this study, 2,841 (16.2%) were recalled for further evaluation; of these, 2,291 (80.6%) returned to the hospital appointment, and 1,647 (71.9%) underwent biopsy. Regarding those men with reported literacy status, PCa was diagnosed in 649 men, leading to a cumulative cancer detection rate of 3.7%. Among those biopsied, the main indication was a PSA > 4.0 ng/mL (54.9%), whereas in 19.7% of the men the indication was suspicious DRE findings in the presence of normal PSA levels (< 4.0 ng/mL).

Most prostate cancers (79.3%) were diagnosed in men aged 50 to 74 years. Overall, 285 (43.7%) had a PSA between 4 to 10 ng/mL, and 32.5% of the men had tumors of Gleason score > 7. Most screen-detected tumors (93.4%) were clinically localized (cT1-cT2).

The effect of literacy on the probability of previous screening tests is shown in Table-1. Of all men, 28.9% had received a previous DRE. Whilst 25.2% of illiterate men had undergone a previous DRE evaluation, the proportion of literate men who had undergone a previous DRE was greater (29.4%) ($p < 0.0001$). Likewise, 29.0% of the men had been previously tested for PSA at least once. Illiterate men were less likely to have been tested for PSA (23.4%) than men with higher education levels (30.4%) ($p <$

0.0001). Illiterate men were also less likely to comply with follow-up recommendation (69%) than literate men (87%) ($p < 0.0001$). Of 2,841 men who were recalled for further evaluation due to altered PSA and/or DRE, ten had missing data on literacy status, and were excluded from evaluation. Of 706 illiterate men who were recalled, 482 men (68.3%) complied with the requirements for further evaluation, whereas of 2,125 literate men who required further evaluation, 1,733 (81.6%) returned for reevaluation ($p < 0.01$).

The effect of literacy status on some of the most commonly reported independent prognostic variables of progression (PSA, clinical stage, biopsy Gleason) is shown in Table-2. A greater proportion of illiterate men had PSA levels > 10 ng/mL than literate men (36.6% versus 27.6%; $p = 0.03$). The probability of a locally advanced cancer (T3-T4) was also higher among illiterate men than among literates (11% vs. 7.7%; $p < 0.005$). Similarly, illiterate men had a greater proportion of cancers with biopsy Gleason scores > 7 (10.9% versus 5.9%; $p = 0.02$).

DISCUSSION

The introduction of PSA-based screening strategies has led to an increase of prostate cancer incidence in countries where it was adopted (1,10). Screen detected cancers tend to be of lower volume and stage, theoretically more amenable to curative therapies (11,12). Decreasing prostate cancer-specific mortality rates have been reported in countries with more widespread screening policies (1,2,13). Nevertheless, a lack of conclusive

Table 1 - Probability of previous DRE and PSA testing, and compliance with follow-up recommendation, according to literacy status.

Epidemiologic characteristics		Literacy status n total: 17,558		p value
		Illiterate n (%)	Literate n (%)	
Men with previous DRE	No (%)	2,546 (74.8)	9,994 (70.6)	$p < 0.001$
	Yes (%)	857 (25.2)	4,161 (29.4)	
Men with previous PSA	No (%)	2,608 (76.6)	9,849 (69.6)	$p < 0.001$
	Yes (%)	795 (23.4)	4,306 (30.4)	

Table 2 - Distribution of prognostic variables in cases of prostate cancers according to literacy status.

Clinical findings		Literacy Status n = 649		p value
		Illiterate n (%)	Literate n (%)	
PSA	0-10 (%)	99 (63.4)	357 (72.4)	p = 0.03
	> 10 (%)	57 (36.6)	136 (27.6)	
Clinical stage	T1 (%)	100 (64.5)	389 (77.1)	p < 0.005
	T2 (%)	38 (24.5)	75 (15.2)	
	T3-4 (%)	17 (11.0)	30 (7.7)	
Gleason score	≤ 7 (%)	139 (89.1)	464 (94.1)	p = 0.02
	8-10 (%)	17 (10.9)	29 (5.9)	

data from ongoing prospective, randomized trials of prostate cancer screening still poses it as a controversial issue (3,4).

Epidemiological data on prostate cancer in Latin America are still limited (14-16). In Brazil, where PSA-based screening is recommended by most specialty societies, the incidence of prostate cancer is considered intermediate. Due to both economic and demographic disparities, however, healthcare access is problematic for those men entirely dependent on the public health system. A recent report of our comparative data between screened and non-screened patients has shown that screened patients have less aggressive disease at diagnosis (17). However, determinants and motivations for screening are not always well understood.

One of the most widely used measurements of both social and economic status in health studies is the level of education, since it is relatively easy to ascertain, and potentially measurable (9). Education can translate the access to resources that may influence health status and more widespread use of preventive services. It is also recognized that the level of education influences health through the acquisition of certain higher cognitive functions and through effects in the adoption of healthier lifestyles (18,19). It is thus quite possible

that literacy status may influence the results of cancer screening. In fact, low literacy rates affect patient communication, leading to substandard medical care. Illiteracy is associated with poor understanding of written or spoken medical advice, adverse health outcomes, and under utilization of preventive services. In addition, advice on health issues may be ineffectively transmitted due to limited knowledge of cancer screening concepts, human anatomy and specific vocabulary (18,19). Cultural barriers and lack of adequate information have also been identified as potential factors affecting PCa screening, especially concerning DRE, since misconceptions about masculinity may prevent adequate evaluation (20). Men with low levels of information may also opt to leave decisions related to participation on PCa screening to their primary physician (8,21).

A common problem in analyzing results from PCa screening studies is screening contamination or number of men pre-screened before trial stated: a variable proportion of the participants are previously tested with PSA or DRE, which may give rise to misleading results and conclusions. In the Prostate, Lung, Colorectal and Ovarian (PLCO) trial about half of the patients in the screening group had already been tested at least once with

either PSA or DRE (3). In the European Randomized Study of Screening for Prostate Cancer (ERSPC), contamination was lower (ranging from 8.6% to 36.6%) (4,22). Regarding pre-screened men rates, in our study illiterate men had lower probability to have had a previous prostatic evaluation compared with more educated patients. We believe that the lack of knowledge about the benefits of screening and the poor availability of healthcare access were the most important factors accountable for this finding. The fact that about a third of our illiterate men did not comply to the recommendation of further evaluations at the university hospital (compared to 13% of literate men) may in part be due to the difficulty in understanding the implications of the initial findings.

Pre-treatment PSA is an independent surrogate marker of prognosis for prostate cancer after initial treatment (23-25). Patients with PSA < 4 ng/mL have an 80-90% probability of localized disease compared to 60-70% when PSA is > 10 ng/mL (23,25). In our screen-detected illiterate patients, the probability of having a PSA > 10 ng/mL was 25% higher than that of more educated men.

Stage migration is the most striking and consistent phenomenon associated with PCa screening (1,13,24,26). Data from ERSPC show a significant stage migration with screening, with a 7-fold reduction in the risk of locally advanced disease (4). Skip data from reference centers in the U.S. showed that lower education levels were independent predictors of higher stage at diagnosis (19). In our screening study, the differences in clinical stage among illiterate and literate patients were significant. About thirty-five percent of higher risk T3-4 tumors were observed among illiterate patients. Pre-treatment Gleason score is also considered an important predictor of pathologic features and of biochemical and clinical recurrence after the initial treatment of PCa (27,28). In our series, illiterate men had an almost 2-fold chance of harboring an aggressive tumor. It's difficult to explain these findings of more aggressive and advanced disease in illiterate men based in biological or genetic causes. More probably, the lower rate of previous screenings and the longer period of tumor evolution have contributed to the development of a disease of higher volume and tumor

aggressiveness. However, environmental, behavioral or other intangible factors associated with lower education rates may also be interacting in this clinical scenario.

Our study has several limitations. It was not a randomized, prospective study, and although literate and illiterate men were contemporary and of similar age range, intrinsic differences in their characteristics cannot be fully ascertained. Additionally, data on ethnicity were not available, since such these classifications in the largely multiethnic Brazilian population are difficult to attribute, as previously shown in genetic studies in Brazilian volunteers for prostate screening (29-31). Another limitation is that income data is not available, but the screening program was targeted to an underprivileged, uninsured population living in rural areas, with all work up and treatment performed by the public health system. Moreover, the outcome variables evaluated are at their best prognostic factors or surrogate markers of disease progression, and may not reflect real future reductions in cancer specific and overall mortality rates. This said, we acknowledge that differences associated with literacy status can only be at this point hypothesis-generating findings, and should not be seen as conclusive under this study design. In countries with a limited health budget, it is important to optimize costs and identify a population to which screening efforts may be more beneficial.

CONCLUSIONS

Illiterate screened men are somewhat less amenable to have been previously screened for PCa, and are less likely to follow recommendations of additional follow-up. Illiterate men have a significantly greater risk of harboring a more advanced and aggressive PCa. In our opinion, this is a priority population to whom PCa screening should be strongly considered.

ABBREVIATIONS

CBUG = Cooperative Brazilian Uro-oncology Group
PCa = Prostate cancer
BCH = Barretos Cancer Hospital

MCPU = Mobile Cancer Prevention Unit
 DRE = Digital rectal examination
 PSA = prostate-specific antigen
 %f/tPSA = percentage of free/total PSA
 PLCO = Prostate, Colorectal, Lung and Ovarian cancer screening
 ERSPC = European Randomized Study of Screening for Prostate Cancer

CONFLICT OF INTEREST

None declared.

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Correspondence address:

Dr. Eliney Ferreira Faria,
Division of Urology,
Hospital do Cancer de Barretos
Rua Antenor Duarte Vilela, 1331
Barretos, SP, 14784-400, Brazil



Correlation between Beta1 integrin expression and prognosis in clinically localized prostate cancer

José Pontes-Júnior, Sabrina Thalita Reis, Felipe S. Bernardes, Luiz C. N. Oliveira, Érika Aparecida Felix de Barros, Marcos Francisco Dall'Oglio, Luciana M. S. Timosczyk, Leopoldo A. Ribeiro-Filho, Miguel Srougi, Kátia R. M. Leite

Laboratory of Medical Investigation – LIM 55, Urology Department, University of Sao Paulo Medical School (JPJr, STR, FSB, LCNO, MFA, LMST, LARFilho, MS, KRML) and Universidade Nove de Julho (JPJr, LCNO, EAFB), Sao Paulo, Brazil

ABSTRACT

Integrins are transmembrane glycoprotein receptors that regulate cell-matrix interactions, thus functioning as sensors from the environment. They also act as cell adhesion molecules that are responsible for the maintenance of the normal epithelial phenotype. Some studies have reported a correlation between carcinogenesis and changes in integrin expression, especially $\beta 1$ integrin, however its role in prostate cancer (PC) is unclear. The aim of our study was to evaluate the expression of $\beta 1$ integrin in localized PC and to correlate the pattern of expression with recurrence after surgical treatment. Methods For this case-control study, we retrospectively selected surgical specimens from 111 patients with localized PC who underwent radical prostatectomy. Recurrence was defined as a PSA level exceeding 0.2ng/mL after surgery, and the median follow-up was 123 months. Integrin expression was evaluated by immunohistochemistry in a tissue microarray containing two samples from each tumor. We employed a semiquantitative analysis and considered a case as positive when the expression was strong and diffusely present.

Results: There was a loss of 11 cases during the tissue micro array assembling. $\beta 1$ expression was positive in 79 of the 100 evaluated cases (79%). The univariate and multivariate analyses showed that the negative expression of $\beta 1$ integrin was associated with biochemical recurrence ($p = 0.047$) and time to recurrence after radical prostatectomy ($p = 0.023$). When $\beta 1$ was negative, the odds ratio for recurrence was 2.78 times higher than that observed in the positive cases [OR = 2.78, $p = 0.047$, IC 95% (1.01-7.66)].

Conclusions: The loss of $\beta 1$ integrin immune expression was correlated with biochemical recurrence in patients treated with radical prostatectomy for localized PC.

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Prostatic Neoplasms; Cell Adhesion Molecules; Prognosis; Integrins

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INTRODUCTION

Prostate cancer (PC) is the most common solid cancer and the second leading cause of death in males in western countries (1). Radical prostatectomy (RP) is a method of treating organ-confined tumors that demonstrates good results in large series of patients; however, up to a quarter of them experience biochemical recurrence after surgery (2).

Prior identification of patients with poor prognosis is important in clinical practice in order to improve the treatment outcome. The classic prognostic parameters, such as the Gleason score and serum levels of prostate specific antigen (PSA), can estimate, to some extent, aggressiveness and recurrence but they are often deficient, even when used in combination (3,4). This deficiency is probably due to the heterogeneous nature of PC. Consequently,

the discovery of new markers is necessary to identify those patients with worse prognoses and increased likelihood of tumor recurrence more effectively, which can improve the management of PC patients.

Many aspects of oncogenesis involve changes in intercellular adhesion, and it was shown that altered expression of the cell adhesion molecule (CAM) correlates with invasion and progression in PC and other neoplasms (5-7). Integrins are transmembrane glycoprotein receptors that regulate cell-matrix interactions, thereby functioning as sensors of the environment. These CAMs form heterodimers composed of one α and one β subunit. Currently, 18 α and 8 β subunits have been identified, and different combinations of these subunits are known to dictate both their specificity for the extra cellular matrix (ECM) proteins and their signaling properties.

Integrins have several functions in cell homeostasis that involve the adhesion of cells to the ECM proteins as well to other cells. As receptors, integrins mediate the anchoring and migration of cells via the recognition of various ECM molecules. Moreover, intracellular signals generated by integrins influence gene expression and thus regulate growth, proliferation and survival (8). The existence of alternative splicing forms of the messenger RNA of some integrins further increases the diversity within the integrin family.

Despite the existence of previous research examining $\beta 1$ integrin in PC, no study to date has evaluated $\beta 1$ integrin in clinical specimens as a prognostic marker for a clinical outcome. Most prior studies in PC have primarily focused on describing integrin expression rather than on examining its correlations to tumor recurrence or biochemical free survival (9-11). The aim of this case-control study was to assess the expression profile of $\beta 1$ integrin in surgical specimens of clinically localized PC through the use of immunohistochemistry by utilizing a tissue microarray (TMA), and to evaluate the association between integrin expression and biochemical recurrence following RP.

MATERIALS AND METHODS

Case selection

We retrospectively evaluated 954 patients with clinically localized PC who underwent RP

with a curative intention between January 1994 and April 2000, all performed by the same surgeon. For this case-control study, we selected 51 patients with low, intermediate or high risk PC that had biochemical recurrence after surgery (case group). Then, 60 patients without biochemical recurrence ten years after surgery and matched to the first group, according to the PC stratification of risk, were selected as controls.

We stratified the PC patients into three groups of risk according to a combined evaluation of pre operative PSA serum level, pathologic stage and surgical specimen's Gleason score. Low risk PC patients was characterized by PSA level lower than 10ng/mL, Gleason score ≤ 6 and pT2a; the risk was intermediate when PSA level was 10 to 20ng/mL, Gleason score 7 and pT2b and higher risk when PSA level was ≥ 20 ng/mL, Gleason score ≥ 8 and pathologic stage was higher than pT2c. This risk stratification is similar to that one proposed by D'Amico; the difference is the adoption of pathologic stage and Gleason score in our study, which are more reliable in terms of prognostic strength, when compared to the clinical parameters employed in D'Amico criteria (12).

The median follow-up time was 123 months. Regarding race, 97.3% of the patients were Caucasian and 2.7% were Asian. The demographic and clinical data according to recurrence are shown in Table-1.

Tumor recurrence was defined as a PSA level exceeding 0.2ng/mL during follow-up. The tumor-node-metastasis (TNM) staging designations were assigned according to the TNM 2010 classification. All subjects provided informed consent to participate in the study and to allow their biological samples to be analyzed. Approval for the study was given by the Institutional Board of Ethics (n°1074/04).

Immunohistochemistry

Histological examination of the prostatectomy specimens were performed in formalin-fixed and totally paraffin-embedded sections stained with hematoxylin and eosin. The slides containing the primary tumor for each patient were selected by sampling the area representative of the final pathologic Gleason score. Two areas from each

Table 1 - Demographic and clinical data: recurrence vs. no recurrence.

	Biochemical recurrence (n = 51)	No biochemical recurrence (n = 60)	P-value
Age (years)			0.687 #
Median (Q1 - Q3)	65.0 (60.0 - 68.0)	64.5 (59.2 - 69.0)	
Minimum - Maximum	45 - 74	41 - 79	
PSApre (ng/mL)			0.752 ##
PSA < 10	30 (58.8%)	39 (65.0%)	
PSA 10-20	15 (29.4%)	16 (26.7%)	
PSA > 20	6 (11.8%)	5 (8.3%)	
Pathologic stage			0.521 ##
T2	41 (80.4%)	51 (85.0%)	
T3	10 (19.6%)	9 (15.0%)	
Gleason score			0.095 ##
≤ 6	9 (17.6%)	20 (33.3%)	
= 7	17 (33.3%)	21 (66.7%)	
≥ 8	25 (49.0%)	19 (31.7%)	
PC stratification of risk			0.266 ##
Low	6 (11.8%)	10 (16.7%)	
Intermediate	15 (29.4%)	24 (40.0%)	
High	30 (58.8%)	26 (43.3%)	

- Mann-Whitney Test

- Chi-square test

tumor were marked with permanent ink; these areas were included in the tissue microarray assay (TMA) (13).

The samples underwent a heat antigen retrieval process using citrate buffer (1mM, pH 6.0). The slides were incubated overnight at 4°C with the anti-β1 integrin monoclonal antibody (Dako Cytomation, CA) in a 1:50 dilution. The LSAB system was used for the immunostaining (Dako Cytomation, CA). Color was developed through a reaction with a 3,3'-diaminobenzidine substrate-chromogen solution followed by counterstaining with Harris hemato-

xilin. The slides were dehydrated, coverslipped and observed under a light microscope.

β1 integrin expression was evaluated as positive or negative through immunohistochemistry in a TMA containing two samples from each case. We employed optical microscopy and the expression was considered positive when it was strong and diffusely present in the tumor tissue (i.e., +++). In order to confirm the integrity of the antigen determinants, we also evaluated the expression of PSA and cytokeratin 18 (CK18) in the same TMA. All of the cases were evaluated by a single uropathologist (KRML).

Statistical analysis

Statistical analyses were performed using SPSS 17.0 for Windows (version 17.0.0). All of the reported p-values were two-sided. The Mann-Whitney, Pearson chi-square and Fisher Exact tests were used to explore the bivariate associations between the status of tumor recurrence and other continuous and categorical covariates.

An approximation of the risk association between integrin expression and recurrence was estimated by odds ratios (ORs) and 95% confidence intervals (95% CI) using unconditional logistic regression analysis. Kaplan-Meier curves were used to illustrate biochemical recurrence free survival. We performed the log-rank test to show differences between the curves.

RESULTS

The PSA and CK18 expression levels were strongly positive in all cases, confirming that the antigens were preserved in our samples. There was a loss of 11 cases during the TMA assembling. As such, $\beta 1$ expression was evaluated in a total of 100 cases.

The cases and controls exhibited similar characteristics regarding the D'Ámico stratification of risk, which was the adopted criterion used to ma-

tch the two groups, attesting to the homogeneity of this case control study (Table-1). The mean and median follow-up for the entire sample was 116 and 123 months, respectively.

The $\beta 1$ integrin was expressed in a non-polarized manner and was mainly located in the cytoplasm and the cell membrane. Positive staining of the $\beta 1$ integrin was found in 79 cases (79%). The $\beta 1$ integrin expression profile was compared with classic prognostic factors, such as Gleason score, PSA pre-surgical levels and pathological stage. These results are shown in Tables 2-4.

There was an association between higher pre-surgical PSA levels and the absence of $\beta 1$ integrin expression ($p = 0.03$). There was no correlation between $\beta 1$ integrin immune expression and Gleason score or between $\beta 1$ integrin and pathological stage.

Correlating the expression with biochemical recurrence after surgery, we found that patients with downregulated $\beta 1$ integrin expression levels had a higher probability of tumor recurrence. Fifty-eight percent of patients whose tumors showed positive $\beta 1$ integrin were free of recurrence after surgery compared to only 33% of those with negative $\beta 1$ integrin expression (Table-5). In a univariate analysis, the chance of biochemical recurrence was 2.8 times higher in patients with

Table 2 - $\beta 1$ integrin expression according to Gleason score.

	G 6	G7	G8-9	p-value
$\beta 1$ integrin				0.120
Positive	23 (92.0%)	27 (79.4%)	29 (70.7%)	
Negative	2 (8.0%)	7 (20.6%)	12 (29.3%)	Chi-square test

Table 3 - $\beta 1$ integrin expression according to pathological stage.

	pT2	pT3	p-value
$\beta 1$ integrin			0.523
Positive	66 (80.5%)	13 (72.2%)	
Negative	16 (19.5%)	5 (27.8%)	Fisher exact test

Table 4 - $\beta 1$ integrin expression according to PSA serum level.

PSA ng/mL	< 4	4-10	> 10	p value
$\beta 1$ integrin				0.030
Positive	7 (87.5%)	51 (86.4%)	21 (63.6%)	chi-square test
Negative	1 (12.5%)	8 (13.6%)	12 (36.4%)	

Table 5 - Univariate analysis: recurrence vs. no recurrence.

	Biochemical recurrence		OR	95% CI	p-value
	yes	no			
$\beta 1$ integrin					
Positive	33 (41.8%)	46 (58.2%)	2.79	1.01-7.67	0.042
Negative	14 (66.7%)	7 (33.3%)			
					Chi-square test

negative $\beta 1$ expression [OR = 2.79, p = 0.042, IC 95% (1.01-7.67)].

The Kaplan-Meier curve of biochemical free survival according to $\beta 1$ integrin immune expression is shown in Figure-1, which confirms the association between $\beta 1$ integrin and time to recurrence in localized PC treated with radical prostatectomy. The mean biochemical recurrence free survival time was 112 and 77 months for the positive and negative cases, respectively (p = 0.023 - Log-Rank test).

In the multivariate analysis, including Gleason score and $\beta 1$ integrin expression, only $\beta 1$ integrin remained independently associated with biochemical recurrence after surgery [OR = 2.78, p = 0.047 IC 95% (1.01-7.66)].

DISCUSSION

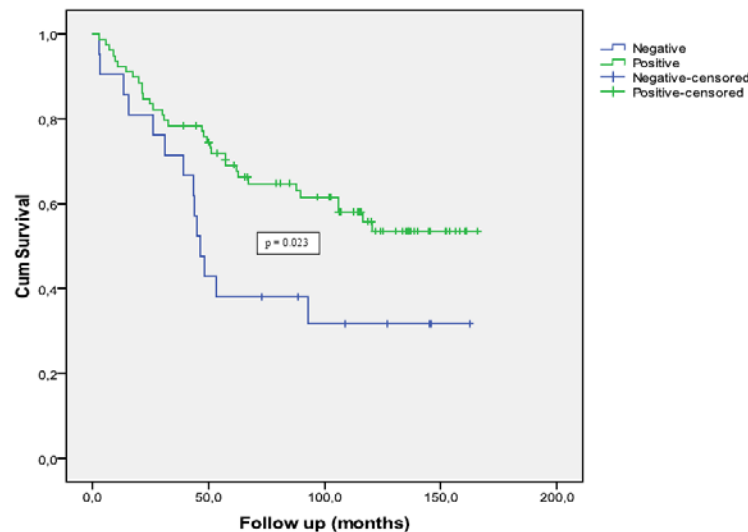
This is the first study in literature to show that the loss of $\beta 1$ integrin expression was independently associated with biochemical recurrence after RP. The strong points associated with these findings were the extended follow-up, along with the fact that all subjects underwent surgery by the same surgeon and had their integrin expression

evaluated by the same uropathologist. The employment of a TMA for integrin expression analysis also confers homogeneity to our results.

The ability of PC cells to proliferate and invade depends on their interactions with the surrounding cells and the ECM, which is partially mediated by integrins. Alterations of integrin expression have been implicated in many aspects of tumorigenesis, including cell survival, invasion and dissemination, making them potential prognostic markers. In PC, the literature indicates that with the exception of $\alpha 6$, $\alpha 3$ and $\beta 1$, there is a down regulation of most integrins (9,14-17). In two previous publications evaluating primary and metastatic PC to the lymph nodes, we also demonstrated that a globally downregulated expression of integrin expression was a characteristic of PC progression (6,7).

Special attention has been given to $\beta 1$ integrin and its splice variants because they modulate cell adhesion and migration (18). $\beta 1$ integrin has a widespread distribution and is known to complex with 10 different α subunits, which have variable binding specificities for different ECM components. As a result, this integrin may act to either prevent or promote cell migration (19).

Figure 1 - Kaplan-Meier curve for biochemical recurrence free survival in 111 primary PC patients according to $\beta 1$ integrin expression. (Log-Rank test $p = 0.023$).



The consistent $\beta 1$ expression in PC and other tumor indicates a role of this CAM in carcinogenesis, and also its potential utility as a prognostic marker (9). In kidney cancer, the expression repertoire of $\beta 1$ integrin was shown to influence the metastatic potential of cancer cells, making them attractive targets for future therapeutic strategies (20).

While there are data suggesting a role of $\beta 1$ integrin in carcinogenesis, its precise functional role in PC is not well understood (21). Previous studies have indicated that $\beta 1$ integrin and its splicing variants act by modulating cell adhesion, proliferation and survival (11,22). In vitro data in a PC3 cell line study showed that alterations in $\beta 1$ integrin expression allowed prostate tumor cells to become more invasive and increased the propensity for metastasis (15).

$\beta 1$ integrin is known to localize in focal contacts and to mediate spreading and cytoskeleton rearrangement in normal cells (16). Furthermore, $\beta 1$ integrin also promotes the activation of selective signaling pathways that support PC progression. Goel et al. observed reduction of $\beta 1$ integrin expression from well to poorly differentiated

PC in animal models, suggesting that its loss is a characteristic of PC progression (23).

Chen et al. evaluated $\beta 1$ integrin expression using immunohistochemistry in 30 PC and 30 controls and found that the loss of expression was associated with a worse prognosis in terms of Gleason score and clinical TNM stage (24). In our study, we did not find a relationship between $\beta 1$ integrin immune expression and Gleason score or between $\beta 1$ integrin and pathological stage. However, we did observe a relationship between the absence of $\beta 1$ integrin expression and higher pre-operative PSA serum levels, which is a well-known prognostic factor.

Conversely, Murant et al. showed that higher $\beta 1$ integrin expression was associated with lower E-cadherin expression and higher Gleason scores (19). This contradictory finding may be explained by the relatively small sample involving only 40 cases and the specific characteristic of their specimens, which comprised tumor tissues from transurethral resections of prostate. That sample may have represented advanced PC cases, whereas our sample came from localized tumors of patients who underwent surgery with curative intent.

Until now, five $\beta 1$ integrin splicing variants have been described, however only variants A and C are expressed in the prostate. They have been shown to differentially affect receptor localization and function (22). Variant $\beta 1C$ normally maintains adhesion and inhibits cell cycle progression, thereby inhibiting cell proliferation and migration (10,22,25). It is expressed by normal prostate tissues and is downregulated in PC; Perlino et al. evaluated 33 PC and 5 normal prostate tissues and observed a down regulation of the mRNA and protein levels of $\beta 1C$ in 94% and 100% of cancer cases, respectively (21). In contrast, variant A was maintained in PC where its translation rates had increased more than twofold, which supports its role in cell proliferation and cancer cell invasion (21-23).

We did not evaluate $\beta 1$ variants in our study because we employed an antibody that recognizes the whole $\beta 1$ repertoire. We may, however, speculate that the loss of $\beta 1$ that resulted in worse prognosis in our study probably indicates the loss of $\beta 1C$ variant, leading to the unfavorable clinical outcome observed.

Although previous studies have described alterations in $\beta 1$ integrin expression in PC, no study to date has explored the expression of $\beta 1$ integrin in a group of patients who were homogenously treated and followed for more than 10 years. Moreover, no other study has correlated expression with tumor outcomes. According to our results, although integrin $\beta 1$ was positively expressed in 79% of our cases, its downregulated expression was associated with worse outcome, indicating a role of this integrin in PC progression. We believe that the loss of $\beta 1$ expression may be important for both progression and invasion and therefore maybe useful as a prognostic marker in PC.

If confirmed, our results could be applied in clinical practice to improve the prognostic evaluations of patients with PC. In combination with other molecular and biochemical assays, test for integrin $\beta 1$ immune-expression may help to identify and select patients with greater likelihood of recurrence for adjuvant treatment, thereby leading to improved disease management. At the same time, this assay may spare many patients from unnecessary adjuvant therapies and their associated complications.

We are aware that these conclusions may not apply to other races, such as African descendant men, because our study was comprised mainly by Caucasian patients (97%). In addition, our results should be regarded as hypothesis generating due the retrospective nature, the case control design and the sample size of the present study. Therefore prospective analysis with larger series, employing another method of protein expression evaluation and containing patients of other races are necessary to validate our results.

CONCLUSIONS

In the present study, we showed that the downregulated expression of $\beta 1$ integrin was significantly correlated with biochemical recurrence after RP for localized PC and with higher levels of pre-operative PSA; thereby making this integrin a potential marker of prognosis. Analyses of the different $\beta 1$ integrin isoforms may confirm our initial findings and can help to establish the role of the $\beta 1$ integrin in PC.

DISCLOSURE

The study was supported by FAPESP (Fundação de Amparo a Pesquisa do Estado de São Paulo) process number 2010/19525-8. This study received institutional review board approval by the Ethical Board of the HCFMUSP under the protocol 1074/04.

ABBREVIATIONS

PC = prostate cancer
 RP = radical prostatectomy
 CAM = cell adhesion molecule
 ECM = extracellular matrix
 TMA = tissue microarray
 TNM = tumor-node-metastasis
 GS: Gleason score

CONFLICT OF INTEREST

None declared.

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Correspondence address:

Dr. José Pontes Jr.
 Faculdade de Medicina da USP
 Av. Dr. Arnaldo 455, 2° andar, CJ 2145
 São Paulo, SP, 01246-903, Brazil
 Phone/Fax: + 55 11 3061-7183
 E-mail: docjpjr@uol.com.br

EDITORIAL COMMENT

This is an elegant case-control study done in 111 patients from a cohort of almost 1000 patients underwent Radical prostatectomy to treat localized prostate cancer (PC).

Nowadays the immunohistochemical analysis are performed in several places with acceptable costs. Then if this results be reproduced by other groups or validated externally, this new marker integrine could became a valuable tool in defining

prognostic in localized PC patients, like as in breast cancer, currently (1).

Unfortunately in this series there were not black or mulato patients. We do not know if the racial factors can influence this marker's expression. I think that this marker could be tested in large multicentric cohorts containing Black and mulatos patients, as is the racial distribution in Brazil. In several series, worldwide, Black men and Afro descendants with PC present worst outcomes than the Caucasian ones (2-5).

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Dr. Stênio de Cássio Zequi

*Membro Titular Sociedade Brasileira de Urologia
Mestre e Doutor em Oncologia-Fundação Antônio Prudente-
SP*

*Rua Batataes, 391 - 4o. andar
Jardim Paulista, SP, 01423-010, Brazil
E-mail: steniozequi@uol.com.br*



Validation of the Brazilian version of the Expanded Prostate Cancer Index Composite (EPIC) for patients submitted to radical prostatectomy

Enaury Alves, Rebecca Medina, Cássio Andreoni

Department of Urology, Escola Paulista de Medicina da Universidade Federal de São Paulo, EPM/UNIFESP - Sao Paulo, SP, Brazil

ABSTRACT

Objectives: Validation of the Expanded Prostate Cancer Index Composite (EPIC) questionnaire translated to Portuguese. This is an evaluation tool of the effects of treatment on quality of life of patients with prostate cancer.

Materials and Methods: In order to translate and validate, several recommended methodological techniques in the literature were included: initial translation, synthesis of translation, board committee review and back translation. Sample included 40 patients with localized prostate cancer submitted to surgical retropubic radical prostatectomy from 2008 to 2010.

Results: The internal consistency analysis of the scales of the questionnaire resulted in alpha Cronbach coefficients "very good" (> 0.9) and "good" (> 0.8) to 8 of 14 domains. The higher coefficients (0.94) were assigned to sexual score, subscales incontinence and sexual function. Post-operative follow-up ranged from 3 to 35 months, median 18.7 months.

Conclusions: The Brazilian version of EPIC is reliable and valid, and is a useful tool to evaluate the impact of retropubic radical prostatectomy on quality of life of Brazilian patients with localized prostate cancer, in national and international studies.

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INTRODUCTION

Prostate cancer is the sixth most common cancer in the World, representing 10% of all tumors. In Brazil, it is the second most common cancer among men (behind non-melanoma skin cancer), and it is 52.350 new cases are estimated in 2010, or an estimated risk of 54 new cases per 100.000 men. The rise of incidence is due to the diagnosis of asymptomatic patients, the rise of life expectancy and the improvement of the information systems of the country. The incidence raised mainly in areas of the country where

surveillance is common (1). The diagnosis of prostate cancer is made through rectal exam and prostate specific antigen essay (PSA) and confirmation with ultrasound-guided prostate biopsy. According to the recommendations of the Brazilian Society of Urology, the preventive exam must be done after 45 years of age, and for those with family history of prostate cancer after 40 years of age (2,3).

Prostate cancer treatment depends of the staging of the disease, histological grade, patient age and general status of the patient. To localized tumors, surgery is an option (radical pros-

tatectomy), as well as external radiotherapy or brachytherapy. To localized advanced tumors, it has been used surgery or radiotherapy combined with hormone therapy, and to metastatic disease, the treatment of choice is hormone therapy (4).

However, all therapeutic options have collateral effects (radical prostatectomy, brachytherapy and external radiotherapy), such as urinary incontinence, erectile dysfunction and intestinal alterations. The evaluation of the treatment must address not only the survival but the negative impact on quality of life of patients (5,6).

Quality of life evaluation related to health includes all aspects of patient evaluation and can verify the impact of the disease, health and treatment (7). The used questionnaires to evaluate quality of life are useful to transform subjective measures in objectives evaluations in order to quantify and analyze specific and global aspects (8,9).

World Health Organization (WHO) defines quality of life as the individual perception of his role in life, in relation to his (her) objectives, expectations, patterns and worries, in culture and values contexts. It is a broad and complex concept that encompass physical health, psychological status, independence level, social relations, personal beliefs, and relation to environment (10,11).

MATERIALS AND METHODS

EPIC questionnaire is a self-administered tool and is intended to evaluate the impact of treatment on quality of life of patients with prostate cancer. It includes 50 questions, of four domains: urinary, intestinal, sexual and hormonal; it evaluates the last four weeks and includes a Likert scale of answers of 5 options. Each domain contains two subscales (function and nuisance). The urinary domain still has two additional subscales: incontinence and irritative/obstructive symptoms (12).

Procedures for translation and validation

The translation and validation of the questionnaire was performed in four steps: initial translation, translation synthesis, board committee of specialists review and back translation, according to the criteria described by Guillemin et al (13).

Initial translation

Question translation was performed by two independent translators, fluent in English and aware of the study objectives.

Translation Synthesis

Production of a synthesis of the two translated versions carried out by the researcher and leader, maintaining the same fundamental characteristics of the original questionnaire.

Review by a Board of Specialists Committee

A committee of specialists included five urologists fluent in English that evaluated for each question the semantic, idiomatic, cultural and conceptual equivalents between the original and the translated version.

Semantic equivalence refers to the meaning of the words (grammar and vocabulary), while idiomatic equivalence refers to colloquial expressions that are equivalent to the original idiom. Cultural equivalence refers to the lived experiences in the cultural context of society. The conceptual equivalence refers to the words with different cultural meaning (14).

A first final version in Portuguese was proposed during the meeting of the panel of specialists, the leader and the researcher, after joint evaluation of instructions, questions and format.

Back Translation

During back translation, the questionnaire was translated again, but this time from Portuguese to English, by two native English translators with domain of the Portuguese language, who did not know the objectives of the study or the original questionnaire. Afterwards, they compared the translation with the original and did not find any discrepancy.

Population and Sampling

The sample included 40 male patients randomly selected in the ambulatory of Urology of Hospital São Paulo, with the following inclusion criteria: a) diagnosis of localized prostate cancer; b) clinical stage T1 or T2; c) did not receive any previous treatment for prostate cancer; d) therapeutic indication of retropubic radical prostatectomy.

Ethical Aspects

Initially, an e-mail authorization was referred to the authors of EPIC in order to translate and validate the original to Portuguese. After that authorization, the research was approved by the Ethical Committee of Research of the Federal University of São Paulo. All patients signed an Informed Consent.

Statistical analysis

In this phase, the same procedure adopted by the original EPIC study was performed, allowing the evaluation of a total sample of heterogeneous patients regarding the impact of treatment on the domains: urinary, intestinal, sexual and hormonal. The tests were performed using the quality of life scores obtained after treatment.

To internal consistency analysis, in order to verify the reliability of the instrument (15), it was used the statistical alpha test of Cronbach (16). This index varies from 0 to 1. The closer to 1, the higher the reliability of the instrument. Internal consistency was considered: very good, if superior to 0.9; good, between 0.8 and 0.9; reasonable, between 0.7 and 0.8; weak, between 0.6 and 0.8; and poor, between 0.6 and 0.7; it was considered inadmissible if inferior to 0.6. If there is no variance between the individual items, the alpha score is 1: all items of the instrument are totally homogeneous, producing exactly the same variance (17,18).

The significance level for the statistical tests was 5%, or $p < 0.05$. Data analysis was made using the software SPSS - Statistical Package for Social Science.

RESULTS

Socio-demographic characteristics of the 40 patients are described in Table-1. Median age was 63.8 ± 8.6 years, varying from 46 to 78 years, and 70% of patients were married or had a fixed partner.

Table-2 depicts the results of internal consistency, median and standard deviation for the 14 domains (4 summaries and 10 subscales) of the Brazilian version of EPIC. The internal consistency analysis of the scales of the questionnaire resulted in Cronbach alpha coefficients "very good" above 0.9 and "good" above 0.8 to 8 of them, and the higher

scores were obtained for the sexual score, subscales incontinence and sexual function. When we compared these data with the original version and the Spanish version (Table-3), it is observed that they are similar, without significant differences.

Table-4 shows the data for patients older than 65 or with less than 65 years and EPIC scores. It is observed that as the age rises, there is a decline of the scores "sexual domain", "sexual function", "incontinence" and "urinary distress".

Table 1 - Socio-demographic profile.

Age	
Median	63.8%
Standard Deviation	8.6
Minimum	46
Maximum	78
Colour / Race	
White	42.5%
Black	42.5%
Brown	15.0%
Marital Status	
Married/fixed partner	70.0%
Single	12.5%
Widow	15.0%
Divorced	2.5%
Schooling	
Illiterate / Incomplete basic school	35.0%
Complete basic school/Incomplete high school	35.0%
Complete high school /Incomplete higher education	20.0%
Complete higher education	10.0%

Table 2 - Descriptive measures of EPIC.

HRQOL Domain	Number of itens	Mean Score (sd)	Internal consistency reliability
HRQOL Domain Summary Scores			
Urinary	12	82.2 (12.1)	0.87
Bowel	14	96.7 (2.1)	0.81
Sexual	13	35.2 (16.3)	0.94
Hormonal	11	94.0 (9.2)	0.74
Domain-Specific HRQOL Subscales			
Urinary Subscales			
Function	5	82.9 (14.9)	0.69
Bother	7	81.7 (11.0)	0.84
Incontinence	4	73.0 (8.7)	0.94
Irritative/Obstructive	7	89.3 (9.5)	0.78
Bowel Subscales			
Function	7	96.2 (2.5)	0.53
Bother	7	97.1 (1.6)	0.87
Sexual Subscales			
Function	9	29.1 (14.0)	0.94
Bother	4	48.9 (13.1)	0.93
Hormonal Subscales			
Function	5	89.7 (12.8)	0.57
Bother	6	97.6 (2.0)	0.66

Post-operative follow-up varied from 3 to 35 months, median 18.7 months, with minimal significant correlations of the following EPIC scores: “domain and urinary function”, “irritative/obstructive”, “domain, function and intestinal distress” and “hormonal distress”). It is observed that as the time passes, the scores tend to decrease (Table-5).

DISCUSSION

The literature highly recommends the adaptation of scales and questionnaires previously validated, due to reduction of costs, a standardization of measure for investigation in different cultures, allowing international studies to compare the same phenomena in several cultures (19).

Table 3- Comparison of EPIC data of original, spanish and brazilian versions.

HRQOL Domain	Number of items	Original Version		Spanish Version		Brazilian Version	
		Mean Score (sd)	Internal consistency reliability	Mean Score (sd)	Internal consistency reliability	Mean Score (sd)	Internal consistency reliability
HRQOL Domain Summary Scores							
Urinary	12	80.2 (17.5)	0.88	74.8 (21.1)	0.73	82.2 (12.1)	0.87
Bowel	14	86.6 (15.7)	0.92	92.2 (9.7)	0.75	96.7 (2.1)	0.81
Sexual	13	33.1 (23.6)	0.93	36.5 (22.0)	0.89	35.2 (16.3)	0.94
Hormonal	11	86.6 (13.8)	0.82	90.0 (11.4)	0.66	94.0 (9.2)	0.74
Domain-Specific HRQOL Subscales							
Urinary Subscales							
Function	5	86.5 (16.7)	0.69	72.3(24.5)	0.68	82.9 (14.9)	0.69
Bother	7	75.8 (20.4)	0.85	76.5 (22.7)	0.74	81.7 (11.0)	0.84
Incontinence	4	83.2 (22.9)	0.89	72.2 (35.7)	0.85	73.0 (8.7)	0.94
Irritative/Obstructive	7	79.7 (18.5)	0.81	77.5 (19.2)	0.64	89.3 (9.5)	0.78
Bowel Subscales							
Function	7	87.9 (13.6)	0.75	89.2 (10.7)	0.43	96.2 (2.5)	0.53
Bother	7	85.3 (18.8)	0.9	95.2 (10.7)	0.75	97.1 (1.6)	0.87
Sexual Subscales							
Function	9	29.5 (24.0)	0.92	24.2 (19.4)	0.88	29.1 (14.0)	0.94
Bother	4	41.1 (30.1)	0.84	64.0 (43.0)	0.98	48.9 (13.1)	0.93
Hormonal Subscales							
Function	5	84.0 (15.3)	0.51	84.9 (14.2)	0.25	89.7 (12.8)	0.57
Bother	6	88.7 (13.6)	0.73	94.3 (11.1)	0.59	97.6 (2.0)	0.66

Table 4 - Correlation of EPIC score and age.

HRQOL Domain	< 65 years (a) (n = 23)		≥ 65 years (b) (n = 17)		p value between (a) and (b)
	Mean	Sd	Mean	Sd	
HRQOL Domain Summary Scores					
Urinary	81.2	7.5	75.2	13.5	0.08
Bowel	95.7	3.1	96.2	1.7	0.55
Sexual	40.4	15.7	28.1	17.7	< 0.03
Hormonal	90.9	11.3	90.7	9.1	0.95
Domain-Specific HRQOL Subscales					
Urinary Subscales					
Function	81.4	10.5	75.0	17.4	0.16
Bother	81.2	5.9	75.2	12.1	< 0.04
Incontinence	77.6	8.1	66.9	9.4	< 0.00
Irritative/Obstructive	85.9	4.2	86.5	12.9	0.84
Bowel Subscales					
Function	94.0	3.5	96.3	2.5	< 0.03
Bother	97.0	2.0	96.2	0.8	0.13
Sexual Subscales					
Function	35.3	14.1	20.7	14.5	< 0.00
Bother	51.9	14.0	44.9	12.1	0.11
Hormonal Subscales					
Function	86.1	15.5	84.8	9.8	0.76
Bother	95.7	0.9	94.9	1.0	< 0.01

After careful review of literature, in order to identify all available instruments for the evaluation of the impact of treatment of prostate cancer in quality of life, EPIC questionnaire was chosen, since it was the one that best approached our goals and was highly accepted in the international scientific community, and easy to use. Since EPIC was develo-

ped to the American population, it was necessary to translate it and adapt it to Brazilian context and to test its measure proprieties.

Final version of translated EPIC (Appendix) maintained the English original title, since it is internationally recognized. It was added "Brazilian Version" to the title to facilitate the search in data bases.

Tabela 5 - Correlation between the score and the EPIC segment postoperatively.

HRQOL Domain	< 12 months (a) n = 14		≥ 12 months (b) n = 26		p value between (a) and (b)
	Mean	Sd	Mean	Sd	
HRQOL Domain Summary Scores					
Urinary	73.9	9.5	81.6	10.7	< 0.03
Bowel	99.2	1.6	95.0	3.0	< 0.00
Sexual	35.1	14.9	35.2	17.3	0.99
Hormonal	91.5	11.1	92.8	9.5	0.70
Domain-Specific HRQOL Subscales					
Urinary Subscales					
Function	70.6	7.3	83.2	14.4	< 0.00
Bother	75.6	10.7	80.3	7.6	0.11
Incontinence	69.5	6.4	74.9	10.2	0.08
Irritative/Obstructive	79.5	11.3	88.1	7.0	< 0.01
Bowel Subscales					
Function	98.2	2.5	94.4	3.6	< 0.00
Bother	99.9	0.0	95.6	2.5	0.00
Sexual Subscales					
Function	29.9	13.6	28.7	14.7	0.80
Bother	46.9	11.6	50.0	14.2	0.49
Hormonal Subscales					
Function	88.4	16.1	89.2	11.9	0.86
Bother	94.6	1.5	97.4	1.4	< 0.00

The patients that answered the questionnaire were invited to answer open question regarding each item in order to identify questions with difficult understanding. In general, the ins-

trument was easy to apply and well accepted by the patients, with good efficacy.

The results obtained in the current study are consistent with the original and indica-

te equivalency with the Brazilian version, with adequate reliability, high sensitivity to alterations. However, it is important to state that the questionnaire was used only for patients submitted to retropubic radical prostatectomy. In the present, another study comparing “open” to robotic-assisted laparoscopic radical prostatectomy is being carried out.

This questionnaire, after comparison of treatments, will be a resourceful tool for clinicians and researchers, to evaluate the impact of different treatments on quality of life of patients with prostate cancer.

CONCLUSIONS

Brazilian version of EPIC is valid and reliable as a new tool for the evaluation of the impact of retropubic radical prostatectomy in quality of life of Brazilian patients with localized prostate cancer, for national and international studies.

APPENDIX

The Expanded Prostate Cancer Index Composite (EPIC) – Brazilian version – Available at:
<http://www.uroepm.com.br/setores/robotica.html>

CONFLICT OF INTEREST

None declared.

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Correspondence address:

Dr. Cássio Andreoni
Disciplina de Urologia - UNIFESP/EPM
Rua Napoleão de Barros, 715 - 2º andar.
CEP: 04024-002 - Vila Clementino - São Paulo / SP
Tel.: (55) 11 - 5576-4086 - Fax: (55) 11 - 5572-6490
E-mail: cassio.andreoni@globo.com



Prognostic factor for Korean patients with renal cell carcinoma and venous tumor thrombus extension: application of the new 2009 TNM staging system

Min Chul Cho, Jung Kwon Kim, Kyung Chul Moon, Hyeon Hoe Kim, Cheol Kwak

Department of Urology, Dongguk University (MCC); Department of Urology (JKK, HHK, CK), College of Medicine, Seoul, Korea and Department of Pathology (KCM), Seoul National University College of Medicine, Seoul, Korea

ABSTRACT

Purpose: To study the surgical outcomes of radical nephrectomy with thrombectomy and to determine prognostic factors for survival of Korean patients with renal cell carcinoma (RCC) and venous tumor thrombus.

Materials and Methods: A total of 124 patients with RCC and venous tumor thrombus who underwent radical nephrectomy and thrombectomy were included in this retrospective study. Cancer-specific survival (CSS) and recurrence-free survival (RFS) rates were analyzed retrospectively according to various prognostic factors.

Results: The median overall follow-up period for all patients was 29.0 months; the median survival period was 50.0 months. The 2-, 5- and 10-year CSS rates for all patients were 64.2%, 47.1% and 31.7%, respectively. Those for 76 patients (pN0/xM0) without metastasis at presentation were 80.9%, 64.5% and 44.9%, respectively. For all patients, lower body mass index (BMI), higher Fuhrman grade, presence of symptoms, perinephric fat invasion, invasion of inferior vena cava (IVC) wall, lymph node (LN) involvement and distant metastasis at presentation were independent predictors for decreased CSS on multivariate analysis, while thrombus level was not. For non-metastatic patients, lower BMI, presence of symptoms and tumor size were independently associated with decreased CSS. In terms of RFS, lower BMI, presence of perinephric fat invasion were prognostic factors for recurrence.

Conclusions: Our data suggest that obesity is independently associated with better survival or lower risk of tumor recurrence in Korean patients undergoing radical nephrectomy with tumor thrombectomy. Also, our results indicate that Fuhrman grade, presence of symptoms, perinephric fat invasion and invasion of IVC wall, LN involvement and distant metastasis at presentation are independent predictors for survival.

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INTRODUCTION

About 5-10% of renal cell carcinoma (RCC) extends as tumor thrombus into the venous system such as renal vein or inferior vena cava (1). Despite advances in radiation, chemotherapy,

and immunotherapy, surgical resection is still the standard treatment for RCC with tumor thrombus (2). However, the range of 5-year survival rates for RCC patients with venous tumor thrombus treated with radical nephrectomy and tumor thrombectomy is only 35% - 45% despite the developments

in surgical techniques and perioperative care (3-5). Furthermore, even the 5-year survival rate for patients without evidence of nodal or distant metastasis at presentation is just 45% - 65% (4,6-8).

Although the prognostic significance of many factors has been tested, only a few are widely used in clinical practice (9). Currently, pathologic stage (T stage), lymph node (LN) status (N stage), and histologic grade represent the main prognostic variables in patients with RCC (10). Accordingly, the American Joint Committee on Cancer (AJCC) TNM classification is regularly revised and a new 2009 AJCC TNM stage classification system has been recently proposed (11).

It has been demonstrated that RCC has different clinical characteristics according to ethnicity, the presenting symptoms, course of disease, and outcomes after standard treatment varied significantly between patients of Caucasian, Hispanic, African-American, and Asian backgrounds (12). To date, there has been sparse data on surgical outcomes and prognostic factors of survival after radical nephrectomy and thrombectomy in Asian populations with RCC and venous tumor thrombus since most studies have been performed in Western countries. The aim of this study was to examine the surgical outcomes of radical nephrectomy with thrombectomy. We also evaluated the prognostic factors that influence survival of Korean patients with RCC and tumor thrombus extension into renal vein or inferior vena cava (IVC), with application of the newly revised 2009 AJCC TNM staging system.

MATERIALS AND METHODS

Study design

This study was approved by the Institutional Review Board of our institution. From February 1988 to April 2009, 1457 patients underwent nephrectomy for RCC at our institution. Among these, a total of 124 patients who underwent radical nephrectomy and thrombectomy for RCC and venous tumor thrombus were included in this retrospective study. The exclusion criteria included the presence of von Hippel-Lindau disease, tuberous sclerosis syndrome, and synchronous bilateral tumor. Pathologic slides from all specimens were re-reviewed by a sin-

gle uropathologist (KCM) who had no knowledge of patient outcomes.

A retrospective review was performed to evaluate patient demographics, presence or absence of clinical symptoms at initial presentation, laboratory findings, American Society of Anesthesiologists (ASA) scores, TNM stage, tumor size, laterality, perioperative complications, and pathologic features. Pathologic staging was determined according to the newly revised 2009 AJCC TNM classification system (11). Tumor grade and histological classification were determined according to Fuhrman's nuclear grading system and 2004 WHO classification system, respectively. Body mass index (BMI) was categorized according to the cutoff points for Asian populations recommended by the WHO (normal; < 23kg/m², overweight; 23 - 25kg/m², obese; ≥ 25kg/m²) (13). Preoperative evaluation included medical history, physical examination, routine laboratory work, chest x-ray, computed tomography (CT) of the abdomen and pelvis, and selective use of chest CT and bone scanning. Postoperative follow-up included blood tests, chest x-ray, and abdominal CT. Chest CT, brain MRI, and bone scan were performed as recommended.

Surgical technique

All patients underwent radical nephrectomy and thrombectomy through a flank, Chevron and thoracoabdominal approach. In one patient with right atrial thrombus, median sternotomy was performed to assess the right atrium. The venous tumor thrombus was removed according to its extent. Liver mobilization was performed in three patients with infrahepatic IVC thrombus, five with retrohepatic IVC thrombus and two with supradiaphragmatic IVC thrombus. A cardiopulmonary bypass was required for three of five patients with retrohepatic IVC thrombus and two with supradiaphragmatic IVC thrombus. An IVC filter was placed in three patients with IVC thrombus. In nine (7.3%) patients in whom invasion of the IVC wall was observed, the wall was partially resected with a negative margin and reconstructed with synthetic grafts as appropriate.

Data analysis

The statistical endpoints in our analysis were cancer-specific survival (CSS) and recurrence-

-free survival (RFS) of the patients. Patients with evidence of metastases at presentation were excluded from analysis of RFS. Survival analyses were performed according to the Kaplan-Meier method. Cox proportional hazard regression model was used to evaluate the prognostic factors for outcomes and their hazard ratio (HR). Variables with p-values less than 0.05 in the univariate analysis were included in the multivariate model and their HR with a 95% confidence interval (CI) was calculated. A 5% level of significance was used for all analyses and all statistical tests were two-sided. The Statistical Package for the Social Sciences (version 17.0) was used for analysis.

RESULTS

General characteristics

Baseline clinicopathological and tumor characteristics of the patients are summarized in Table-1. The median follow-up period for all patients was 29.0 months (range, 1 to 222), while that for survivors at the last follow-up was 49.0 months (range 1 to 222). At the time of analysis, 65 patients (52.4 %) died of RCC at a median follow-up period of 14.0 months (range 3 to 134), 2 patients (1.6%) died of non-cancer-related causes at 76 and 111 months postoperatively, respectively, 21 patients (16.9%) survived with the disease, and 36 patients (29.0%) were alive with no evidence of disease.

In two patients with left-sided RCC involving retrohepatic or infrahepatic IVC, a pulmonary embolism occurred intraoperatively. One of the patients died 1 day after operation, and overall perioperative mortality (death within one month after surgery) was 0.8%.

Survival, recurrence, and prognostic factors

The 2-, 5- and 10-year CSS rates for all patients were 64.2%, 47.1% and 31.7%, respectively. The median CSS time was 50.0 months for all patients. For 48 patients with metastasis (N1 or M1) at presentation, the 2-, and 5-year CSS rates were 35.9% and 16.9%, respectively, while the median CSS time was 16 months (Figure-1). For all patients as well as patients (pN0/xM0) without metastasis at presentation, the CSS rates in the patients with renal vein thrombus were higher than those in the pa-

tients with IVC thrombus (Figure-1). For 76 patients (N0/xM0) without metastasis at presentation, the 2-, 5-, and 10-year CSS rates were 80.9%, 64.5% and 44.9 %, respectively, and the median survival time was 107.0 months (Figure-1). The 2- and 5-year RFS were 50.7% and 37.3%, respectively, and the median RFS time was 25 months. Tumors recurred in 49 (64.5%) of the patients without metastasis at presentation; 28 of these individuals developed isolated metastases and 21 had disease recurrences at multiple sites. Median time for recurrence was 12 months (range, 1 to 222). Lung was the most common site of first recurrence (39.5%) followed by liver (14.5%), bone (13.2%), and brain (6.8%). Of the 30 patients with lung metastases, 15 developed isolated lung metastases and 15 had metastases in multiple sites.

Multivariate Cox regression analysis showed that lower BMI, presence of symptoms at presentation, higher Fuhrman grade, LN involvement, distant metastasis at presentation, perinephric fat invasion and invasion of IVC wall were independent factors for predicting cancer-specific death in all patients (Table-2). Tumor thrombus level (IVC versus renal vein) was significantly associated with cancer-specific death in the univariate model (Figure-1), but not an independent predictor in the multivariate analysis (Table-2).

For the 76 patients without metastasis at presentation, multivariate model showed that lower BMI, the presence of symptoms at presentation and tumor size correlated independently with reduced CSS rates (Table-3). In terms of RFS, lower BMI and the presence of perinephric fat invasion were the independent prognostic factors for relapse in the multivariate model (Table-3).

DISCUSSION

To date, reports on survival rates and prognostic factors for CSS and RFS after radical nephrectomy and thrombectomy in Asian populations with RCC and venous tumor thrombus have been limited to a small number of series (14,15). Although performed in Western country, a recent study involving a large population based on the California Cancer Registry showed that Asian/Pacific Islanders have a higher survival rate than all other racial and ethnic groups (12). In our study,

Table 1 - Baseline clinical and pathological characteristics on 124 patients.

Variables	No. pts (%) or Mean \pm SD	
	Renal vein thrombus (n = 88)	IVC thrombus (n = 36)
Clinical variables		
Age, yr	57.0 \pm 11.2	55.5 \pm 11.6
BMI, kg/m ²	23.3 \pm 2.6	23.3 \pm 3.4
< 23	37 (42.0)	19 (52.8%)
23 - 25 (overweight)	26 (29.6)	7 (19.4%)
\geq 25 (obese)	25 (28.4)	10 (27.8%)
No. sex (M/F)	60 (68.2) / 28 (31.8)	25 (69.4) / 11 (30.6)
No. ASA score		
1	29 (33.0)	7 (19.4)
2	46 (52.3)	21 (58.3)
3	13 (14.7)	6 (16.7)
4	0 (0.0)	2 (5.6)
No. symptoms (%)		
Gross hematuria	26 (29.5)	12 (33.3)
Pain/discomfort	25 (28.4)	12 (33.3)
Palpable mass	2 (2.3)	4 (11.1)
Weight loss	6 (6.8)	3 (8.3)
Others	6 (6.8)	3 (8.3)
No. preop. renal insufficiency (%) [*]	23 (26.1)	13 (36.1)
Pathological variables		
Laterality (Rt/Lt)	24 (27.3) / 64 (72.7)	27 (75.0) / 9 (25.0)
T classification (3a / 3b / 3c / 4)	84 (95.5) / 0 (0.0) / 0 (0.0) / 4 (4.5)	0 (0.0) / 26 (72.2) / 8 (22.2) / 2 (5.6)
N classification (N0 or Nx / N1)	69 (80.7) / 17 (19.3)	25 (75.0) / 9 (25.0)
M classification (M0 / M1)	64 (72.7) / 24 (27.3)	22 (61.1) / 14 (38.9)

Fuhrman grade

G1 / G2	2 (2.3) / 18 (20.5)	0 (0.0) / 5 (13.9)
G3 / G4	46 (52.2) / 22 (25)	22 (61.1) / 9 (25.0)

Thrombus level

Renal vein only	88 (100.0)	0 (0.0)
Subdiaphragmatic IVC	0 (0.0)	34 (94.4)
Supradiaphragmatic IVC	0 (0.0)	2 (5.6)
No. perinephric fat invasion (%)	43 (48.9)	17 (47.2)
No. adrenal gland invasion (%)	4 (4.5)	1 (2.8)
No. collecting system invasion (%)	31 (35.2)	17 (47.2)
No. renal hilar invasion	23 (27.4%)	13 (38.2%)
No. lymphovascular invasion (%)	10 (11.4)	10 (27.8)
No. sarcomatoid feature (%)†	8 (9.1)	3 (8.3)
No. gross necrosis (%)‡	59 (70.2)	20 (58.8)
No. invasion of renal vein wall (%)	24 (27.3)	10 (27.8)
No. invasion of IVC wall (%)	0 (0.0)	9 (25.0)

No. histological type

Clear cell	78 (88.6)	24 (66.7)
Papillary	3 (3.4)	6 (16.7)
Chromophobe	4 (4.5)	2 (5.6)
Collecting ducts of Bellini	0 (0.0)	2 (5.6)
Xp11.2 translocation	2 (2.3)	1 (2.8)
Unclassified	1 (1.1)	1 (2.8)
Tumor size, cm	9.4 ± 3.3	10.1 ± 3.8

BMI = body mass index; **ASA score** = American Society of Anesthesiologists score; **IVC** = inferior vena cava; **MDRD GFR** = Modification of Diet in Renal Disease glomerular filtration rate (calculated using the abbreviated MDRD study equation)

* Renal insufficiency: preoperative MDRD GFR less than 60 ml per minute per 1.73 m², **MDRD GFR** = Modification of Diet in Renal Disease glomerular filtration rate (calculated using the abbreviated MDRD study equation)

† Sarcomatoid feature: a spindle cell malignancy that had the histological appearance of a sarcoma

‡ Gross necrosis: the presence of necrosis in the gross specimen according to the pathology reports

Figure 1 - Estimated cancer-specific survival (CSS) using Kaplan-Meier curve according to (a) thrombus level (renal vein vs. IVC) in overall patients; (b) presence or absence of metastasis (pN0/xM0 vs. pN1 or M1) in overall patients; (c) thrombus level (renal vein vs. IVC) in non-metastatic (pN0/xM0) patients; (d) presence or absence of metastasis (pN0/xM0 vs. pN1 or M1) in patients with renal vein thrombus only; (e) presence or absence of metastasis (pN0/xM0 vs. pN1 or M1) in patients with IVC thrombus. IVC = inferior vena cava.

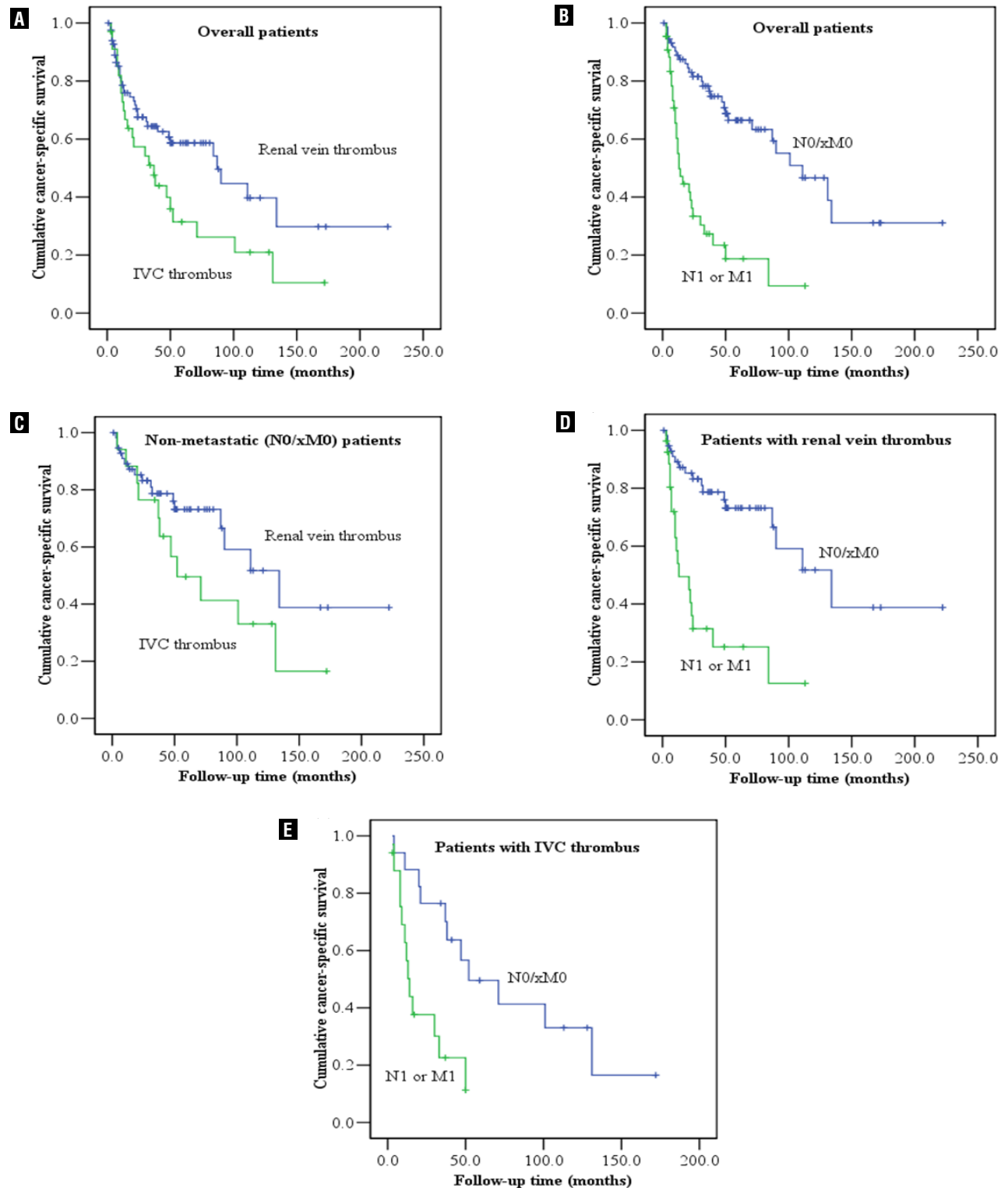


Table 2 - Multivariate Cox proportional hazard analysis for cancer-specific death in all patients.

Covariates	Hazard ratio	95% CI	p-value
BMI			
< 23 kg/m ²	1.000		
23 - 25 kg/m ²	0.768	0.356 - 1.658	0.502
≥ 25 kg/m ²	0.237	0.105 - 0.535	0.001
ASA score			
1 - 2	1.000		
3 - 4	1.949	0.863 - 4.398	0.108
Presence of clinical symptoms at presentation	3.376	1.239 - 9.195	0.017
Tumor thrombus level			
Renal vein thrombus	1.000		
IVC thrombus	1.241	0.580 - 1.908	0.573
LN metastasis	2.143	1.010 - 4.786	0.047
Distant metastasis	3.802	1.803 - 8.017	< 0.001
Tumor size	1.050	0.951 - 1.160	0.334
Fuhrman grade			
G1-2	1.000		
G3-4	5.340	1.455 - 19.593	0.012
Presence of perinephric fat invasion	2.147	1.023 - 4.508	0.043
Presence of sarcomatoid change	1.341	0.429 - 4.190	0.613
Presence of collecting system invasion	1.245	0.598 - 2.590	0.558
Presence of IVC wall invasion	4.496	1.291 - 15.657	0.018

BMI = body mass index; **ASA score** = American Society of Anesthesiologists score; **IVC** = inferior vena cava; **LN** = lymph node

* This table listed only the statistically significant prognostic factors for each outcome in the univariate Cox proportional hazard analysis.

the 2-, 5-, and 10-year CSS rates of RCC patients with tumor thrombus extension into the renal vein only and those with IVC extension were 68.5%, 55.3%, and 36.4%, and 54.2%, 29.4%, and 19.6%, respectively. Similar to our results, previous studies of Asian population have reported 5-year CSS rates between 30% ~ 40% in patients with RCC

involving the IVC (14,15). In our study, the 2-, 5- and, 10-year CSS rates of all patients and those with non-metastatic RCC were 64.2%, 47.1% and 31.7%, and 80.9%, 64.5% and 44.9%, respectively. These rates appear to be a little higher than those of most reports from Western countries (3-5,7,8,16). However, our patient population included a higher

Table 3 - Multivariate Cox proportional hazard analysis for cancer-specific death and recurrence in non-metastatic RCC (pN0/xM0) patients.

Covariates*	Hazard ratio	95% CI	p-value
Cancer-specific death			
BMI			
< 23 kg/m ²	1.000		
23 - 25 kg/m ²	0.305	0.111 - 0.840	0.022
≥ 25 kg/m ²	0.184	0.063 - 0.534	0.002
Presence of clinical symptoms at presentation	5.182	1.199 - 22.391	0.028
Tumor thrombus level			
Renal vein thrombus	1.000		
IVC thrombus	2.179	0.975 - 4.873	0.058
Tumor size	1.113	1.012 - 1.224	0.027
Recurrence			
BMI			
< 23 kg/m ²	1.000		
23 - 25 kg/m ²	0.440	0.210 - 0.922	0.030
≥ 25 kg/m ²	0.406	0.195 - 0.846	0.016
Tumor size	1.080	0.995 - 1.173	0.066
Presence of perinephric fat invasion	1.866	1.002 - 3.484	0.048

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

* This table listed only the statistically significant prognostic factors for each outcome in the univariate Cox proportional hazard analysis.

proportion of individuals with tumor thrombus extension into renal vein only than other studies except for the one by Wagner et al., and may explain these seemingly higher survival rates (16).

One of the significant findings of our study was that obesity was independently associated with better survival or lower risk of tumor recurrence in Korean patients with RCC and venous tumor thrombus. There has been a controversy about a prognostic impact of BMI on survival of patients with RCC (17-19). Recently, we demonstrated that overweight or obese Korean patients with RCC have more favorable pathological fea-

tures and a better prognosis than those with normal BMI, which is in accordance with the result from a study by Awakura et al. evaluating Japanese patients with RCC (20,21). To our knowledge, our study is the first to demonstrate the prognostic impact of obesity on survival and tumor recurrence after radical nephrectomy with thrombectomy for treating RCC and venous tumor thrombus. In our study, there was no difference in clinical or histopathological features between obese patients and those with normal BMI (data not shown). However, the underlying mechanisms for the effect of BMI on RCC survival and prognosis remain to

be elucidated. It has been suggested adipose tissue in obese patients secretes certain proteins and signaling factors including leptin and adiponectin, some of which could be involved in suppressing RCC progression (21,22). Also, lower BMI related to the presence of cachexia might be a possible explanation.

Another significant finding of our study was that a higher Fuhrman grade was independently associated with worse prognosis in Korean patients with RCC and venous tumor thrombus. Fuhrman grade is currently the most widely used grading protocol in North America and Europe, and higher grades are correlated with tumor aggressiveness and increased metastatic potential (23,24). The prognostic significance of histological grade in patients with RCC and venous tumor thrombus is controversial (4,6-8,16,25). In our study, Fuhrman grade was found to be one of most important prognostic factors for survival and relapse in patients with RCC and venous tumor thrombus, supporting the role of histological grade as an important predictor of patient outcomes. This is in accordance with a previous report from Japan demonstrating that tumor grade has more potential prognostic significance compared to other factors (14). Thus, tumor aggressiveness characterized by histological grade as well as the presence of local extension or distant metastasis of tumor may have important prognostic significance as in Asian populations with RCC and venous tumor thrombus (14).

Advanced disease at presentation is another unfavorable prognostic factor in patients with RCC, as confirmed by most studies (4,7,8,16). In accordance with previous Western studies including a recent study by Martínez-Salamanca et al. that evaluated the newly revised 2009 TNM staging system, this study demonstrated that the presence of LN involvement and distant metastasis at presentation were independent predicting factors for survival in Korean patients with RCC and venous tumor thrombus (4,7,8,16).

The newly revised 2009 AJCC TNM staging system stratifies the pT3b group according to tumor thrombus extension (11). However, our study failed to demonstrate any difference in survival between patients with tumor thrombus ex-

tension into the renal vein only and those with IVC extension. This result could have been due to the relatively small size of the group with IVC extension. Furthermore, the presence of invasion of IVC wall, not the level of tumor thrombus, was found to be a significant prognostic factor of survival in several studies (26-28). Thus, invasion of IVC wall by tumor thrombus rather than thrombus level may predict a poor prognosis. In accordance with these facts, our study showed that the presence of invasion of IVC wall was an independent prognostic factor of survival in Korean patients with RCC and venous tumor thrombus.

The presence or absence of symptoms at presentation has been shown to have prognostic significance in patients with RCC (29,30). Thus, the presence of clinical symptoms at presentation may be related to more aggressive tumors and associated with worse clinical outcomes. However, there are limited data on the influence of the presence of symptoms at presentation on survival of patients with RCC and venous tumor thrombus. Our study suggested that the presence of clinical symptoms at presentation was an independent predicting factor of cancer-specific death in both all and non-metastatic RCC patients. Contrary to our result, multivariate analysis in two studies from Western countries failed to demonstrate prognostic significance of the presence of symptoms at presentation in RCC patients with venous tumor thrombus (4,16).

Interestingly, intraoperative pulmonary embolisms only developed in two patients with left-sided RCC involving IVC who did not undergo preoperative placement of an IVC filter. This may be due to the presence of a longer venous tumor thrombus caused by a longer left renal vein in left-sided RCC patients compared to that in right-sided RCC cases, resulting in a higher probability of fractured thrombus producing pulmonary embolisms. Thus, preoperative placement of an IVC filter might be needed in patients with left-sided RCC involving IVC.

Some potential shortcomings of our study warrant consideration. Our study is limited by both its retrospective nature and the fact that it was conducted at a single institution. Nevertheless, our study involved the largest group

of Asian patients with RCC and venous tumor thrombus extension.

CONCLUSIONS

Our study indicates that CSS rate after radical nephrectomy with tumor thrombectomy for Korean patients with RCC and venous tumor thrombus is not lower than that reported in Western populations. Our data suggest that obesity is independently associated with better survival or lower risk of tumor recurrence in Korean patients undergoing radical nephrectomy with tumor thrombectomy to treat RCC with venous tumor thrombus. In addition, Fuhrman grade, the presence or absence of symptoms at presentation, LN involvement, distant metastasis at presentation, perinephric fat invasion and invasion of IVC wall were found to be independent prognostic factors for survival after surgery, while tumor thrombus level (renal vein versus IVC) was not. However, additional studies with larger cohorts including prospective, multicenter, comparative studies among patients of different ethnicities are necessary to fully validate the newly revised 2009 TNM staging in Asian population such as Korean patients with RCC and venous tumor thrombus.

CONFLICT OF INTEREST

None declared.

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Correspondence address:

Dr. Cheol Kwak
Department of Urology,
Seoul National University College of Medicine
28, Yongon-Dong, Chongno-Ku,
Seoul, 110-744, Korea
Fax: + 82 2 742-4665
E-mail: mdrafael@snu.ac.kr



Is TURBT able to cure high risk recurrent superficial or muscle invasive bladder cancer: Factors resulting in pT0 radical cystectomy specimens

Evangelos Mazaris, Shady Nafie, Gregory Boustead

Lister Hospital, East & North Hertfordshire NHS Trust, Stevenage, United Kingdom

ABSTRACT

Purpose: In 76% of radical cystectomy patients there is discrepancy between the initial stage at transurethral resection and the final pathological stage of the cystectomy specimen. More specifically in contemporary series the absence of tumor at radical cystectomy specimens (stage pT0) is estimated at 5-25%. Our aim was to determine which factors contributed to the absence of tumor in our series of radical cystectomy patients.

Materials and Methods: Fifty one patients were submitted to radical cystectomy in our department over the last 10 years (January 2002-January 2012). A thorough analysis of the patients' files with no residual tumor on the cystectomy specimen (pT0) was performed. Possible factors contributing to such a result were described and a systematic analysis of the relevant literature was performed.

Results: Five patients had a pT0 stage after radical cystectomy. Four of them had transitional cell carcinoma and one of them had squamous cell carcinoma of the bladder on the initial transurethral resection. None of the tumors presented lymphovascular invasion. Four patients are still alive and one died 45 months postoperatively from a cardiac cause.

Conclusions: Four factors were identified in our study to contribute towards a pT0 cystectomy result. Those included the absence of lymphovascular invasion, the completeness of transurethral resection, the experience of the surgeon and the use of a standardized technique for the transurethral resection. The time to cystectomy in our series did not have a negative effect on pT0 final pathology result.

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Transurethral Resection of Prostate; Cystectomy; Urinary Bladder Neoplasms

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INTRODUCTION

Radical cystectomy (RC) with pelvic lymph node dissection is the gold standard for treatment of muscle invasive bladder cancer (MIBC) as well as high risk superficial bladder cancer. In about 76% of patients there is discrepancy between the initial clinical T-stage (cT-stage) at transurethral resection of the bladder tumor (TURBT) and the final pathology (pT-stage) after RC (1). Factors that may be responsible for such discrepancy may be

poor sensitivity of current image exams, incomplete TURBT with undersampling of muscle tissue or a long interval between TURBT and RC (2,3). Clinical understaging of the tumor is reported in 40-49% of RC (1,4) while clinical overstaging occurs in 20-27% of RC (1,5). Up to 30% of patients with MIBC at the time of TURBT have non-MIBC at RC specimen (5).

There are several reports on the effect on prognosis of tumor downstaging from MIBC at TURBT to non-MIBC at RC. Although one study

reported no survival advantage (6) most of them reported excellent long-term survival rates for patients with tumor downstaging (1,5,7-11). The inclusion of patients who have been treated with neoadjuvant chemotherapy (NAC) and/or radiotherapy (5,6,8,10) complicates the interpretation of the results of these studies.

Our aim was to perform an analysis in our series (our center is a regional cancer center covering a population over 2 million) of radical cystectomies in order to determine which factors caused downstaging and particularly the absence of tumor at radical cystectomy. That obviously means that such tumors were in fact cured by just a TURBT from a surgical perspective.

MATERIALS AND METHODS

We retrospectively reviewed the final pathological result of all 51 radical cystectomies performed in our institution over the last 10 years (January 2002 until January 2012). Five pT0 radical cystectomies were discovered while patient and operative notes were thoroughly reviewed by two reviewers. Emphasis was placed when reviewing the notes on the initial TURBT pathology specimen, the surgeon who performed the TURBT and the details of the operation, whether any neo-adjuvant treatment (e.g. chemotherapy) was administered etc. Special attention was placed on the TURBT pathology specimen and thorough re-discussion with an uro-pathologist about all specimens was done. Factors that might have contributed to a pT0 cystectomy specimen were disclosed and a systematic review of the available literature (all articles in PubMed were searched including non-English publications using the keyword 'pT0 radical cystectomy') was performed in order to present possible factors leading to such favorable cystectomy specimens.

RESULTS

Five patients in our series (Table-1) submitted to radical cystectomy for bladder cancer had a final pathological stage of pT0 (no residual tumor). In all patients neither preoperative work-up (chest and computed tomography urogram) nor post-operative full lymph node dissection revealed any

lymph node or metastatic spread. Four TURBT specimens were transitional cell carcinomas (TCC) and one was squamous cell carcinoma (SCC). The estimated macroscopic size of the TURBT tumor ranged from 5 mm to 4 cm. All operative notes from the TURBT reported a complete macroscopic clearance of the tumor with deep resection and additional biopsies from the tumor bed. No lymphovascular invasion was noted in none of the TURBT specimens. All the TURBTs were performed by two experienced senior urologists and the radical cystectomies were performed by 2 urological surgeons. The time from TURBT to radical cystectomy ranged from 120-210 days. Neo-adjuvant chemotherapy was administered to two patients. Follow-up after cystectomy ranged from 6 months to 69 months. Four patients are doing very well on follow-up. One died 45 months after the operation from other cause (cardiac event) and had no cancer recurrence.

The literature review resulted in 11 articles (Table-2) after excluding case reports and small series as well as irrelevant publications and duplicates.

DISCUSSION

Our series of pT0 radical cystectomies indicated a few factors that may be contributing to such a result (no tumor found on final cystectomy specimen). Obviously it would be very important to know if there are factors after a TURBT that should prompt treatment with bladder preservation strategies for muscle invasive or recurrent high risk superficial bladder cancer. This would obviously prevent patients from having a major operation like a radical cystectomy with urinary diversion, which have high morbidity and a deterioration in the quality of life. Herr (12) found a 10-year disease-specific survival of cT2 patients who were treated with re-TURBT of 76% (57% had eventually their bladder preserved) compared with 71% for those who had immediate radical cystectomy. Although initially an older study (6) found no advantage in cancer-specific survival for pT0 cystectomy patients (the study had low number of patients to draw statistically valid results) all subsequent studies (Table-2) have shown an advantage in recurrence-free survival or cancer-specific survival.

Table 1 - Our series of pT0 radical cystectomies.

Patient (Male/Female)	Age at cystectomy	Co-morbidities/ Smoking	TURBT pathology/size	Neoadjuvant chemotherapy	Time to cystectomy (days)	Follow-up / Recurrence
1-Male	65	Hypertension	G2-3 cT2 TCC with no lymphovascular invasion (41 gm resected tissue)/4cm on MRI scan	Yes	162	9 months doing well
2-Female	71	Abdominal aortic aneurysm, myocardial infarction/Heavy smoker	G2 cT2 SCC with no lymphovascular invasion/3cm on cystoscopy calcified lesion	No	120	45 months post-op died from other cause (cardiac)
3-Male	76	Non-smoker	G2-3 cT2a at least with no lymphovascular invasion and tumour necrosis/3.5cm on CT scan	No	100	6 months doing well
4-Male	69	Hypertension	Recurrent G3 cT1 and CIS (no lympho- vascular invasion)/ Failed intravesical therapy	No	125	68 months doing well
5-Male	80	Atrial flutter ablated, poor performance status	G3 cT2 (no lympho- vascular invasion)/ quite small on cystoscopy 5mm	Yes	210	24 months doing well

It seems that a macroscopic complete resection as reported by the surgeon in the operative notes was a factor contributing to a pT0 specimen. It has been suggested in other studies (11,13) that a thorough and complete TURBT may be warranted in most patients who have even got an appearance of invasive tumor as when re-evaluating with a re-TURBT they may be candidates for bladder preservation especially if no residual tumor is present. Furthermore, a study (9) confirmed that patients with a cT2a tumor stage on TURBT had significantly better cancer-specific survival when a pT0 stage was achieved at RC (with the use of

neo-adjuvant chemotherapy) compared with those who had residual cancer on RC. As others (14) have suggested a radical TURBT is probably not causative of the improved cancer-specific survival in pT0 cystectomy patients but rather individual tumor characteristics allow for complete tumor eradication, including small tumor size, unifocality and stage T2a.

Another factor that was evident, even in large tumors measuring 3-4 cm, was the absence of lymphovascular invasion which seemed to consistently produce final pT0 radical cystectomy specimens in our series. Another study (15) has

Table 2 - Series pT0 cystectomy patients.

Author	No. of pT0	Total No. of cystectomy patients	Neoadjuvant Chemotherapy (NAC)	Neoadjuvant Radiotherapy	Staging TURBT Pathology	Median Follow Up (months)	CSS / DSS	OS	RFS
Van Dijk PR et al. (13)	62	375	--	--	--	92	93.9% (5-year)	--	91.9% (5-year)
Kassouf W et al. (15)	120	1104	Yes (77)	--	cT1 (21), cT2 (65), cT3b (20), cT4a (11), cT4b (3)	32	88% (5-year)	84% (5-year)	84% (5-year)
Chromiecki TF et al. (17)	433	5018	Yes (41)	--	--	46 (TUR-alone) 34 (NAC)	--	--	87.2% (TUR-alone) 75.6% (NAC)
May M et al. (14)	132	2403	--	--	cTa (5), cTis (9), cT1 (39), cT2 (79)	53 (mean)	92% (5-year)	75% (5-year)	--
Iriarte AL et al. (11)	20	153	--	--	T2 G3 (80%), T2G2 (10%), T1G3 (5%), Tis (5%)	24 (mean)	75%	--	65%
Kaag MG et al. (23)	24	1905	Yes (24)	--	--	27	--	--	100%
Palapattu GS et al. (10)	59	888	Yes (8)	Yes (2)	cTis (9%), cTa (4%), cT1 (32%), cT2 (52%), cT3 (4%)	56	95% (5-year)	88%	90%
Tilki D et al. (19)	228	4430	--	--	cTa-is (13.6%), cT1 (29.8%), cT2-4a (56.2%)	48.2	93.1% (5-year)	--	89.7% (5-year)
Volkmer BG et al. (24)	181	900	--	--	Ta/Tis/ T1 (43.1%), T2a/ T2b (56.9%)	120	86.5%	65.9%	85.2%
Mateo EM et al. (25)	43	420	--	--	T1 (23.3%), T2 (72%), T3 (4.7%)/ G2 (55.8%), G3 (44.2%)	89.3	88.4%	--	83.7%
Cho KS et al. (26)		197	--	--		60	84.1%	--	--

also underlined the role of lymphovascular invasion as an independent factor of advanced tumor stage, grade and shorter overall and recurrence-free survival.

There was a source of bias for two of our patients who received neo-adjuvant chemotherapy and that might have contributed to a favorable result, however, we have to take into account that chemotherapy is a non-invasive treatment and may consist part of a multimodality approach for bladder-preserving techniques. It has been demonstrated in one study (16) that the prognostic significance of a pT0 stage is independent of whether this was achieved by means of TURBT or neo-adjuvant chemotherapy. In the same study TURBT achieved a 15% pT0 rate at radical cystectomy, while neoadjuvant chemotherapy and TURBT achieved a 38% pT0 rate respectively. Others (7) claimed that neoadjuvant chemotherapy is not necessary or beneficial for downstaged cT2 tumors to pT0 by TURBT. On the contrary, another study (17) supported that patients who are pT0 after neoadjuvant chemotherapy are at higher risk of disease recurrence compared with those who achieve pT0 with TURBT alone (the explanation was that an increased rate of non-organ confined clinical stage was selected for chemotherapy in the study).

Furthermore, it has been found from studies that delay > 90 days for cystectomy is unfavorable (18) regarding prognosis. That was not confirmed in our study since even extreme delay up to 210 days did not affect the outcome. It seems that in three of our patients the TURBT cleared the tumor and in two more the addition of neo-adjuvant chemotherapy either consolidated such a result or eradicated any residual tumor. Other factors that have been mentioned in a study (19) which are associated with a worse outcome for pT0 cystectomy patients were females and patients with nodal spread, however, these could not be assessed in our series. Also, the presence of concomitant carcinoma in situ is associated with disease progression but could be treated with intravesical BCG and closer follow-up (20).

Experience of the surgeon performing the TURBT might also play a role. All our patients were operated by experienced surgeons

(> 50 procedures) and a standardized procedure was performed with the aim of completely removing the whole tumor including deep resection and also biopsy of its base. The base was then thoroughly diathermised with a rolleyball. It has been suggested that a 'radical' transurethral resection is justified when the tumor is clinically limited to the muscular layer and when all biopsies of the periphery and the base of the tumor are negative for further muscular invasion (20). This offered an 80.5% cancer-specific survival in 5 years with a bladder preservation rate of 82.7% (20).

Bladder sparing techniques have included re-TURBTs (12), the use of chemotherapy and/or radiotherapy and also the use of re-TURBT with adjuvant radiotherapy and laparoscopic lymphadenectomy for high risk tumors (Grade 2 or 3) (21). One very important multi-institutional trial (SPARE trial) comparing selective bladder preservation versus radical excision was abandoned in 2010 due to poor accrual (22). The urological community has to learn by these mistakes and conduct relevant trials that will answer the important issue of bladder preservation.

The limitations of the study are that the number of patients is obviously small to extract valid statistical results but our aim was to identify in our series factors that would be worth investigating in a multi-institutional setting in order to increase validity. More than one surgeon performed the procedures but a standard operative technique was used as described earlier.

CONCLUSIONS

Four factors were identified in our study and could be investigated further: the absence of lymphovascular invasion and a complete resection which do have a favorable role, surgical experience and adherence to standardized techniques according to guidelines also contribute to such results. On the contrary, the time to cystectomy did not seem to have affected outcomes in our case series and that also remains to be confirmed. All these issues need to be clarified by the urologic community by designing and conducting multi-institutional randomized studies.

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RC = radical cystectomy

MIBC = muscle-invasive bladder cancer

TURBT = transurethral resection of bladder tumor

TCC = transitional cell carcinoma

SCC = squamous cell carcinoma

NAC = neo-adjuvant chemotherapy

CONFLICT OF INTEREST

None declared.

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Correspondence address:

Dr. Evangelos Mazaris
908 Cavalier House,
46-50 Uxbridge Road,
London W5 2SU, UK
E-mail: evmazaris@yahoo.gr



Can the learning of laparoscopic skills be quantified by the measurements of skill parameters performed in a virtual reality simulator?

Natascha Silva Sandy, José Arnaldo Shiomi da Cruz, Carlo Camargo Passerotti, Hiep Nguyen, Sabrina Thalita dos Reis, Eder Maxwell Gouveia, Ricardo Jordao Duarte, Homero Bruschini, Miguel Srougi

Urology Department, University of Sao Paulo Medical School (NSS, JASC, CCP, EMG, RJD, HB, MS); Laboratory of Medical Investigation (LIM55), Urology Department, University of Sao Paulo Medical School (CCP, STR); Urology Department, Nove de Julho University (UNINOVE) (CCP), Sao Paulo, Brazil and Urology Department, Children's Hospital Boston (HN), Boston, Massachusetts, USA

ABSTRACT

Purpose: To ensure patient safety and surgical efficiency, much emphasis has been placed on the training of laparoscopic skills using virtual reality simulators. The purpose of this study was to determine whether laparoscopic skills can be objectively quantified by measuring specific skill parameters during training in a virtual reality surgical simulator (VRSS).

Materials and Methods: Ten medical students (with no laparoscopic experience) and ten urology residents (PGY3-5 with limited laparoscopic experience) were recruited to participate in a ten-week training course in basic laparoscopic skills (camera, cutting, peg transfer and clipping skills) on a VRSS. Data were collected from the training sessions. The time that individuals took to complete each task and the errors that they made were analyzed independently.

Results: The mean time that individuals took to complete tasks was significantly different between the groups ($p < 0.05$), with the residents being faster than the medical students. The residents' group also completed the tasks with fewer errors. The majority of the subjects in both groups exhibited a significant improvement in their task completion time and error rate.

Conclusion: The findings in this study demonstrate that laparoscopic skills can be objectively measured in a VRSS based on quantified skill parameters, including the time spent to complete skill tasks and the associated error rate. We conclude that a VRSS is a feasible tool for training and assessing basic laparoscopic skills.

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Key words:

Virtual Reality Exposure Therapy; Education; Laparoscopy

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INTRODUCTION

Among the most important advances made in surgery in the last three decades, one of the most significant is the clinical introduction of minimally invasive interventions through laparoscopy and, more recently, robotic-assisted laparoscopic surgery.

Initially restricted to a select few areas of surgery (1), the use of laparoscopy rapidly spread, necessitating capacitation as its use became adopted in many areas as the ideal form of surgical treatment.

The implementation of minimally invasive surgery requires specific and persistent training

and is accompanied by a long learning curve (2-4). More psychomotor training and practice is required to perform laparoscopic interventions due to the two-dimensional representation of the surgical field through the monitors, the absence of orientation by digital touch, and the manipulation of structures by clamps and long work tools (5,6). During the long learning process, the number and severity of intraoperative complications are often far greater than those observed in open surgery (7,8). Thus, different clinical and laboratorial tools have been employed to combat this challenge, including the guidance of experienced mentors in patient interventions and training in animals or inanimate models (9,10). This "ex-vivo" learning is viewed as particularly valuable because it eliminates the ethical constraint involved in teaching surgery in animal models or humans, is considered efficient in the acquisition of psychomotor skills, is secure, and has a reasonable cost (11). For these reasons, with the goal of improved patient safety and surgical efficiency, much emphasis has been placed on training laparoscopic skills using simulators that replicate the conditions encountered during actual surgeries.

The Surgical Council on Resident Education (SCORE), an agency dedicated to the development of training programs for surgical residents in the United States, has recommended the use of virtual reality surgical simulators in the laparoscopic training curriculum (12). However, it is not known if the use of these simulators results in a quantifiable improvement in laparoscopic surgical skills. The purpose of this study is to determine if laparoscopic skills can be objectively quantified by the specific skill parameters measured when performing in a virtual reality laparoscopic simulator.

MATERIALS AND METHODS

This prospective study was developed in the Laboratory of Experimental Minimally Invasive Surgery, Faculty of Medicine, University of Sao Paulo. The virtual reality surgical simulator "LapVR™" (Immersion Medical, San Jose, California, USA) was used for a ten-week training program (one session per week). Ten medical students (from the 1st through 4th years) with no previous expe-

rience in laparoscopy were recruited from the University of Sao Paulo Medical School, and ten urology residents (PGY3-5) with limited laparoscopic experience were recruited from the University of Sao Paulo Medical School. Informed consent was obtained from every participant.

The training consisted of the execution of the following basic laparoscopic skills: camera navigation, cutting, peg transfer and clipping. For each skill, there were three levels of difficulty. During each training session, all tasks were performed at each level of difficulty. The length of the training was ten weeks, and each weekly session lasted approximately one to two hours. The number of sessions (n = 10) was chosen based on the previous demonstration that a plateau in the learning curve occurs within 8 sessions of using the simulator (13). Each of the four tasks was performed three times during each session with increasing levels of difficulty (1 to 3). To insure uniformity, all participants were assisted by the same technician, who controlled the camera during the exercises except the camera navigation task.

The sessions were recorded, and an assessment of the performance was made by the simulator's software, which quantifies the time required to perform the task and the number of errors committed. Individual and group learning curves were obtained for each task and level of difficulty.

Analysis of variance (ANOVA) was performed with repeated measures of two factors, using the group as the fixed factor (Group I [students] x Group II [residents]) and the week as the repetition factor as well as assuming correlation matrices and unstructured and symmetric components between the weeks. For measures that showed statistically significant interactions between the groups and weeks, a comparison was made between the groups for each week. Contrasts were used to verify in which weeks the groups differed and in which weeks they began to be coincident. A 95% confidence interval was established.

RESULTS

The average times (in seconds) and the standard deviations for the completion of each skill

(difficulty levels 1 to 3) were obtained weekly for both groups. A portion of these data are represented in Table-1 (weeks 1, 5 and 10; level 2). As shown in this table, there was a reduction in the time task execution in both groups; this trend was not always followed by a similar variation in the standard deviation. In the first contact with the VRSS, the mean time to complete the tasks was in general (all three levels of difficulty) significantly different between the groups ($p < 0.05$), with the residents performing up to 2 or 3 times faster than the students on average. The residents' group also completed the tasks with fewer errors in the first week.

When we analyzed the tasks and the levels of difficulty separately, a statistically significant difference - with residents completing the exercises in a shorter amount of time - was observed at the first level of difficulty only in the first week for all four tasks (all with $p < 0.05$). Residents performed the level 1 camera navigation task more quickly ($p = 0.02$). At the second difficulty level, a

significant p-value for task completion time was obtained only in the first week for the clipping skill ($p = 0.04$), in the first two weeks for the peg transfer skill ($p = 0.03$) and up until week eight for the cutting skill ($p = 0.026$). At the third level of difficulty, there was a significant difference in completion time between the groups up until week seven for both the clipping ($p = 0.01$) and camera navigation ($p = 0.029$) tasks.

We analyzed the errors during the clipping and cutting skills, the number of dropped pegs in the peg transfer exercise, and the ability to focus on the target in camera navigation task. Group II (residents) performed statistically better than group I (students) in the first two weeks in the level 1 ability to find the target with the camera ($p = 0.019$). Similarly, group II performed better in the first week for the level two dropped pegs task ($p = 0.026$). The most significant difference in the superior group II performance was observed for level three tasks: in the clipping skill in weeks

Table 1 - Average time (in seconds) for completion of the exercise and standard deviation. - Level 02.

Skill	Week	Group I - Students	Group II - Residents	P
Camera	1	427.6 ± 97.67	269.0 ± 119.27	0.004
	5	388.4 ± 155.03	188.4 ± 103.51	0.002
	10	205.0 ± 115.77	188.2 ± 79.95	0.737
Clipping	1	149.5 ± 48.72	109.9 ± 36.59	0.043
	5	100.3 ± 36.48	86.60 ± 34.89	0.377
	10	69.55 ± 20.82	68.80 ± 31.79	0.833
Cutting	1	413.8 ± 132.8	224.0 ± 94.06	0.001
	5	240.4 ± 83.73	183.7 ± 60.14	0.026
	10	156.5 ± 32.09	156.9 ± 49.96	0.353
Peg Transfer	1	656.9 ± 316.8	339.1 ± 72.69	0.049
	5	292.9 ± 78.33	263.6 ± 100.7	0.453
	10	214.3 ± 80.83	203.2 ± 65.43	0.283

one through six ($p = 0.05$); up until week seven in target locating with the camera ($p = 0.026$); and up through week eight in the number of errors in camera navigation ($p = 0.048$).

The majority of the medical students and residents exhibited a statistically significant improvement in task completion time and error rate between the first 5 and the last 5 training sessions. Comparing the final performance of both groups, we found no statistically significant difference in any of the four basic skills for all three levels of difficulty.

DISCUSSION

Although minimally invasive surgery has revolutionized surgical techniques, laparoscopic skill acquisition has proven to be a significant and technically difficult challenge, requiring a different 'skill set' than traditional open surgery as well as presenting differences in cost, safety and ethical issues (14). Among the technical aspects, learning laparoscopic skills demands three-dimensional interpretation of a two-dimensionally represented scenario and the manipulation of endoscopic instruments (15). In addition, laparoscopic surgery has the further challenges of reduced depth perception and attenuations in the tactile sensations and end-force of the instruments (16). Such factors produce the broader base and greater slope of the learning curve, which presents a challenge in the OR environment (17-20). In this sense, VRSS has emerged as an intriguing and promising tool, enabling apprentices to acquaint themselves with this novel reality, to practice basic skills and to practice specific procedures of minor complexity. This device offers convenient and unlimited practice with the advantages of low cost and the absence of ethical concerns (21). However, it is unclear whether the use of VRSS results in a quantifiable improvement in laparoscopic surgical skills.

Previous studies have demonstrated that VRSS is a useful tool to discriminate novices from experts (22,23). In the present study, our general analysis was similarly able to distinguish medical students with no laparoscopic experience from residents with laparoscopic experience. In a deeper analysis, when we examined each basic skill se-

parately at the three difficulty levels, the ability to discriminate between the two groups was not always present. A significant difference was observed at the first level of difficulty for all tasks but could not be observed for camera navigation at the second level or for cutting and peg transfer at the third difficulty level. When we assessed these differences throughout our training, we observed the trend that the performances gradually reached a plateau and became similar, so that by the end of the training, there was no significant difference between the groups. Our findings of the time to achieve plateau were consistent with previous studies, which shown that ten weeks is more than sufficient to ensure that a plateau in the learning curve was reached, even for beginners.

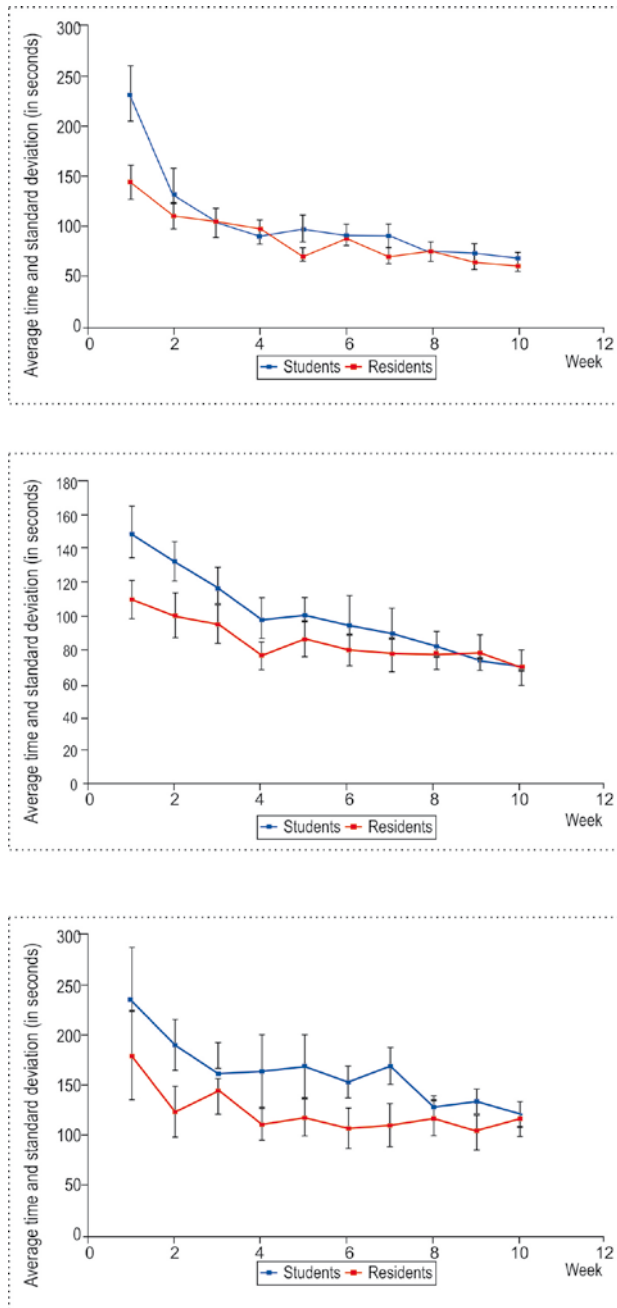
Learning is indirectly represented by the improvement in motor performance, which is inferred in terms of the speed and task precision in repetitive trials. In our study, we observed the skill learning in both groups through numerical and graphical displays (Figure-1 - learning curves of time on clipping skill, for all three levels). In support of these findings, previous studies have demonstrated that VRSS training results in a decrease in task conclusion time coupled with an increase in task execution accuracy for trainees without previous experience as well as for trainees with limited laparoscopic experience (24).

We recognize several limitations in this study. The number of participants used in our study may have been insufficient to demonstrate greater significant differences between the groups over the weeks of training. It is possible that increasing our sample size could empower the study and show larger differences in the trends found.

CONCLUSIONS

The many potential benefits of VRSS have prompted their emergence as optimal tools for training outside operating room. Our findings demonstrate that basic laparoscopic skills can be objectively measured using the skill parameters performed in a virtual reality surgical simulator by monitoring the time spent to perform the skill tasks and the associated error rate. VRSS has been shown to be a feasible tool for the training and

Figure 1 – Learning curves of time on clipping skill, for all three levels.



assessment of basic laparoscopic skills for trainees without previous laparoscopic experience as well as trainees with limited experience, with more gain for the former group. However, it remains unclear whether improvement in the skill tasks in VRSS

can be directly translated into improvements in actual surgical performance.

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CONFLICT OF INTEREST

None declared.

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Correspondence address:

Dr. Carlo C. Passerotti
Rua Barata Ribeiro, 490 / 76
Sao Paulo, SP, 01308-000, Brazil
Fax: + 55 11 3255-6372
E-mail: carlopasserotti@hotmail.com



Robot-assisted vs. Laparoscopic Partial Nephrectomy: utilization rates and perioperative outcomes

Jesse D. Sammon, Pierre I. Karakiewicz, Maxine Sun, Praful Ravi, Khurshid R. Ghani, Wooju Jeong, Marco Bianchi, Jens Hansen, Paul Perrotte, James O. Peabody, Craig G. Rogers, Shahrokh F. Shariat, Mani Menon, Quoc-Dien Trinh

Vattikuti Urology Institute, Henry Ford Health System (JS, KRG, WJ, JOP, CGR, MM, QDT), Detroit, MI, USA; Cancer Prognostics and Health Outcomes Unit, University of Montreal Health Center (PIK, MS, MB, JH, PP, QDT), Montreal, Canada; Department of Urology, University of Cambridge, Cambridge (PR), United Kingdom; Department of Urology, Università Vita Salute San Raffaele (MB), Milan, Italy; Martini-Clinic, Prostate Cancer Center Hamburg-Eppendorf (JH, SFS), Hamburg, Germany

ABSTRACT

Objectives: To examine the effect of surgical approach on perioperative morbidity and mortality after partial nephrectomy.

Materials and Methods: Within the Nationwide Inpatient Sample, patients who underwent RAPN or LPN between October 2008 and December 2009 were identified. Propensity-based matching was performed to adjust for potential baseline differences between the two groups. The rates of intraoperative and postoperative complications, blood transfusions, prolonged length of stay, and in-hospital mortality, stratified according to RAPN vs. LPN, were compared.

Results: Overall, 851 (72.5%) patients underwent RAPN and 323 (27.5%) underwent LPN. For RAPN and LPN respectively, the following rates were recorded in the propensity-score matched cohort: blood transfusions, 4.5 vs. 6.8% ($p = 0.223$); intraoperative complications, 5.2 vs. 2.6% ($p = 0.096$); postoperative complications, 10.6 vs. 13.5% ($p = 0.268$); prolonged length of stay, 6.8 vs. 9.4% ($p = 0.238$); in-hospital mortality, 0.0 vs. 0.0%.

Conclusions: RAPN has supplanted LPN as the predominant minimally invasive surgical approach for renal masses. Perioperative outcomes after RAPN and LPN are comparable. Interpretation of these findings needs to take into account the lack of adjustment for case complexity and surgical expertise.

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INTRODUCTION

The incidence of renal cell carcinoma (RCC) has steadily risen in the United States in recent years. While the rates of metastatic and regional disease have remained steady, the incidence of organ confined disease has risen (1). Increased utilization of cross-sectional imaging has led to more frequent discovery of suspicious renal masses for

which surgical resection remains the standard of care (2).

Over the past decade, the management of suspicious renal masses in patients with a healthy contra-lateral renal unit has undergone a fundamental evolution; techniques developed for imperative partial nephrectomy have now become the standard of care. Due to the downward stage migration in contemporary years (1,3), utilization

rate of partial nephrectomy (PN) has increased significantly (4). PN has been demonstrated to provide equivalent oncologic control (5), improved renal function, a lower risk of cardiovascular disease, as well as improved overall survival (6-8). In consequence, PN is supported by both American and European guidelines when indicated and technically feasible.

Laparoscopic PN (LPN) is a minimally invasive alternative to open partial nephrectomy (9,10). However, LPN is technically challenging, limiting its widespread adoption. Robot-assisted PN (RAPN) has emerged as a viable alternative to mitigate the technical challenges of LPN, demonstrating perioperative outcomes at least comparable to LPN, with the benefit of reduced warm ischemia time (11-13).

In the current manuscript, we explore the effect of minimally invasive surgical approach (RAPN vs. LPN) on five immediate and short-term PN outcomes. Specifically, we focus on blood transfusions, on intraoperative and postoperative complications, on prolonged length of stay (LOS) beyond the 75th percentile, and on in-hospital mortality. We rely on propensity-score matched analyses to adjust for potential differences between patients undergoing RAPN or LPN, using a contemporary (2008-2009) population-based cohort of individuals.

MATERIALS AND METHODS

Data Source

Data from the Nationwide Inpatient Sample (NIS) were abstracted between October 2008 and December 2009. The NIS includes inpatient discharge data collected via federal-state partnerships, as part of the Agency for Healthcare Research and Quality's Healthcare Cost and Utilization Project. As of the year 2009, the NIS contains data from approximately 8 million hospital stays drawn from more than 1,000 community hospitals in 44 States, approximating 20% of community hospitals within the United States, including public hospitals and academic medical centers. The NIS is the sole hospital database in the United States with charge information on all patients regardless of

payer, including persons covered by Medicare, Medicaid, private insurance, and the uninsured.

Sample population and surgical procedures

Using a previously described extraction methodology (14), patients with a primary diagnosis of cancer of the kidney were identified using the International Classification of Disease, 9th Revision, Clinical Modification (ICD-9-CM) diagnostic code: 189.0; patients who underwent PN (55.4) were abstracted. Secondary diagnostic codes (ICD-9-CM 197.0, 197.7, 198.x) were used to identify patients with metastases, who were subsequently excluded from analysis. Beginning October 1, 2008, the robot-assisted modifier (ICD-9-CM 17.4x) code was recognized by the National Center of Health Statistics and the Centers for Medicare and Medicaid Services and was used to identify RAPN. Laparoscopic surgical approach was identified via concurrent procedure codes for laparoscopic exploration (54.21). The remaining patients, with an absence of RAPN or LPN codes, were determined as having undergone an OPN, and were excluded from further analysis (n = 2921).

Baseline patient and hospital characteristics

Patient characteristics include age at surgery (in years), coded as a continuous variable. Gender and race (white, black and other races including: Hispanic, Asian, Pacific Islander, Native American, or other/unknown) were also examined. Baseline Charlson Comorbidity Index (CCI) was calculated according to Charlson et al. (15), and adapted according to Deyo et al. (16). To ensure uniformity of coding across data sources, detailed insurance categories are combined in the more general groups, namely: private insurance, Medicare, Medicaid, and other (self-pay).

Hospital characteristics include location (rural vs. urban), region (Northeast, Midwest, South, West), and academic status (academic vs. non-academic), and were obtained from the American Hospital Association Annual Survey of Hospitals. Having an American Medical Association (AMA) -approved residency program, membership in the Council of Teaching Hospitals or having a ratio of full-time equivalent interns and residents to beds of 0.25 or higher defined the hospital academic status. Hospital volume

was defined according to the number of partial nephrectomies performed annually.

Intraoperative complications, postoperative complications, and blood transfusions during hospitalization

The NIS records up to 15 diagnoses and procedures per in-hospital stay. The presence of any complication was defined using ICD-9 diagnoses 2 through 15. The specific ICD-9 codes used for complications relied on previously published methodology (17). Intraoperative complication was defined as accidental puncture or laceration during a procedure. Additionally, seven groups of postoperative complications were identified, namely: cardiac, respiratory, neurologic, urinary, vascular, infection, hemorrhage, operative wound, as well as septicemia (18). Blood transfusion recipients were identified using the ICD-9 procedure for transfusion of homologous blood (99.04). For purposes of statistical analysis of overall complication and transfusion rates, we stratified patients by 0 vs. 1 or greater, during hospitalization.

LOS and in-hospital mortality

LOS, provided by the NIS, is calculated by subtracting the admission date from the discharge date. In-hospital mortality information is coded from disposition of patient. Patients with missing or invalid LOS or in-hospital mortality status were not considered within the current study ($n = 10$). Prolonged LOS was defined as a hospitalization beyond the 75th percentile cut-off of five days.

Statistical analysis

Descriptive statistics focused on frequencies and proportions for categorical variables. Means, medians and ranges were reported for continuously coded variables. Chi-square and independent-sample t tests were used to compare the statistical significance of differences in respective proportions and means.

Due to inherent differences between patients undergoing robotic vs. laparoscopic nephrectomy in terms of baseline patient and hospital characteristics, we relied on propensity-score matched analysis (19,20). Cohorts were matched by patient characteristics: age, gender, race, CCI

and insurance status; as well as hospital characteristics: volume, location, region, and academic status. Covariate balance between the matched groups was subsequently examined. Chi-square tests were used to compare the statistical significance of differences between outcomes of RAPN and LPN.

Categorical distributions are reported as counts (%) and continuous variables as means and standard deviations (SD), and medians and interquartile ranges (IQR). Fisher's exact test, Chi-square test, and Mantel-Haenszel test were used to assess differences in distribution among categorical variables. The median test and t -test were used to assess the difference in distributions among continuous variables. All tests were two-sided with a statistical significance set at $p < 0.05$. Analyses were conducted using the R statistical package (the R foundation for Statistical Computing, version 2.14.1).

RESULTS

Description of sample population

Within the NIS, 1174 patients underwent minimally invasive PN between October 2008 and December 2009. Of those, 851 (72.5%) and 323 (27.5%) patients underwent RAPN and LPN, respectively. Characteristics of the patient populations are presented in Table-1a. Patient populations differed by race; a greater percentage of LPN patients were white (57.6% vs. 56.2%). No difference was noted between groups when stratification was performed according to gender, comorbidity, insurance status or income quartile. Hospital characteristics were significantly different between the two groups. Specifically, a higher proportion of RAPNs was performed at non-teaching institutions (26.2 vs. 17.6%, $p = 0.002$). RAPNs were more often performed in the Midwest (39.1%), whereas LPNs were most commonly performed in the Northeast (37.2%).

Propensity-score matching resulted in a cohort of 310 RAPN and 310 LPN patients (Table-1b). The standardized differences between the two groups in patient and hospital characteristics were less than 10% (19), indicating a high degree of similarity in the distribution of both

Table 1a - Demographic characteristics of patients undergoing minimally invasive partial nephrectomy – entire cohort [p values all Chi-Square].

	Overall	Laparoscopic	Robotic	p
Number of patients	1174	323	851	
Year of surgery				0.760
2008	386 (32.9)	104 (32.2)	282 (33.1)	
2009	788 (67.1)	219 (67.8)	569 (66.9)	
Gender				0.121
Male	653 (55.9)	167 (52.2)	486 (57.2)	
Female	516 (44.1)	153 (47.8)	363 (42.8)	
Age				0.545
Mean (SD)	56.9 (14.0)	56.5 (14.9)	57.1 (13.7)	
Median (IQR)	58.0 (48.67)	57.0 (47.67)	58.0 (48.67)	
Race				0.012
White	664 (56.6)	186 (57.6)	478 (56.2)	
Black	55 (4.7)	25 (7.7)	30 (3.5)	
Other	128 (10.9)	33 (10.2)	95 (11.2)	
Unknown	327 (27.9)	79 (24.5)	248 (29.1)	
CCI				0.762
0	762 (64.9)	217 (67.2)	545 (64.0)	
1	295 (25.1)	77 (23.8)	218 (25.6)	
2	70 (6.0)	18 (5.6)	52 (6.1)	
≥ 3	47 (4.0)	11 (3.4)	36 (4.2)	
Income				0.058
1st quartile	210 (18.4)	63 (20.0)	147 (17.8)	
2nd quartile	320 (28.0)	80 (25.4)	240 (29.0)	
3rd quartile	318 (27.8)	76 (24.1)	242 (29.2)	
4th quartile	295 (25.8)	96 (30.5)	199 (24.0)	
Hospital location				0.236
Rural	27 (2.3)	10 (3.2)	17 (2.0)	
Urban	1131 (97.7)	303 (96.8)	828 (98.0)	
Hospital region				< 0.001
Northeast	258 (22.0)	120 (37.2)	138 (16.2)	
Midwest	429 (36.5)	96 (29.7)	333 (39.1)	
South	278 (23.7)	66 (20.4)	212 (24.9)	
West	209 (17.8)	41 (12.7)	168 (19.7)	
Hospital teaching status				0.002
Non-teaching	276 (23.8)	55 (17.6)	221 (26.2)	
Teaching	882 (76.2)	258 (82.4)	624 (73.8)	
Hospital volume				0.085
Mean (SD)	72 (11)	81 (121)	69 (101)	
Median (IQR)	30 (13.59)	27 (12.48)	32 (14.59)	
Insurance status				0.498
Private	714 (60.8)	204 (63.2)	510 (59.9)	
Medicaid	56 (4.8)	16 (5.0)	40 (4.7)	
Medicare	350 (29.8)	86 (26.6)	264 (31.0)	
Other	54 (4.6)	17 (5.3)	37 (4.3)	

Table 1b - Demographic characteristics of patients undergoing minimally invasive partial nephrectomy – propensity-score matched cohort [p values all Chi-Square].

	Overall	Laparoscopic	Robotic	p
Number of patients	620	310	310	
Year of surgery				0.299
2008	194 (31.3)	103 (33.2)	91 (29.4)	
2009	426 (68.7)	207 (66.8)	219 (70.6)	
Gender				0.519
Male	334 (53.9)	163 (52.6)	171 (55.2)	
Female	286 (46.1)	147 (47.4)	139 (44.8)	
Age				0.633
Mean(SD)	56.3 (14.1)	56.5 (14.9)	56.0 (13.3)	
Median(IQR)	57 (48.66)	57 (47.67)	57 (48.65)	
Race				0.043
White	358 (57.7)	176 (56.8)	182 (58.7)	
Black	37 (6.0)	25 (8.1)	12 (3.9)	
Other	56 (9.0)	33 (10.6)	23 (7.4)	
Unknown	169 (27.3)	76 (24.5)	93 (30.0)	
CCI				0.983
0	419 (67.6)	208 (67.1)	211 (68.1)	
1	147 (23.7)	75 (24.2)	72 (23.2)	
2	35 (5.6)	17 (5.5)	18 (5.8)	
≥ 3	19 (3.1)	10 (3.2)	9 (2.9)	
Income				0.211
1st quartile	117 (19.4)	60 (19.9)	57 (18.9)	
2nd quartile	159 (26.3)	74 (24.5)	85 (28.1)	
3rd quartile	159 (26.3)	73 (24.2)	86 (28.5)	
4th quartile	169 (28.0)	95 (31.5)	74 (24.5)	
Hospital location				0.664
Rural	22 (3.5)	10 (3.2)	12 (3.9)	
Urban	598 (96.5)	300 (96.8)	298 (96.1)	
Hospital region				0.002
Northeast	223 (36.0)	120 (38.7)	103 (33.2)	
Midwest	214 (34.5)	86 (27.7)	128 (41.3)	
South	108 (17.4)	66 (21.3)	42 (13.5)	
West	75 (12.1)	38 (12.3)	37 (11.9)	
Hospital teaching status				0.587
Non-teaching	101 (16.3)	53 (17.1)	48 (15.5)	
Teaching	519 (83.7)	257 (82.9)	262 (84.5)	
Hospital volume				0.769
Mean (SD)	84 (120)	83 (124)	86 (116)	
Median (IQR)	31 (13.59)	27 (11.48)	36 (16.60)	
Insurance status				0.769
Private	391 (63.1)	197 (63.5)	194 (62.6)	
Medicaid	27 (4.4)	15 (4.8)	12 (3.9)	
Medicare	173 (27.9)	82 (26.5)	91 (29.4)	
Other	29 (4.7)	16 (5.2)	13 (4.2)	

populations. The matched populations represent the focus of all subsequent analyses.

Intraoperative and postoperative outcomes during hospitalization

Table-2 shows the rate of intraoperative and postoperative outcomes, stratified according to RAPN and LPN, pre (Table-2a) and post-propensity score matching (Table-2b). In the post propensity-score matched cohort, the rates of intraoperative and postoperative complications were found to be equivalent, as was the rate of blood transfusion and prolonged LOS ($p > 0.05$). Statistically significant differences were however seen for individual complications. Specifically, patients undergoing RAPN had fewer neurologic (0.0 vs. 2.3%, $p = 0.008$), urinary (0.0 vs. 1.9%, $p = 0.014$) and bleeding complications (0.6 vs. 2.9%).

DISCUSSION

RAPN was initially described in 2004 (21) and represents an acceptable alternative to open or laparoscopic PN for selected renal masses. It has been hypothesized that RAPN may help overcome the technical challenges of LPN and may offer an easier transition to minimally invasive PN (22). In this retrospective case-control study, we compare short-term perioperative outcomes of RAPN vs. LPN, relying on a contemporary (2008-2009) population-based cohort of individuals undergoing minimally invasive partial nephrectomy. To account for baseline patient and hospital differences between the two procedure cohorts, we relied on propensity-score matched analysis to eliminate the plausible biases associated with treatment selection.

Our findings are several-fold. First, our contemporary analysis shows that RAPN has overtaken LPN as the preferred surgical approach of minimally invasive PN in the United States. In this 20% representative sample of all inpatient admissions between October 2008 and December 2009, nearly three times as many RAPNs were performed compared to LPNs (851 vs. 323), thus accounting for 72.5% of minimally invasive PNs. This finding is both novel and unexpected; the unique identifier for robotic procedures was only

introduced by CMS in 2008; 2009 represents its first full year of utilization. Although objective evidence has shown that the utilization of robot-assisted radical prostatectomy has increased dramatically in recent years (23,24), no previous reported has alluded to a similar phenomenon in the context of PN.

Second, the characteristics of RAPN vs. LPN cases differed regarding several patient and hospital attributes. When compared to LPN patients, patients undergoing RAPN were less likely to be white and less likely to be treated at teaching, high-volume institutions. Moreover, LPN is predominantly performed in the Northeast, while RAPN is chiefly performed in the Midwest. LPN regionalization may be explained by the location of teaching vs. non-teaching hospital within the current database. Specifically, most teaching hospitals were located within the Northeast region, and since the majority of LPN was performed at teaching hospitals, the observed regional variability was likely a reflection of teaching vs. non-teaching hospital status. In consequence, it may be possible that lack of surgical LPN expertise in some areas could have prevented LPN being performed. In this regard, we corroborate previous findings that the use of PN is highly regionalized (25). Taken together, these findings corroborate the hypothesis that the diffusion of laparoscopy has not been uniform and is strongly influenced by patient provider characteristics, such as hospital volume and teaching status (26). Conversely, our findings also indicate that RAPN might have a different dissemination pattern, as this procedure has already been adopted in a higher proportion by surgeons at non-teaching and lower volume institutions than conventional laparoscopy. It is reasonable to presume that introduction of RAPN may in fact accelerate the utilization of PN as patients and surgeons seek to utilize minimally invasive approaches to PN. Unfortunately, temporal trend analyses are not possible since the robot-assisted modifier was only introduced in the fall of 2008.

Nonetheless, these findings emphasize the need for meticulous adjustment of patient differences when comparing perioperative outcomes. Lack of adjustment may result in important confounding of results. Specifically, based on previous

Table 2a - Outcomes of patients undergoing minimally invasive partial nephrectomy – entire cohort (unmatched).

	Overall	Laparoscopic	Robotic	p
Number of patients	1174	323	851	-
Intraoperative complication	43 (3.7)	8 (2.5)	35 (4.1)	0.183
Postoperative complication				
Overall	144 (12.3)	42 (13.0)	102 (12.0)	0.635
Cardiac	14 (1.2)	3 (0.9)	11 (1.3)	0.608
Respiratory	46 (3.9)	18 (5.6)	28 (3.3)	0.072
Neurologic	17 (1.4)	7 (2.2)	10 (1.2)	0.204
Urinary	14 (1.2)	6 (1.9)	8 (0.9)	0.196
Vascular	1 (0.1)	0 (0.0)	1 (0.1)	0.538
Infection	1 (0.1)	0 (0.0)	1 (0.1)	0.538
Hemorrhage	22 (1.9)	9 (2.8)	13 (1.5)	0.155
Operative wound	8 (0.7)	1 (0.3)	7 (0.8)	0.340
Septicemia	1 (0.1)	0 (0.0)	1 (0.1)	0.538
In-hospital mortality	0 (0.0)	0 (0.0)	0 (0.0)	-
Blood transfusion	74 (6.3)	22 (6.8)	52 (6.1)	0.659
Length of stay				
Length of stay > 5 days (median)	82 (7.0)	30 (9.3)	52 (6.1)	0.056

reports, better surgical outcomes are expected in patients treated at teaching (27) and high-volume (28) centers. To minimize bias and control for the baseline differences between RAPN and LPN patients, we relied on propensity-score matching. Propensity-score matching resulted in highly comparable patient and institutional characteristics within the RAPN and LPN groups. In propensity-score matched cohorts, no statistically significant difference between the two groups was recorded with regard to intraoperative and postoperative complications, prolonged LOS and in-hospital mortality. It is noteworthy hemorrhagic complications were significantly lower in the RAPN group ($p = 0.033$); though no statistically significant difference in transfusion rates was recorded ($p = 0.223$).

Based on the diffusion pattern of RAPN, it can be hypothesized that the utilization rates of nephron-sparing surgery will improve. Dulabon et al. (4) used a population-based dataset and identified 18330 localized RCC patients between years 1999

to 2006. PN utilization rates were 21% in 1999 vs. 45% in 2006. These findings strongly contrasts with those of Thompson et al. (29), who showed that PN rates at the Memorial Sloan-Kettering Cancer Center increased from 69 to 89% between 2000 and 2007. The disparities in reported rates highlight the difficult diffusion of LPN for small renal masses, as this technique is relatively challenging and often limited to centers of excellence.

From a practical perspective, our results indicate that on average, similar intraoperative and postoperative outcomes, including transfusion rates, prolonged LOS and in-hospital mortality, are expected whether the patient undergoes RAPN or LPN. However, these results should be interpreted with caution, since the NIS is unable to account for disease characteristics. Specifically, it is not known if complex cases, such as endophytic or hilar tumors, higher stage and grade lesions, are more often performed by the robotic or laparoscopic approach.

Table 2b - Outcomes of propensity-score matched patients undergoing minimally invasive partial nephrectomy. Patients were matched by age, gender, race, CCI and insurance status; as well as by hospital characteristics: volume, location, region, and academic status.

	Overall	Laparoscopic	Robotic	p
Number of patients	620	310	310	-
Intraoperative complication	24 (3.9)	8 (2.6)	15 (5.2)	0.096
Postoperative complication				
Overall	75 (12.1)	42 (13.5)	33 (10.6)	0.268
Cardiac	10 (1.6)	3 (1.0)	7 (2.3)	0.202
Respiratory	29 (4.7)	18 (5.8)	11 (3.5)	0.183
Neurologic	7 (1.1)	7 (2.3)	0 (0.0)	0.008
Urinary	6 (1.0)	6 (1.9)	0 (0.0)	0.014
Vascular	0 (0.0)	0 (0.0)	0 (0.0)	-
Infection	0 (0.0)	0 (0.0)	0 (0.0)	-
Hemorrhage	11 (1.8)	9 (2.9)	2 (0.6)	0.033
Operative wound	1 (0.2)	1 (0.3)	0 (0.0)	0.317
Septicemia	1 (0.2)	0 (0.0)	1 (0.3)	0.317
In-hospital mortality	0 (0.0)	0 (0.0)	0 (0.0)	-
Blood transfusion	35 (5.6)	21 (6.8)	14 (4.5)	0.223
Length of stay				
Length of stay > 5 days (median)	50 (8.1)	29 (9.4)	21 (6.8)	0.238

To the best of our knowledge, the current manuscript represents the first population-based report to compare complication rates after RAPN and LPN. Benway et al. recently published the largest multi-institutional comparison of the two approaches, describing the outcomes of three experienced minimally invasive surgeons (n = 247) (11). They found similar post-operative complication rates of RAPN vs. LPN (8.6 vs. 10.2%), which corroborate the findings of this study.

A significant limitation of the NIS is the lack of capture of tumor stage and pathological characteristics; there was likely some variability in distribution and this would affect our findings. There may have also been unobserved differences in operative characteristics that were not captured in discharge level data, ie. warm ischemia time. Although propensity-based matching was performed,

there may have also been differences amongst the groups concerning important patient variables (personal preferences, education, body mass index and medication utilization), as well as socio-economical determinants. Moreover, our mortality estimates are based on in-hospital rates. It is possible that the true mortality is underestimated as some patients may have died at other institutions where their mortality was not captured. Finally, we were not able to classify complications according to ideal, prospective methodologies, such as the Clavien classification, nor were we able to grade them using the Common toxicity Criteria for Adverse Events (30,31).

CONCLUSIONS

RAPN has supplanted LPN as the predominant minimally invasive surgical approach for renal

masses. Perioperative outcomes after RAPN and LPN are comparable and remain comparable following adjustment for several common confounders. Interpretation of these findings needs to take into account the lack of adjustment for case complexity and surgical expertise.

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CONFLICT OF INTEREST

None declared.

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Correspondence address:

Dr. Jesse D. Sammon
 Vattikuti Urology Institute
 Henry Ford Health System
 2799 W. Grand Boulevard
 Detroit, Michigan, USA, 48202
 Fax: + 1 313 916-4352
 E-mail address: jsammon79@gmail.com



Bilateral Single-Session Retrograde Intrarenal Surgery for the Treatment of Bilateral Renal Stones

Gokhan Atis, Hakan Koyuncu, Cenk Gurbuz, Faruk Yencilek, Ozgur Arıkan, Turhan Caskurlu

Medeniyet University, Goztepe Training and Research Hospital, Urology (GA,CG,OA,TC) and Yeditepe University Medical School, Urology (HK,FY), Istanbul, Turkey

ABSTRACT

Purpose: The aim of the study was to evaluate the efficacy and safety of bilateral single-session retrograde intrarenal surgery in the treatment of bilateral renal stones.

Materials and Methods: From December 2008 to February 2012, 42 patients who had undergone bilateral single-session retrograde intrarenal surgery (RIRS) and laser lithotripsy were included in the study. The procedures were performed in the lithotomy position on an endoscopy table under general anesthesia, beginning on the side in which the stone size was smaller. Plain abdominal radiography, intravenous urograms (IVU), renal ultrasonography (USG) and / or non-contrast tomography (CT) scans were conducted for all patients. The success rate was defined as patients who were stone-free or only had residual fragment less than 4 mm.

Results: A total of 42 patients (28 male, 14 female) with a mean age 39.2 ± 14.2 were included in the present study. The mean stone size was 24.09 ± 6.37 mm with a mean operative time of 51.08 ± 15.22 minutes. The stone-free rates (SFR) were 92.8% and 97.6% after the first and second procedures, respectively. The average hospital stay was 1.37 ± 0.72 days. In two patients (4.7%), minor complications (Clavien I or II) were observed, whereas no major complications (Clavien III-V) or blood transfusions were noted in the studied group.

Conclusions: Bilateral single-session RIRS and laser lithotripsy can be performed safely and effectively with a high success rate and low complication rate in patients with bilateral renal stones.

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INTRODUCTION

Percutaneous nephrolithotomy (PCNL), shock wave lithotripsy (SWL), and retrograde intrarenal surgery (RIRS) are the most widely used treatment modalities for the management of renal stones. The 2012 European Association of Urology (EAU) guidelines on urolithiasis recommends SWL as the first treatment of choice for renal stones < 20mm and PCNL for renal stones > 20mm located within the renal pelvis and upper or middle calices (1). Either PCNL or RIRS are re-

commended for stones > 15mm located within the lower pole due to the limited efficacy of SWL for stones of this size (1).

In the literature, different surgical procedures have been determined to treat bilateral renal calculi such as staged PCNL, synchronous bilateral PCNL, synchronous PCNL with contralateral ureterorenoscopy (URS), staged bilateral SWL and simultaneous SWL (2-5). Additionally, Chung et al. demonstrated the feasibility of simultaneous

bilateral RIRS (sb-RIRS) in four patients with bilateral renal stones and significant comorbidities (6). However, the safety and efficacy of bilateral single-session RIRS has been poorly investigated. In this study, we assessed our treatment outcomes in patients undergoing bilateral single-session RIRS for bilateral renal stones.

MATERIAL AND METHODS

We performed a retrospective analysis of 42 evaluated patients with bilateral renal calculi, who underwent bilateral single-session RIRS at two referral hospitals in Turkey from December 2008 to February 2012. The selection criteria for this intervention were patients preference, failure of other treatments and multicalyceal stones. All patients were evaluated with serum biochemistry, urinalysis, urine culture, plain radiography of kidney-ureter-bladder (KUB), IVU, renal USG and/or CT. The stone size was determined by measuring its maximum diameter using KUB. Patients who had positive urine cultures were treated with the appropriate antibiotics before surgery. In all patients, the procedures were performed in the lithotomy position under general anesthesia, beginning on the side in which the stone size was smaller. To dilate the ureter and insert a hydrophilic guidewire to the renal pelvis, semirigid ureteroscopy was initially performed. A ureteral access sheath (UAS) was placed through the hydrophilic guidewire in all cases. The dilation of the ureteral orifice was performed using balloon dilators when the ureteroscope did not pass with ease. A flexible ureteroscope (f-URS) was placed through the UAS and the stones were fragmented using the Ho:YAG laser with a 273 μ laser fiber at 0.6–1.0 J energy and 5–10 Hz frequency levels. The relocation of the lower pole stones to the renal pelvis or upper pole was performed by basketing, when achievable, to facilitate better visualization during the lithotripsy. At the end of the procedure, a pig-tail stent was placed, according to the surgeon's preference. The same procedures were then performed for the contralateral side of the renal unit that contained calculi.

At the follow-up evaluation, serum biochemistry, a post-operative plain film and renal

ultrasonography were conducted at postoperative day one and repeated serum biochemistry, ultrasonography and IVU were performed at one month after the surgery to determine the presence of obstructions, clinically significant renal fragments and ureteral strictures. The success rate was defined as patients who were stone-free or only had residual fragment < 4mm. CT was conducted only in patients with residual stones, which were present in 3 patients (7.1%).

Statistical analysis was performed using SPSS, version 17.0. A paired sample t-test was used to compare the pre-operative and post-operative serum creatinine levels. A p value < 0.05 was considered statistically significant.

RESULTS

The pre-operative characteristics of the patients are summarized in Table-1. A total of 42 patients (28 male, 14 female) with a mean age 39.2 ± 14.2 were included in the present study. The localizations of the 134 renal stones were as follows: 51 (38.05%) in the lower calyx of the kidney, 41 (30.59%) in the middle calyx, 22 (16.41%) in the renal pelvis, and 20 (14.92%) in the upper calyx. In 12 (28.5%) of the 42 patients, the following previous interventions were noted: PCNL in 4 (9.5%) patients, pyelolithotomy in 3 (7.1%) patients, SWL in 3 (7.1%) patients and more than one of these procedures in 2 (4.7%) patients.

The mean stone size was 24.09 ± 6.37 mm. The mean operative time was 51.08 ± 15.22 minutes, and the mean fluoroscopy screening time was 39.26 ± 9.87 seconds. The SFR after the first procedure was 92.8%. Three patients with residual stones underwent additional RIRS. Two of them became stone-free, resulting in overall SFR of 97.6%. Stone analyses were conducted in 30 patients (71.4%) and the most frequent stone composition was noted as calcium oxalate.

The dilation of the ureteral orifice was performed in 26 of 84 renal units (30.95%) and UASs were placed in all renal units. Bilateral stents were placed in 30 patients (71.4%) and unilateral stents were placed in 12 patients (28.6%). Stent(s) were removed approximately two weeks after the procedure. The average hospital stay was 1.37 ± 0.72

Table 1 - Patient and Stone characteristics.

Variable	Value
Age (y)	39.2 ± 14.2
Gender (n)	
Female	14 (33.3%)
Male	28 (66.6%)
Previous renal intervention (n)	
SWL	3 (7.1%)
PCNL	4 (9.5%)
Open surgery	3 (7.1%)
More than 1	2 (4.7%)
Pre-operative serum creatinine (mg/dL)	1.21 ± 0.37
Stone size (mm)	24.09 ± 6.37
Stone location (n)	
Renal pelvis	22 (16.41%)
Upper calyx	20 (14.92%)
Middle calyx	41 (30.59%)
Lower calyx	51 (38.05%)

days. In two patients (4.7%), minor complications (Clavien I or II) were observed, whereas no major complications (Clavien III-V) or blood transfusions were noted in the studied group (Table-2).

The mean serum creatinine levels before and after one month following the procedures (after two weeks following pigtail stent removal) were 1.21 ± 0.37mg/dL and 1.26 ± 0.41 mg/dL, respectively. There was no statistically significant difference between pre-operative and post-operative serum creatinine levels ($p = 0.89$).

DISCUSSION

Several published articles have outlined the treatment modalities used for patients with

Table 2 - Operative and postoperative outcomes.

Variable	Value
Mean operation time (min)	51.08 ± 15.22
Mean fluoroscopy time (sec)	39.26 ± 9.87
Stone clearance rate	
After first session	39 (92.8%)
After second session	41 (97.2%)
Post-operative serum creatinine (mg/dL)	1.26 ± 0.41
Minor complication rate	2 (4.7%)
Mean hospitalization time (day)	1.37 ± 0.72

bilateral renal stones and one of the primary surgical modalities used to treat these stones is PCNL. This procedure can be administered in either a staged, synchronous or simultaneous manner (2,7,8). It has been reported that bilateral simultaneous PCNL has some unique advantages such as shorter hospital stay, less radiation exposure, reduced anesthesia and medication requirements, as well as cost-effectiveness (2,7,9). However, the complication rates are similar to bilateral staged or unilateral PCNL (2,10). Handa et al. examined the effects of simultaneous bilateral PCNL on bilateral renal function in pigs, and they reported that bilateral functional responses of kidneys were comparable to those observed after unilateral PCNL (11). Despite the reported efficacy and safety of bilateral PCNL procedures, some major complications, such as drops in hemoglobin that require blood transfusions and hydropneumotoraces, may still occur (7,12).

SWL is another treatment modality used to manage bilateral renal calculi, which can also be applied in a simultaneous or staged manner (4,5). In the study of Pienkny et al., the investigators compared the effects on renal function between simultaneous versus staged SWL, and they found no difference between the two groups (4). Perry et al. evaluated 120 patients who had undergone bilateral synchronous SWL (5). They reported a bilateral

SFR of 60% after a single treatment without any major complications, such as renal failure or bilateral renal obstruction, however, 16% of the patients required additional procedures for residual stones.

PCNL and contralateral URS can also be used to treat bilateral urolithiasis. Mason et al. reported a efficacy rate of 92.3% and 100% after one and two sessions, respectively, in patients who underwent synchronous PCNL and contralateral URS (3). Although these complication rates were similar to those observed with unilateral PCNL in this cohort of patients, 7.7% of them experienced major complications after this treatment modality.

The 2012 EAU urolithiasis guidelines do not recommend fURS as first-line treatment for stones > 15mm in the renal pelvis and upper or middle calices, because of the decreased SFR after fURS as well as the requirement for multiple procedures to treat these stones (1). At the other site, the guidelines recommend PCNL or fURS for stones >15mm that are located in the lower pole, given that SWL has limited efficacy in the treatment of these stones (1). Despite these recommendations, there have been various published articles reporting high success rates with fURS, even for high stone burdens in any kidney location (13-15). The retrograde intrarenal stone surgery is gaining more popularity day by day, given that it offers similar SFR and lower complication rates compared with PCNL, as well as and higher success rates compared with SWL (13-15).

Only a few studies have examined the safety and efficacy of RIRS in treating bilateral renal stones. In 2005, Chon et al. first reported the efficacy of SB-RIRS (16). In another study by the same investigators, they assessed their treatment outcomes in four patients with significant comorbidities who had undergone SB-RIRS, and the authors observed no major complications (6). In these studies, the procedures were performed by two surgeons who operated simultaneously. The disadvantage of this technique is the requirement of two sets of equipment such as the fURS and laser lithotripter as well as two surgeons. In the present study, we performed single-session RIRS in 42 patients with bilateral renal calculi. Because the smaller stone size was associated with lower operative times, the procedures were initiated at

the side in which the stone size was smaller. After completing one side, the RIRS was performed for the other side. We did not have to stop the procedure once the initial side was completed. On the other hand, it may be possible to end the procedure after completing one side, so the procedures can be also initiated at the symptomatic side. Our technique is advantageous because it requires only one set of equipment and a single surgeon. Furthermore, the present technique allows for the treatment of bilateral renal calculi in one anesthetic session.

Huang et al. examined bilateral RIRS in 25 patients with bilateral renal stones and reported an overall SFR of 70%, 92% and 92% after first, second and third procedures, respectively (17). Similarly, the SFRs in our study group were 92.8% and 97.2% after the first and second sessions of RIRS, respectively. Although the mean stone size was smaller than that reported in the previously published articles that have evaluated the outcomes of bilateral PCNL, we achieved a similar SFR to those studies following a bilateral PCNL. Additionally, our SFR was higher when compared with the published articles on bilateral SWL, and only 7.2% of patients in our study group required additional procedures, which is lower than the typical rates following SWL.

Bilateral single ureteroscopic procedures for ureteral calculi have been performed by several investigators. Some of these investigators have reported that bilateral calculi can be managed by bilateral URS, with low complication and high success rates (18,19). In contrast, Hollenbeck et al. reported a complication rate of 26% in same session of ureteroscopic procedures (20). In their study, the post-operative complications included post-operative pain, urosepsis, urinoma and pulmonary embolus resulting in death. In the present study, only 2 patients (4.7%) experienced minor complications. One patient had fever post-operatively and was treated with antibiotics. The other patient exhibited bleeding without the need for a transfusion. None of the patients experienced any major complications. In any of the semirigid and flexible ureteroscopic procedures, an increase in the renal pelvic pressure may occur through irrigation. The increased renal pelvic pressure can lead to infec-

tious complications due to intrarenal, pyelovenous and pyelolymphatic backflow (21). To decrease the pelvic pressure, the use of UAS has been suggested, as well as irrigation with isoproterenol and limiting the operative time (22,23). Although the UAS may fail in some circumstances such as ureteral stenosis or kinking, we were able to place the UAS in all renal units in the present study group. Our relatively low complication rate may be due to our use of UAS and our low mean operating times.

The reported mean operative times for bilateral PCNL may vary from 46 to 240 minutes (3). Additionally, Mason et al. reported an average of 149 minutes operation time for bilateral calculi that were treated with synchronous PCNL and contralateral URS (3). In the present study, the mean operative time was 51.09 ± 15.22 minutes which is lower than that reported for other techniques. While there is no need to reposition the patient from the supine to prone position in RIRS, it provides an advantage for minimizing operative time as compared with PCNL. At the contralateral site, the short operative time may also be due to our lower mean stone size as compared with those in other PCNL cases.

According to the recommendations of EAU guidelines on urolithiasis, the placement of a ureteral stent after an uncomplicated URS is optional and it should be inserted in cases with a risk of postoperative complications, such as perforation, bleeding, urinary tract infection, residual fragments and pregnancy (1). After the bilateral ureteroscopic procedures, bilateral ureteral edema, leading to acute renal failure, may develop (18). Although we did not observe any intraoperative complications, at least an unilateral pigtail stent was placed to prevent acute renal failure, and none of the patients experienced such complication after the procedure. Because the operative time was too short, we placed only an unilateral stent in 12 patients. In the remaining 30 patients, bilateral pigtail stents were placed due to the above concerns.

The present study has certain limitations. First, this study was a retrospective analysis of the patients who underwent bilateral same session RIRS for bilateral renal stones, which were performed by more than one surgeon. Second, we did not evaluate the cost-analysis of the technique

and post-operative pain scores and did not compare them to the other techniques that can be used to manage bilateral renal stones. Third, a post-operative plain film and renal ultrasonography were used in most of the cases to assess residual fragments, which may overestimate our SFRs post-operatively. Despite these limitations, the present study is the largest series in the literature that evaluates the outcomes of bilateral RIRS in the treatment of bilateral renal calculi.

CONCLUSIONS

Bilateral RIRS is a safe and effective treatment choice for the management of bilateral renal calculi. The procedure has a high success rate with minimal morbidity, and precludes the need for multiple procedures. Multiple studies comparing the outcomes of PCNL, SWL and RIRS are needed in this population of patients.

ABBREVIATIONS

RIRS = Retrograde intrarenal surgery
 IVU = Intravenous urograms
 USG = Ultrasonography
 CT = Computed tomography
 PCNL = Percutaneous nephrolithotomy
 SWL = Shock wave lithotripsy
 EAU = European Association of Urology
 URS = Ureterorenoscopy
 sb-RIRS = Simultaneous bilateral RIRS
 KUB = Kidney-ureter-bladder
 UAS = Ureteral access sheath
 f-URS = Flexible ureteroscopy
 SFR = Stone-free rate

CONFLICT OF INTEREST

None declared.

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Correspondence address:

Dr. Gokhan Atis
Tahrili Sitesi Şafakyeli Apt. D 36
Atasehir, Istanbul, Turkey
Fax: + 90 21 6521-8608
E-mail: gokhanatis@hotmail.com



Prostatic disorders in acromegalic patients experience of a Brazilian center

Lívia L. Corrêa, Giovanna A. Balarini Lima, Suzana A. Cavallieri, Luiz Carlos D. de Miranda, Mônica R. Gadelha

Service of Endocrinology, University Hospital Clementino Fraga Filho (HUCFF), Federal University of Rio de Janeiro (UFRJ) (LLC, GABL, MRG); Department of Urology, University Hospital Clementino Fraga Filho (HUCFF), Federal University of Rio de Janeiro (UFRJ) (LCDM) and Labs D'Or Laboratory and Imaging (SAC), Rio de Janeiro, RJ, Brazil

ABSTRACT

Introduction: Published data suggest that patients with acromegaly have an increased prevalence of prostate disorders.

Objective: To evaluate prostatic disorders in acromegalic patients comparing these results after one year of treatment of acromegaly and with a group of healthy men.

Materials and Methods: This study was composed of two parts: sectional study comparing patients with healthy controls (baseline) and prospective, longitudinal study (at baseline and after one year of treatment). Forty acromegalic patients were enrolled and evaluated at baseline and after one year with the application of international prostatic symptoms score (IPSS), digital rectal examination, measurements of growth hormone (GH), insulin-like growth factor-I (IGF-I), insulin-like growth factor-binding protein-3 (IGFBP-3), sex hormone-binding globulin (SHBG), prolactin, luteinizing hormone (LH), follicle-stimulating hormone (FSH), total testosterone, total and free prostate-specific antigen (PSA) levels and prostate ultrasonography (US). Thirty healthy men were selected as control group.

Results: We stratified patients and controls according to age, considering 40 years-old as cut off. Healthy controls under 40 had IPSS values lower than acromegalic patients. When considering only older patients and controls prostate hyperplasia and structural abnormalities were more frequent in acromegalics. After one year of treatment there was significant decrease in GH, IGF-I and prostate volume in acromegalics over 40 years-old.

Conclusions: Acromegalics under 40 have more urinary symptoms according to IPSS and above 40 years-old higher frequency of structural changes and increased prostate volume than healthy men. Significant reduction of GH and IGF-I levels during treatment of acromegaly leads to decrease in the prostate volume.

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INTRODUCTION

Acromegaly is a rare disease caused by GH hyper secretion (1,2). It is well known that GH promotes a stimulatory effect on IGF-I and IGFBP-3. IGF-I stimulates cell proliferation (3),

however, IGFBP-3 stimulates apoptosis (4). Therefore, it is not well established whether acromegaly is associated or not with increased relative risk for cancer development.

Several epidemiologic studies have suggested that high-normal serum IGF-I levels may

be concordant with a higher risk of prostate cancer in the general population, and that high-normal serum IGFBP-3 levels are concordant with a lower risk (5-8). Published data suggest that the relationship between prostatic carcinoma and acromegaly is infrequent (9).

Patients with acromegaly have an increased prevalence of prostatic disorders compared to age-matched healthy subjects. Increased size of the whole prostate, together with an elevated incidence of other structural changes, such as nodules, cysts, and calcifications were shown in a large proportion of patients (10,11). The presence of an enlarged prostate in acromegalic patients under 40 years suggests a possible role of GH-IGF-I axis on this gland growth (10,11). Only two studies evaluated the effects of acromegaly treatment on prostate volume (PV), and demonstrated that well controlled patients presented significant reduction in PV after treatment of acromegaly (10,12).

The IPSS is a symptom index for benign prostatic hyperplasia (BPH), developed and validated by a multidisciplinary measurement committee of the American Urological Association (AUA) (13). The IPSS is widely used to evaluate the severity of urinary symptoms (14,15). Until today, there is no report of the use of IPSS in the evaluation of acromegalic patients.

In a recent guideline for acromegaly management (16) there was no information about the BPH evaluation. The aim of this study was to evaluate the prostate of acromegalic patients through digital rectal examination, transrectal US and IPSS, comparing these results with a group of healthy men, above and below 40 years, and after one year of acromegaly treatment.

MATERIALS AND METHODS

Patients

Forty acromegalic patients, aged 45.2 ± 11.3 years (24-69 years), were recruited from the outpatient endocrinology clinic of the University Hospital Clementino Fraga Filho - HUCFF, of the Federal University of Rio de Janeiro - UFRJ, over a 24-month period. The diagnosis of acromegaly was based on the following criteria: 1) a lack of suppression of GH to below 1 ng/mL after oral administration of 75g

glucose or 2) high serum IGF-I levels. Reasons for ineligibility included patients with previous treatment of prostate cancer or BPH. All subjects entered the study after obtaining written informed consent according to a protocol approved by the Ethics Committee of HUCFF.

At baseline, 36 patients had active disease (10 were de novo patients) and four had acromegaly cure or control for less than one year before the inclusion in this study. Twenty-six patients were previously submitted to surgery, nine to radiotherapy and 25 to medical treatment with octreotide LAR and/or cabergoline. Hypogonadism, based on low testosterone levels, was present in 27 (67.5%) patients. Seventeen of these patients were not receiving testosterone as replacement therapy because of severe sleep apnea.

As control group, thirty healthy and education-matched men were included. Fourteen of them were less than 40 years-old and were paired to the 14 acromegalic patients younger than 40 years. The other 16 were proportionally age-matched with the 26 acromegalics older than 40 years-old.

Study design

This study was composed of two parts: a) sectional study comparing patients (at baseline) with healthy controls; b) prospective, longitudinal study (at baseline and after one year of treatment). The study protocol included application of IPSS, digital rectal examination, measurements of GH, IGF-I, IGFBP-3, SHBG, prolactin, LH, FSH, total testosterone, total and free PSA levels and transrectal US, both at baseline and after one year of treatment for patients and on baseline for controls. The free testosterone and the bioavailable testosterone were calculated according to the Vermeulen's formula (17).

Urologic evaluation

The IPSS is a survey composed of seven questions related to incomplete bladder emptying, urinary frequency, intermittency, urgency, weak stream, straining and nocturia. To each answer is attributed a value in scale (0-5 points), to a maximum of 35 points. A total score of 0-7 indicates mildly symptomatic; 8-19 moderately symptomatic; 20-35 severely symptomatic patients (13). This survey was validated to the Portuguese language in 1999 (18).

The digital rectal examination was performed by the same urologist (LCDM).

Hormone assays

Serum GH, IGF-I, IGFBP-3, SHBG, prolactin, LH, FSH and total testosterone levels were determined by chemiluminescence immunometric assays. Total and free PSA serum levels were measured by electrochemiluminescence assay.

The low detection limit of GH by the Immulite 2000 kit (DPC- Diagnostic Products Corporation, Los Angeles, CA) is 0.01 ng/mL and its linear working range is 0.01-40 ng/mL. Standards are calibrated against the International Standard WHO 98/574. The intra-assay CVs at the respective concentrations of 1.7, 7.8 and 31.0 ng/mL are 5.3%, 6.0% and 6.5%, while the inter-assay CVs at 3.0, 9.3 and 18.0 ng/mL are 5.7%, 6.2% and 6.1%, respectively. The low detection limit of IGF-I measured by Immulite 2000 kit DPC is 20 ng/mL and the intra and inter-assay CVs are 3.6 and 6.6%, respectively. The standards are calibrated against the first International Reference Reagent WHO 87/518 and IGF-I was expressed in mass units and age-related standard deviation scores (SD-scores).

All serum samples were collected in the early morning after an eight-hour fasting period.

Transrectal US

Transrectal US was performed with a HDI 11XE, Phillips, 2008, using a 5.0-7.5 MHz transducer by the same radiologist (SAC). The prostate examination included the anterior-posterior (AP), transversal (T) and longitudinal (L) diameters, the morphology of boundaries, texture, the occurrence of calcifications and/or nodules, and the evaluation of seminal vesicles and bladder. Prostate volume was calculated by the elliptical shape volume formula ($\pi/6 \times \text{APD} \times \text{TD} \times \text{LD}$). Prostate hyperplasia was defined as a PV exceeding 30 mL (19-21).

Statistical analysis

Analyses were performed by SAS System (version 6.11; SAS Institute North Caroline). The results were expressed as median (min-max). Comparisons between categorical variables were done by χ^2 test and between numerical variables were

carried out using the Mann Whitney test. Comparisons between related samples were done by Wilcoxon test. McNemar's test was used to compare paired proportions. Correlations were sought by calculating the Spearman's rank correlation coefficient. P values < 0.05 were considered statistically significant.

RESULTS

Comparison between acromegalic patients and control group under 40 years at baseline

Because it is well established that prostate enlargement starts approximately at the age of 40 (22,23), we stratified patients and controls according to age, considering 40 years-old as cut off. When considering only younger patients and controls (Table-1), the acromegalics presented high levels of GH (4.96 vs 0.05, $p = 0.0001$), IGF-I (914.50 vs 183.00, $p = 0.0002$), IGFBP-3 (6.96 vs 4.36, $p = 0.0008$) and IPSS (2.00 vs 0.00, $p = 0.003$) and presented low levels of LH (2.07 vs 4.85, $p = 0.0009$), total testosterone (135.00 vs 567.50, $p = 0.0001$), free testosterone (4.17 vs 13.40, $p = 0.0001$), bioavailable testosterone (97.70 vs 314.00, $p = 0.0001$) and SHBG (11.00 vs 25.20, $p = 0.0005$). The mean PV was not different between these two groups (18.50 vs 15.00, $p = 0.10$).

Prostate hyperplasia was not found in patients or controls under 40. Structural abnormalities found at US were: calcifications in one patient and one control (7% vs 7%, $p = 1.0$) and hyperechogenic foci suggesting corpora amylacea in 4 patients and 3 controls (28.6% vs 21.4%, $p = 1.0$).

Comparison between acromegalic patients and control group above 40 years at baseline

When considering only patients and controls ≥ 40 years-old (Table-2), the acromegalics presented higher levels of GH (4.92 vs 0.05, $p = 0.0001$), IGF-I (466.00 vs 142.00, $p = 0.0001$), IGFBP-3 (5.65 vs 3.93, $p = 0.0005$) and PV (28.50 vs 20.50, $p = 0.048$) and presented lower levels of LH (1.81 vs 3.30, $p = 0.0002$), total testosterone (256.50 vs 542.00, $p = 0.004$), free testosterone (5.96 vs 10.95, $p = 0.002$), bioavailable testosterone (139.50 vs 256.50, $p = 0.002$) and SHBG (20.40 vs 33.00, $p = 0.034$).

Prostate hyperplasia was found in 12 patients and 2 controls. The frequency of BPH was

Table 1 - Characteristics of acromegalic patients at baseline and control group (< 40 years-old).

	Acromegalic patients (n = 14)		Control group (n = 14)		p value
	Median	Min-Max	Median	Min-Max	
Age (years)	34.00	24.00-39.00	33.50	24.00-38.00	0.63
GH (ng/mL)	4.96	0.23-40.00	0.05	0.04-2.10	0.0001
IGF-I (ng/mL)	914.50	114.00-1158.00	183.00	128.00-282.00	0.0002
IGFBP-3 (mcg/mL)	6.96	1.96-10.50	4.36	2.50-5.25	0.0008
PRL (ng/mL)	9.00	0.50-61.00	9.47	4.07-29.10	0.85
FSH (mUI/mL)	4.86	0.10-14.70	4.23	1.20-7.63	0.93
LH (mUI/mL)	2.07	0.10-6.97	4.85	1.60-15.00	0.0009
Total testosterone (ng/dL)	135.00	20.00-289.00	567.50	365.00-1094.00	0.0001
Free testosterone (ng/dL)	4.17	0.73-7.44	13.40	8.70-30.50	0.0001
Bioavailable testosterone (ng/dL)	97.70	17.10-174.00	314.00	204.00-715.00	0.0001
SHBG (nmol/L)	11.00	3.60-35.90	25.20	8.20-47.00	0.0005
Total PSA (ng/mL)	0.65	0.04-1.65	0.65	0.34-2.17	0.71
Free PSA (ng/mL)	0.12	0.05-0.38	0.18	0.07-0.33	0.45
IPSS	2.00	0.00-12.00	0.00	0.00-2.00	0.003
Prostate volume (mL)	18.50	12.00-28.00	15.00	12.00-19.00	0.10

Normal Values: GH (0.06-5 ng/mL), IGF-I (116-358, 117-329, 115-307, 109-284, 101-267, 94-252, 87-238, 81-225, 75-212, 69-200 ng/mL for patients aged 21-25, 26-30, 31-35, 36-40, 41-45, 46-50, 51-55, 56-60, 61-65 and 66-70, respectively), IGFBP-3 (3.4-7.8, 3.5-7.6, 3.5-7.0, 3.4-6.7, 3.4-6.6, 3.3-6.7, 3.4-6.8, 3.4-6.9, 3.2-6.6, 3.0-6.2 mcg/mL for patients aged 21-25, 26-30, 31-35, 36-40, 41-45, 46-50, 51-55, 56-60, 61-65 and 66-70, respectively), PRL (2.1-17.7 ng/mL), FSH (1.4-18.1 mUI/mL), LH (1.5-9.3 mUI/mL), total testosterone (241-827 ng/dL), free testosterone (4.7-23 ng/dL), bioavailable testosterone (140-400 ng/dL), SHBG (13-71 nmol/L), total PSA (less than 4 ng/mL), free PSA (less than 0.92 ng/mL), IPSS (0-35), prostate volume (less than 30 mL).

significantly higher in the acromegalic population when compared with the control group (46.15% vs 12.50%, $p = 0.015$). Structural abnormalities were also more frequent in acromegalics: calcifications in 11 patients and one control (42.31% vs 6.25%, $p = 0.018$) and hyperechogenic foci suggesting corpora amylacea in 18 patients and 5 controls (69.23% vs 31.25%, $p = 0.036$). A utricular cyst of 0.4 cm was found in one patient.

Comparison of the acromegalic patients under 40 years at baseline and after one year of treatment

The main characteristics of the acromegalic population under 40 years before and after treatment are presented in Table-3.

After one year of treatment, there was a significant reduction in GH (4.96 vs 3.28, $p = 0.011$). Biochemical control of acromegaly, based on random GH < 1 ng/mL and normal IGF-I (24), was achieved

Table 2 - Characteristics of acromegalic patients at baseline and control group (≥ 40 years-old).

	Acromegalic patients (n = 26)		Control group (n = 16)		p value
	Median	Min-max	Median	Min-Max	
Age (years)	50.00	42.00-69.00	52.00	40.00-69.00	0.74
GH (ng/mL)	4.92	0.28-68.90	0.05	0.05-1.70	0.0001
IGF-I (ng/mL)	466.00	104.00-1600.00	142.00	83.20-206.00	0.0001
IGFBP-3 (mcg/mL)	5.65	3.24-11.90	3.93	2.32-6.13	0.0005
PRL (ng/mL)	5.80	0.50-86.39	7.43	3.70-14.40	0.043
FSH (mUI/mL)	4.10	0.10-15.40	4.48	2.15-12.30	0.58
LH (mUI/mL)	1.81	0.10-4.68	3.30	2.16-6.10	0.0002
Total testosterone (ng/dL)	256.50	20.00-1688.00	542.00	206.00-790.00	0.004
Free testosterone (ng/dL)	5.96	0.23-66.90	10.95	5.10-15.20	0.002
Bioavailable testosterone (ng/dL)	139.50	5.39-1570.00	256.50	120.00-356.00	0.002
SHBG (nmol/L)	20.40	6.00-79.50	33.00	12.60-54.00	0.034
Total PSA (ng/mL)	0.63	0.04-2.50	0.93	0.31-2.80	0.18
Free PSA (ng/mL)	0.15	0.04-0.57	0.27	0.06-0.55	0.066
IPSS	4.50	0.00-21.00	4.00	0.00-16.00	0.19
Prostate volume (mL)	28.50	10.00-84.00	20.50	11.00-40.00	0.048

Normal Values: GH (0.06-5 ng/mL), IGF-I (116-358, 117-329, 115-307, 109-284, 101-267, 94-252, 87-238, 81-225, 75-212, 69-200 ng/mL for patients aged 21-25, 26-30, 31-35, 36-40, 41-45, 46-50, 51-55, 56-60, 61-65 and 66-70, respectively), IGFBP-3 (3.4-7.8, 3.5-7.6, 3.5-7.0, 3.4-6.7, 3.4-6.6, 3.3-6.7, 3.4-6.8, 3.4-6.9, 3.2-6.6, 3.0-6.2 mcg/mL for patients aged 21-25, 26-30, 31-35, 36-40, 41-45, 46-50, 51-55, 56-60, 61-65 and 66-70, respectively), PRL (2.1-17.7 ng/mL), FSH (1.4-18.1 mUI/mL), LH (1.5-9.3 mUI/mL), total testosterone (241-827 ng/dL), free testosterone (4.7-23 ng/dL), bioavailable testosterone (140-400 ng/dL), SHBG (13-71 nmol/L), total PSA (less than 4 ng/mL), free PSA (less than 0.92 ng/mL), IPSS (0-35), prostate volume (less than 30 mL).

in five patients, two of them under 40 years, and the four patients initially considered cured/controlled at baseline, persisted with cure/control criteria. The inclusion of these four patients did not influence the outcomes of the research. No significant reduction in PV was observed after treatment (18.50 vs 18.50, $p = 0.75$) and there was no significant difference in the frequency of prostate hyperplasia (0% vs 7.14%, $p = 0.50$) neither in the frequency of structural ab-

normalities found at transrectal US. The reduction in IPSS achieved borderline statistical significance (2.00 vs 2.00, $p = 0.056$).

Comparison of the acromegalic patients above 40 years at baseline and after one year of treatment

The main characteristics of the study population above 40 years before and after treatment are presented in Table-4.

Table 3 - Characteristics of acromegalic patients at baseline and after one year of treatment (< 40 years-old).

	Baseline (n = 14)		1 year (n = 14)		p value
	Median	Min-Max	Median	Min-Max	
GH (ng/mL)	4.96	0.23-40.00	3.28	0.21-20.30	0.011
IGF-I (ng/mL)	914.50	114.00-1158.00	522.50	131.00-1345.00	0.133
IGFBP-3 (mcg/mL)	6.96	1.96-10.50	6.72	3.33-9.26	0.638
PRL (ng/mL)	9.00	0.50-61.00	9.70	0.30-85.59	0.363
FSH (mUI/mL)	4.86	0.10-14.70	2.95	0.30-10.50	0.013
LH (mUI/mL)	2.07	0.10-6.97	1.23	0.07-6.95	0.03
Total testosterone (ng/dL)	135.00	20.00-289.00	170.00	31.00-424.00	0.079
Free testosterone (ng/dL)	4.16	0.73-7.44	5.14	1.10-12.80	0.035
Bioavailable testosterone (ng/dL)	97.00	17.10-174.00	120.50	25.70-301.00	0.035
SHBG (nmol/L)	11.00	3.60-35.90	12.00	5.00-28.30	0.47
Total PSA (ng/mL)	0.65	0.04-1.65	0.48	0.01-2.27	0.28
Free PSA (ng/mL)	0.12	0.05-0.38	0.16	0.01-0.41	0.78
IPSS	2.00	0.00-12.00	2.00	0.00-6.00	0.056
Prostate volume (mL)	18.50	12.00-28.00	18.50	11.00-32.00	0.753

Normal Values: GH (0.06-5 ng/mL), IGF-I (116-358, 117-329, 115-307, 109-284, 101-267, 94-252, 87-238, 81-225, 75-212, 69-200 ng/mL for patients aged 21-25, 26-30, 31-35, 36-40, 41-45, 46-50, 51-55, 56-60, 61-65 and 66-70, respectively), IGFBP-3 (3.4-7.8, 3.5-7.6, 3.5-7.0, 3.4-6.7, 3.4-6.6, 3.3-6.7, 3.4-6.8, 3.4-6.9, 3.2-6.6, 3.0-6.2 mcg/mL for patients aged 21-25, 26-30, 31-35, 36-40, 41-45, 46-50, 51-55, 56-60, 61-65 and 66-70, respectively), PRL (2.1-17.7 ng/mL), FSH (1.4-18.1 mUI/mL), LH (1.5-9.3 mUI/mL), total testosterone (241-827 ng/dL), free testosterone (4.7-23 ng/dL), bioavailable testosterone (140-400 ng/dL), SHBG (13-71 nmol/L), total PSA (less than 4 ng/mL), free PSA (less than 0.92 ng/mL), IPSS (0-35), prostate volume (less than 30 mL).

After one year of treatment, there was a significant reduction in GH (4.92 vs 1.85, $p = 0.015$) and IGF-I (466.00 vs 362.50, $p = 0.020$) levels. Significant reduction in PV was observed after treatment (28.50 vs 25.50, $p = 0.001$), however there was no significant difference in the frequency of prostate hyperplasia (46.15% vs 30.76%, $p = 0.43$) neither in the frequency of structural abnormalities found at transrectal US. There was no reduction in IPSS (4.50 vs 4.50, $p = 0.65$).

The findings of digital rectal examination, both in acromegalics under and above 40 years, were in accordance with transrectal US.

DISCUSSION

There are three important studies in the literature, all of them European, reporting the prevalence of benign prostate hyperplasia and structural abnormalities in acromegalic patients

Table 4 - Characteristics of acromegalic patients at baseline and after one year of treatment (≥ 40 years-old).

	Baseline (n=26)		1 year (n=26)		p value
	Median	Min-Max	Median	Min-Max	
GH (ng/mL)	4.92	0.28-68.90	1.85	0.16-68.40	0.015
IGF-I (ng/mL)	466.00	104.00-1600.00	362.50	129.00-959.00	0.020
IGFBP-3 (mcg/mL)	5.65	3.24-11.90	5.36	2.33-9.09	0.131
PRL (ng/mL)	5.82	0.50-86.39	5.16	0.20-43.87	0.135
FSH (mUI/mL)	4.06	0.10-15.40	3.65	0.10-17.40	0.367
LH (mUI/mL)	1.81	0.10-4.68	2.53	0.07-6.30	0.191
Total testosterone (ng/dL)	256.50	20.00-1688.00	252.00	14.00-661.00	0.751
Free testosterone (ng/dL)	5.95	0.23-66.90	6.05	0.18-12.00	0.568
Bioavailable testosterone (ng/dL)	139.50	5.39-1570.00	142.00	4.15-281.00	0.568
SHBG (nmol/L)	20.40	6.00-79.50	20.00	9.00-63.20	0.696
Total PSA (ng/mL)	0.63	0.04-2.50	0.93	0.03-3.91	0.049
Free PSA (ng/mL)	0.15	0.04-0.57	0.18	0.01-0.54	0.501
IPSS	4.50	0.00-21.00	4.50	0.00-25.00	0.649
Prostate volume (mL)	28.50	10.00-84.00	25.50	12.00-69.00	0.001

Normal Values: GH (0.06-5 ng/mL), IGF-I (116-358, 117-329, 115-307, 109-284, 101-267, 94-252, 87-238, 81-225, 75-212, 69-200 ng/mL for patients aged 21-25, 26-30, 31-35, 36-40, 41-45, 46-50, 51-55, 56-60, 61-65 and 66-70, respectively), IGFBP-3 (3.4-7.8, 3.5-7.6, 3.5-7.0, 3.4-6.7, 3.4-6.6, 3.3-6.7, 3.4-6.8, 3.4-6.9, 3.2-6.6, 3.0-6.2 mcg/mL for patients aged 21-25, 26-30, 31-35, 36-40, 41-45, 46-50, 51-55, 56-60, 61-65 and 66-70, respectively), PRL (2.1-17.7 ng/mL), FSH (1.4-18.1 mUI/mL), LH (1.5-9.3 mUI/mL), total testosterone (241-827 ng/dL), free testosterone (4.7-23 ng/dL), bioavailable testosterone (140-400 ng/dL), SHBG (13-71 nmol/L), total PSA (less than 4 ng/mL), free PSA (less than 0.92 ng/mL), IPSS (0-35), prostate volume (less than 30 mL).

(10-12). Two of these studies compared acromegalics to age-matched healthy subjects (10,11). Until today, there is no report of the use of IPSS in the evaluation of acromegalic patients.

Although physiological development and growth of the prostate depend on testosterone and dihydrotestosterone (DHT), androgens action alone seems to be insufficient to explain prostatic diseases (22,25). The existence of important cross-talk

among androgens, growth factors and IGF binding proteins at prostatic level has been suggested (26). Patients with acromegaly are an interesting population model to study the possible involvement of IGF-I in the development of prostatic diseases. In our study, the group of acromegalic patients above 40 years presented a higher proportion of BPH (46.15 vs 12.50%) and structural prostatic changes, such as calcifications and corpora amylacea, when

compared to age-matched healthy men. Colao et al., in a study that included 30 acromegalics with active disease and a control group, demonstrated that the prevalence of BPH was significantly higher in the group of acromegalics (58% vs 26.6%) and structural abnormalities were shown in a large proportion of these patients (11). Probably, we found less prostate hyperplasia than Colao's group because our patients were younger. Nonetheless, these data suggest a possible role of GH-IGF-I axis on prostate growth.

Only one study evaluated the effects of acromegaly treatment on prostatic disorders (12). This study included 23 acromegalics and evaluated PV and structural prostatic abnormalities before and after two years of acromegaly treatment with surgery and/or lanreotide. Considering the whole group of patients, there was no significant change in PV after two years of treatment (34.6 vs 32.5 mL, $p = 0.3$). However, when analyzing only well controlled patients ($n = 16$), there was a significant reduction in PV (29.3 vs 25.4 mL, $p = 0.03$). Regarding the eleven patients that presented BPH at baseline, four had PV < 30 mL at the end of the 2-year follow-up, all of them with controlled acromegaly. In our study, when we analyzed the acromegalics above 40 years, there was a significant reduction in the PV after one year of treatment (28.50 vs 25.50 mL, $p = 0.001$), independently of disease control. Of the twelve patients that had BPH at baseline, five presented PV < 30 mL at the end of the study and only one of them had the disease controlled. This finding suggests that a significant reduction in GH and IGF-I levels is sufficient to promote reversion of BPH, even though biochemical criteria of acromegaly control was not achieved.

It is well established that prostate enlargement starts approximately at the age of 40 (22). In a community-based group of 502 men aged 55 to 74 years without prostate cancer, the prevalence of BPH was 19% using the criteria of a PV above 30 mL together with a high IPSS score (15). Berry et al. (19) described that the PV in men aged 21 to 30 years is approximately 20 ± 6 mL. Analyzing our patients according to age, 14 (35%) were younger than 40 years-old and prostate hyperplasia was not found at baseline. Five out of 12 patients with prostate hyperplasia (41.6%) were aged from 40 to 50 years-old. Besides, our data shows that PV in

acromegalics and the control group younger than 40 years-old is not different, however, IPSS score in the acromegalics is higher than in the control group. In contrast, probably there was no difference in IPSS score in acromegalics and the control group older than 40 years-old because at this age both groups are already exposed to the onset of urinary symptoms. This is the first report of the use of IPSS in the evaluation of acromegalic patients.

CONCLUSIONS

This was the first Brazilian study that assessed prostate disorders in patients with acromegaly. We demonstrated that acromegalic patients above 40 years-old have a higher frequency of structural changes and increased prostate volume in comparison to age-matched healthy men. In this group, significant reduction of GH and IGF-I levels during treatment of acromegaly leads to decrease in the prostate volume.

ABBREVIATIONS

AUA = American Urological Association
 BPH = Benign Prostate Hyperplasia
 DHT= Dihydrotestosterone
 DPC = Diagnostic Products Corporation
 FSH = Follicle-Stimulating Hormone
 GH = Growth Hormone
 HUCFF = Hospital Universitário Clementino Fraga Filho
 IGF-I = Insulin-Like Growth Factor-I
 IGFBP-3 = Insulin-Like Growth Factor-Binding Protein-3
 IPSS = International Prostatic Symptoms Score
 LH = Luteinizing Hormone
 PSA = Prostate-Specific Antigen
 PV = Prostate Volume
 SHBG = Sex Hormone-Binding Globulin
 UFRJ = Universidade Federal do Rio de Janeiro
 US = Ultrasonography

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CONFLICT OF INTEREST

None declared.

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Correspondence address:

Dr. Livia Lugarinho Corrêa de Mello
Avenida General San Martin, 900 / 603, Leblon
Rio de Janeiro, RJ, 22441-014, Brazil
Fax: + 55 21 2421-5335
E-mail: livia.lugarinho@terra.com.br



Methods and Rates of Punishment Implemented by Families to Enuretic Children in Turkey

M. Ihsan Karaman, Orhan Koca, Eyup Veli Kucuk, Metin Ishak Ozturk, Mehmet Akyuz

Department of Urology, Haydarpasa Numune Training and Research Hospital, Istanbul, Turkey

ABSTRACT

Purpose: Nocturnal enuresis is a serious health problem affecting a significant portion of the population. In this study, we investigated the frequency of punishment methods in nocturnal enuresis (NE) in Turkey and its relationship with other parameters.

Materials and Methods: A total of 501 children (301 boys and 200 girls) who were admitted to our outpatient clinic due to nocturnal enuresis were included in the study. Mean age was 9.39 years (range 5-18). Prepared questionnaire form inquiring educational status of the family, frequency and implementation and duration of punishment methods was applied to patients and families.

Results: At least one punishment method was applied to 291 (58.1%) of children with NE. Punishment methods of parents were detected as condemnation (257 patients, 51.3%), depriving desires of the child (120 patients, 23.9%), humiliating the child in the presence of other children (113 patients, 22.6%), reprimanding- threatening with punishment (203 patients, 40.5%). This application was found to continue for longer than 1 year in 52% of punished children. Families graduated of high school and above were found to use punishment methods significantly more than others.

Conclusion: According to the results of our study, a quite high proportion of enuretic children were detected to be exposed to punishment methods. Even, some parents consider that these methods are a part of nocturnal enuresis treatment. We, the doctors, should endeavor more for raising awareness of the community in order to diminish this worrisome behavior.

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Nocturnal Enuresis;
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INTRODUCTION

Enuresis is synonymous to intermittent nocturnal incontinence in discrete episodes while asleep. Monosymptomatic nocturnal enuresis is a symptom and a condition without any other lower urinary tract symptoms (LUTS) and without a history of bladder dysfunction (1).

Monosymptomatic nocturnal enuresis is a symptom rather than a disease and a severe health problem affecting a significant proportion of the community. Approximately 15% of children

aged 5 years still have night-wetting and these complaints improve 15% each year (2). In the studies conducted in our country, NE was found in varying incidences between 11.5-19.03% (3,4). Maturation deficiency in central nervous system development, stress affecting the child especially between 2-4 years, depth of sleep, genetic and rarely organic factors play roles in etiology of enuresis (5). A variety of treatment methods are tried in nocturnal enuresis. Every physician or medical center may have different therapy protocols differing according to the patient and the family.

Behavioral therapies, medical treatments, enuretic alarm devices are some of these methods.

Reactions of the families differ against this condition which concerns such a great majority of the community and leads to various social and psychological problems. Parents try different treatment methods in order to solve this problem by themselves. Threatening and punishing the child is one of these treatment methods (6). In this study, we investigated the frequency of punishment methods implemented in nocturnal enuresis and their relationship with other parameters.

MATERIALS AND METHODS

A total of 501 children (301 (60%) boys and 200 (40%) girls) who were admitted to our outpatient clinic with nocturnal enuresis for the first time and detected to have nocturnal enuresis (NE) as the result of evaluations were included in the study, in a period of three years.

Questionnaire forms prepared for this purpose were filled out by the families of children who complaint of nocturnal enuresis (Table-1). Parental education level, applied punishment methods and frequency and duration of application were inquired with this questionnaire. Depriving of desires of the child, humiliating the child in the presence of other children, reprimanding-threatening with punishment were accepted as mild punishment methods, depriving of sleep, searing the child's penis, leaving the child wet, locking up in the room or the house, mildly beating, beating with hands or a bat were grouped as severe punishment methods. This discrimination was made mainly on cultural features other than scientific data. All forms were given to the mother or the father with closed envelopes and asked to fill at home or in the hospital by themselves. Questionnaire forms of the illiterate parents were filled out by their relatives by asking to them.

Spearman correlation and chi-square test were used for statistical analysis. A p value of < 0.05 was accepted as statistically significant.

RESULTS

A total of 501 children (301 boys-60% and 200 girls-40%) with mean age of 9.39 ± 3.58 ye-

ars (range 5-18) who were admitted to outpatient clinic with complaint of nocturnal enuresis were evaluated in our study.

At least one punishment method had been applied to 291 (58.1%) children with NE. Of these children, 153 (30.5%) were exposed to at least one severe punishment method. Punishment methods were condemnation in 257 patients (51.3%), depriving of the desires of the child in 120 patients (23.9%), humiliating the child in the presence of other children in 113 patients (22.6%), reprimanding-threatening with punishment in 203 patients (40.5%), depriving of sleep in 17 patients (3.4%), searing the child's penis in 5 patients (1%), leaving the child wet in 29 patients (5.8%), locking up in the room or the house in 26 patients (5.2%), mildly beating in 63 patients (12.6%), beating with hands or a bat in 77 patients (15.4%) (Table-2).

This punishment method was continued for longer than 1 year in 52% of children, between 6 months and 1 year in 14%, shorter than 6 months in 20% and only for a few times in 14%. Parents stated that they applied these punishment methods constantly to 24.2% of children and sometimes to 75.8% of children. 195 of parents (38.9%) used punishment in order to cease NE, on the other hand 96 of parents (19.2%) used punishment just for punishing the behavior.

When educational status of mothers was analyzed, it was seen that while 22 (4.4%) were graduates of college, 133 (26.5%) were graduates of high-school, 22 (4.4%) were graduates of intermediate school, 291 (58.1%) were graduates of elementary school, 33 (6.6%) were illiterate. Educational status of fathers was as follows: 100 (20%) were graduates of college, 158 (31.5%) were graduates of high school, 26 (5.2%) were graduates of intermediate school, 203 (40.5%) were graduates of elementary school, 14 (2.8%) were illiterate (Table-3). If at least one parent was high school graduated or above, It was seen that application of punishment methods were more frequent compared to others ($p = 0.018$) (Table-2). Maternal education level was found to be correlated more significantly than paternal educational level ($p < 0.038$). However, it was seen that while parents who were high school graduates or above applied mostly mild punishment methods, parents whose

Table 1 - Questionnaire for nocturnal enuresis.

1 - How old is your child?	
2 - What is your child gender?	
a) Boy	b) Girl
3 - Did you use any punishment method because of nocturnal enuresis?	
a)Yes	b)No
4 - What kind of punishment method did you use?	
a) condemnation	b) depriving of the desires of the child
c) depriving of sleep	d) reprimanding-threatening with punishment
e) searing the child's penis	f) humiliating the child near others
g) leaving the child wet	i) locking up in the room or the house
j) mildly beating	k) beating with hands or a bat Other
5 - How often did you use this punishment method?	
a) sometimes	b) constantly
6 - How long did you use this punishment method?	
a) only for a few times	b) shorter than 6 month
c) 6 months-1 year	c) longer than 1 year
7 - Why did you use this punishment method?	
a) in order to treat	b) in order to punish
8 - What is the educational status of the mother:	
a) illiterate	b) elementary school
c) intermediate school	d) high-school graduated
e) college graduated	
9 - What is the educational status of the father?	
a) illiterate	b) elementary school
c) intermediate school	d) high-school graduated
e) college graduated	

Table 2 - Distribution of punishment methods implemented by parents.

		*high school and above (N:281)	**low educational status (N:220)	P	Total
Mild punishment #139 (27.9%)	Condemnation	153 (30.5%)	104 (20.8%)	0.012	257 (51.3%)
	Depriving of the desires of the child	73 (14.6%)	47 (9.4%)	0.028	120 (23.9%)
	Humiliating the child near others	55 (10.0%)	68 (13.6%)	> 0.05	113 (22.6%)
	Reprimanding-threatening with punishment	123 (24.6%)	80(16.0%)	0.042	203 (40.5%)
Severe punishment ##152 (30.2%)	Depriving of sleep	9 (1.8%)	8 (1.6%)	> 0.05	17 (3.4%)
	Leaving the child wet	15 (3.0%)	14 (2.8%)	> 0.05	29 (5.8%)
	Locking up in the room or the house	9 (1.8%)	17 (3.4%)	0.038	26 (5.2%)
	Mildly beating	29 (5.8%)	34 (6.8%)	> 0.05	63 (12.6%)
	Beating with hands or a bat	15 (3.0%)	62 (12.4%)	< 0.001	77 (15.4%)
	Searing the child's penis	0 (0%)	5 (1.0%)	a	5 (1%)

a: Statistical analysis was not performed in the patients because of the small number of patients

*: Families with at least one parent is high school graduated and above

**: Families that both parents are graduated of intermediate school and lower

#: Exposed to no severe punishment method

##: Exposed to at least one severe punishment method

Table 3 - Educational status of parents (percent).

	Illiterate	Elementary school	Intermediate school	High school	College
Mother	33 (6.6%)	291 (58.1%)	22 (4.4%)	133 (26.5%)	22 (4.4%)
Father	14 (2.8%)	203 (40.5%)	26 (5.2%)	158 (31.5%)	100 (20%)

educational level was lower applied more severe punishment methods.

There were no statistically significant relation between sex of children, method of punishment, duration and frequency of punishment.

While there was no statically significant relation between age of children and mild punishment methods ($p > 0.05$), severe punishment methods were significantly more frequent for children aged 12 or older ($p = 0.028$).

DISCUSSION

NE is bed wetting of a child with no bladder dysfunction and other lower urinary tract symptoms who is expected to be continent by his or her age and neurological development (7). NE has negative effects on both child and parents. This situation creates serious stress on family and causes loss of self confidence of child (5). Reactions of families to this condition which concerns vast majority of society and causing psychological problems may differ in many ways. Parents try to solve this situation with different methods on their own.

Expectations of parents about bed-wetting and its treatment differ from those of the doctors. Unfortunately, the most common punishment methods includes behavior models towards punishment and humiliating the child in the presence of other people throughout history. In a study, one-third of American families who have enuretic children were emphasized to exhibit punitive attitudes (8). In the study of Ouédraogo et al. carried out in France, they reported that 27% of families punished their enuretic children (6). In our study, 58% of families applied at least one punishment method to their children. 24.2% of the families that applied punishment methods reported that this was done constantly. It should be kept in mind that application of these punishment methods could lead to guilt and reduction in self-confidence in children.

In a study of Byrd et al., it was reported that when alarm device was used due to failure of other methods, awakening and disturbance of other family members besides the enuretic child could increase the punishment of the child and physical abuse (8). In the study of Redsell et al., they reported that tolerance level of mothers of enuretic children was lower (9). In the study of Can et al., they detected child abuse in 86.4% of enuretic children (4). Incidence of spanking were found to be 42.1% in the these families. In our study, parents of children with PMNE applied punishment methods more as education measure. Possible reason for this condition was considered to be lower endurance of parents.

In a study of Butler et al., they stated that parents of enuretic children can be extremely guarding and even some of them could be angry and prefer punishment for coping with enuresis (10). In the study of Sapi et al., they stated that all of enuretic children were exposed to domestic violence and detected physical punishment in 48.5% (11). In our study, 30% of families who applied punishment methods exhibited behaviors that could lead to severe psychological and physical traumas like beating the enuretic child. Searing the child's penis (1% of children with NE) in our study is a quite thought-provoking and unacceptable condition. It is a known reality today that punishing is wrong and rewarding is a more appropriate approach. Additionally, behavioral methods, medical treatments, acupuncture and hypnotherapy-like methods which are still in investigation phase are gradually gaining importance as our knowledge about etiopathogenesis of nocturnal enuresis increases (12,13).

Our study has some limitations. First of all, it is a single-center study and we did not question the severity of NE. Selecting the sample from parents who referred to hospital and omitting the others who didn't seek any help for this problem is another limitation. Also we didn't question whether or not the parents have any previous the experience about NE.

The first and the most important step in treatment of enuresis is motivating the child for treatment and you need to empathize with the child, provide the understanding and support of the family, assure that the problem will be solved to achieve this.

CONCLUSIONS

According to the results of our study, a quite high proportion of children were exposed to punishment methods. Even some parents consider that these methods are a part of NE treatment. Definitely a large part of these results arise from insufficient knowledge. However, an interesting data of our study is that punishment rates increased as educational level of parents increased. While mild punishments were preferred by the parents who

have high educational level, families with lower educational status preferred severe punishment methods. Informing only the parents who apply to outpatient clinic is not sufficient for raising awareness of the community in order to reduce the trauma that the children are exposed to. Since it is not possible for all children with NE to be treated by health institutions, other means (media etc.) should be tried to create awareness not to use punishment to heal NE by parents.

CONFLICT OF INTEREST

None declared.

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Correspondence address:

Dr. Orhan Koca
Department of Urology
Haydarpasa Numune Training and Research Hospital
Tıbbiye cad. no:2 Üsküdar, 34718, Istanbul, Turkey
Fax: + 90 216 345-5982
E-mail: drorhankoca@hotmail.com



A wet dressing for hypospadias surgery

Ana Gabriela Santos Martins, Salvador Vilar Correia Lima, Luiz Alberto Pereira de Araújo, Fábio de Oliveira Vilar, Niedson Thiago Pereira Cavalcante

Urologic Department (SVCL, FOV), Pediatric Surgery (AGSM, LAPA, NTPC), Hospital das Clínicas - Federal University of Pernambuco (HC-UFPE), PE, Brazil

ABSTRACT

Introduction: One of the main problems faced by surgeons involved in male genitalia surgeries, in particular in children with hypospadias, is the type of dressing and its use during the post-operative period.

Materials and Methods: From a multidisciplinary project involving the use of sugarcane biopolymer membrane developed in the last 10 years, produced by bacterial action over sugarcane molasses, we developed a multiperforated pellicle that, when applied around the penis, protects the surgical field. It is a proven inert material that does not induce any reaction on the surgical field and can be left in situ maintaining the same characteristics during a long period of time without the need of replacement. This multiperforated tape can involve several times the penis shaft and due to its adhesiveness it hardly loosens. We compared the use of this dressing with a commercial one (made by polyurethane). Thirty patients with hypospadias were randomly selected for the use of this new type of dressing in the last 18 months. A similar group of patients used a similar commercial dressing made of polyurethane (Tegaderm®) according to the same criteria of use. For safety reasons, we applied one or two sutures without the inclusion of the skin using an absorbable suture in order to prevent early detachment. A small gauze was left for 24 hours in order to absorb any possible bleeding. We recommended the irrigation of the dressing with water or saline at least three or four times a day and the patient was allowed to bath. The dressing did not need any special care and if not removed it usually detached spontaneously after 10 to 14 days.

Results: The tolerance to the material was satisfactory and there were no adverse reactions on the penile surface. In two cases of the biopolymer group the dressing detached spontaneously on the first and third days, respectively. In two cases of the polyurethane group it was observed major edema. The grade of satisfaction of the patients and their relatives was excellent on the biopolymer group.

Conclusion: This dressing produced by a polysaccharide is a promising alternative for the treatment of children and adolescents submitted to genital surgery. Its main advantage is the possibility of several washes along the day without the need of any other manipulation.

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Hypospadias; Biopolymers; canecystatin protein, sugarcane [Supplementary Concept]; Polysaccharides

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INTRODUCTION

Surgical correction of male genitalia anomalies in children and adolescents has evolved in the last years with the use of new techniques,

instruments and sutures, that somehow have contributed to better results. In spite of that, the correct choice of dressing is still challenging. Several materials have been used with various results, and there is no consensus about their use (1-9).

The clinical indication of a specific dressing is based upon the protective function and mechanical barrier of the tissues against contamination and reduction of the edema caused by the surgical trauma. The structure material should present physical characteristics including elasticity, resistance and flexibility, and must adjust tightly on the surface of the tissues. The chosen dressing must present minimal adverse reactions when in contact with living tissues or organic fluids and must be easily removable.

The main objective of the study was to prove that the biopolymer tape made of sugarcane molasses is biocompatible and protects the affected area in the same or better manner than the commercial available polyurethane tape (Tegaderm®) that is used nowadays for dressing after surgical correction of hypospadias.

MATERIALS AND METHODS

The target population included children and adolescents with hypospadias, and the parents were correctly informed about the study and were invited to join it. Thirty patients used the biopolymer tape as dressing and a similar group of patients used the polyurethane pellicle Tegaderm®. The groups ages varied between 1 and 18 years old, with a median age of 6.6 years for the group that used the sugarcane biopolymer and 7.4 for the group that used the polyurethane tape.

The comparison of the two materials was made with the use a question form that allowed the evaluation of the results until complete recovery of the patients submitted to surgical correction of hypospadias. It was also analyzed local inflammatory reaction, edema, hyperemia, transudation and adhesiveness of the material to the surgical field until its removal.

The evaluation of adverse reactions as skin irritability was categorized by the researcher observation as absent, limited to the foreskin, or extended to other areas.

Grade of adhesiveness: the researcher observed the adhesiveness as with areas of detachment, adhesiveness without detachment or without adhesiveness.

Grade of discomfort: the patients or their relatives described it during the questionnaire as “very troubled”, “troubled” or “not troubled” with the use of the dressing.

Grade of adhesiveness and fixedness “in situ”: this aspect was evaluated after the patients or their relatives cleaned the dressing with water three times a day and informed the researcher.

Sample size

This was an experimental controlled and randomized clinical trial and the size of the sample included 60 patients, divided between two groups: group 1 (n = 30), patients submitted to surgical correction of hypospadias using biopolymer tape made of sugarcane molasses and group 2 (n = 30), patients that used the polyurethane pellicle as dressing.

All patients were submitted to anamnesis including questions about previous surgeries, physical and urological exam and classification of the hypospadias grade, along with pre-operative routine exams. There were 21 proximal hypospadias and 9 distals in the group that used the biopolymer tape. In the group that used the polyurethane pellicle, 19 presented proximal hypospadias and 11 distal.

The patients were assigned randomly to the groups (use of biopolymer made of sugarcane molasses or polyurethane), through sealed envelopes containing numbered randomized distributions generated by computer in a 1:1 manner in groups of 10. The envelopes were opened at the surgical room by a nurse right before the surgical procedure. All volunteer patients were operated by one or more members of the surgical team. To make sure that all procedures were done properly all patients were hospitalized for 8 days.

The polyurethane pellicles sized 4.4 x 4.4 and 6.0 x 7.0, were waterproof and protected against external contaminants, and were adequately fitted with comfort of all patients (Figure-1).

The biopolymers tapes of sugarcane presented the same measures of the polyurethane pellicles and could be cut out according to the size of the patient organ (Figure-2). They were sterilized using gamma rays.

Figure 1 - Polyurethane pellicle molded to the penile shaft.



Figure 2 - Biopolymer pellicle made of sugarcane molded to the penile shaft.



Technique of pellicle application

After completion of the surgical correction of the hypospadias according to several techniques, the area was washed with saline and dried with gauze. Then, the dressings were applied over the surgical field involving all penile surface (Figures 3a,b,c and d). There was no tension over the surgical field (Figure-4). Although they adhered correctly, three sutures were passed on the ends of the dressings using catgut 6-0, not including the skin, as a safety measure. No creams were used in both groups. In the group of patients with up to 7 years and that used a catheter, it was used a double diaper layer; the catheters were passed from a first to a second diaper through an opening, preventing the urine to be in touch with the dressing. In the group of boys more than 7 years old the urine drainage included a urethral catheter attached to a closed collecting system.

Ethical Aspects

This study did not present ethical risks and was in accordance to the determinations of the Na-

tional Council of Health, the Helsinki Declaration and the Nuremberg Code for human experiment. The project was approved by the local Ethical Committee of the Institution. This was a randomized and controlled clinical experimental and observational trial.

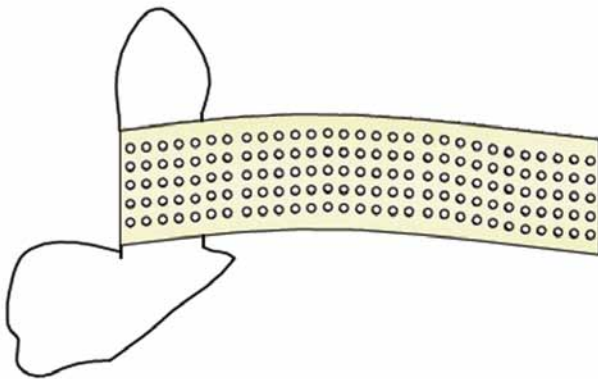
RESULTS

The follow-up evaluations were made by one of the members of the team daily until the removal of the dressing in the eighth day after the surgery before discharge (Figure-5). Eight parents of patients of the biopolymer group decided not to remove the dressing, allowing it to spontaneously fall, which occurred after 10 to 14 days, maintaining the recommendations of regular washes (Figure-6). All patients tolerated well the dressings in both groups. In one patient of the biopolymer group it was observed spontaneous fall of the dressing, one during the first day after the surgery and the other after three days of the surgery. There was significant edema in two patients of the Tegaderm® group, along

Figure 3 a, b, c and d - Placement of the biopolymer pellicle of sugarcane, involving all penile surface.



Figure 4 - Schematic drawing of the application of the biopolymer pellicle of sugarcane.



with a foul odor resembling urine, according to relatives. Such cases were not observed in the biopolymer group.

DISCUSSION

There is no consensus about the correct dressing material for use during the post-operative period of male patients with genital anomalies. Some groups advocate not to use any dressings and others use complex dressings with different grades of handling difficulties (10,11).

In a study from Canada, three groups of patients were compared. The median age was 2.2 years and all patients were submitted to surgical correction of different grades of hypospadias and used three different dressings: one with the same material used in the present study, one group using a porous material and a third group that did not use dressings. All patients used an ointment and the surgeries were made in a day hospital. The study did not establish the correct number of patients of each group. The dressing was removed by the parents after three days and the most important aspects was the urine odor and concerning about feces contamination. It concluded that dressings were not necessary in hypospadias surgeries (12). It is known that in minimal grades of hypospadias presenting minimal detachment of the foreskin, the same approach similar to post-tectomy can be applied, as stated by Leclair et al. (11). In the present study, there was no need of dressing exchange and it was possible to wash it as many times as needed, including the daily bath; the post-operative handling was similar to those patients without dressings and the advantage that the surgical field is protected against external agents as feces. The fact that the studied material has already been studied regarding cytotoxicity and that it was used in other areas (including as

Figure 5 - Sugarcane biopolymer pellicle on the 8th day, before removal.



Figure 6 - Spontaneous loss of the biopolymer tape after the 10th day.



implants) makes it a safe option, with minimal undesired effects when applied to operated surfaces (13,14). It is important to reinforce that this material has been used in an uncontrolled manner for more than 5 years with good results (15). The present controlled study also studied prospectively the material and compared the results with previous reports. Since this material is multiperforated and transparent it is possible the observation of hematomas or areas of necrosis by the parents and doctors, that are very troublesome in male genitalia surgeries. The early withdrawal of the dressing as advocated by some (4,12) does not seem correct, since the development of edema will increase the tension in the sutured area and possible dehiscence. Another important aspect in the randomized trials is that the median age of the patients is low (less than 3 years old) in which group there seldom is observed erection (11,12). It seems possible that the use of a dressing with adequate contention of distension limits the occurrence of edema and its consequences on the surgical surface. As stated, the median age of the patients in our study was superior than the others.

CONCLUSIONS

Based on the present study it is possible to conclude that the proposed material is a highly satisfactory alternative. Since it is a product obtained from a renewable and natural source, possibly cheaper, its use is adequate for surgical dressing of hypospadias repair.

CONFLICT OF INTEREST

None declared.

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Correspondence address:

Dr. Salvador Vilar Correia Lima
 Hospital das Clínicas - Federal University of Pernambuco
 (HC-UFPE)
 Avenida Parnamirim, N 95, Parnamirim
 Recife, Pernambuco, 50.060-901, Brazil
 Fax: +55 81-3031-3846
 E-mail: salvador@ufpe.br



Integration of collagen matrices into the urethra when implanted as onlay graft

Kleber Sayeg, Luiz G. Freitas-Filho, Ângela Flávia Logullo Waitzberg, Victor Eduardo Arrua Arias, Marcus Laks, Fernanda Mattos Egydio, Andréia Silva Oliveira

Department of Surgery (KS, LGFFilho, ML, FME), Department of Nephrology (ASO) and Department of Pathology (AFLW, VEAA) – Federal University of São Paulo, Brazil

ABSTRACT

Objective: To assess the integration of decellularized heterologous collagen matrices into the urethra, when implanted with no cells or when seeded with autologous smooth muscle cells.

Materials and Methods: Eighteen New Zealand rabbits were randomly assigned to two groups: Group I (n = 9) - animals undergoing urethral segment resection with interposition of a patch of heterologous collagen matrix seeded with autologous smooth muscle cells; Group II (n = 9) - animals undergoing resection of a urethral segment with interposition of a decellularized heterologous collagen matrix patch. Two animals from each group were sacrificed on postoperative days seven, fourteen and twenty-eight; three animals from each group were sacrificed at the end of three postoperative months. At the end of the third month one animal from each group underwent urethroscopy for urethral integrity assessment and one animal from each group had its microcirculation image captured by a SDF device (Side-stream Dark Field - Microscan Analysis Software). One animal from each group in each euthanasia period underwent cystourethrography so as the urethra could be viewed at flow time. The matrices integration was assessed through histological examination using hematoxylin and eosin (H&E), Masson trichrome (MT), Picrosirius red and Von Willebrand staining. In a blind study with two pathologists all the slides were studied.

Results: The matrices whether seeded or not with autologous muscle cells were able to restore the architecture of the urethra, but were eliminated from the first week on, before incorporation. Microcirculation of the neourethra, at the end of the third month, showed the same characteristics as a normal urethra in both groups of animals.

Conclusion: Natural heterologous matrices implanted in the urethra as onlay graft were not incorporated into its walls but were able to fully restore the cell architecture of the organ, regardless of being seeded or not with autologous muscle cells.

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INTRODUCTION

Urethral reconstruction is frequently used in the treatment of both congenital and acquired anomalies (1-6). When the urethra needs to be repaired or reconstructed, either partially or totally,

different surgical approaches can be used such as anastomosis of the urethral stumps, flaps or grafts. Although such techniques are still largely employed, in several occasions adverse effects such as fistulas, stenosis, infections, stone formation, graft contracture or diverticula formation may

appear. In addition to morbidity at the donor site, there is tissue scarcity when such patients undergo a number of surgical interventions (1,2,4-12).

Tissue engineering techniques are an alternative for such difficulties. A decellularized matrix, either natural or synthetic, may serve as a pattern for implantation of individual cells previously obtained by harvesting a full fragment from the very organ. The different cell layers are separated by using different techniques, expanded “in vitro”, and then seeded in the matrix before reimplantation (5). The different researchers that use cell-seeded matrices describe the process of matrix incorporation into the new urethra, however, not observing the manner in which this incorporation actually takes place in the early phases of tissue repair.

This paper aimed to study the evolution of decellularized heterologous matrices integration along time, whether or not seeded with autologous smooth muscle cells and implanted into the urethra.

MATERIALS AND METHODS

All experimental procedures were approved by the local Research Ethics Committee (CEP - 0939/07) and conducted in strict conformity with local institutional guidelines and with international standards for manipulation and care of laboratory animals.

Eighteen New Zealand male rabbits, 6 to 8 months old, weighing approximately 3 kg, from the Center for Development of Experimental Models for Medicine and Biology of the Federal University of São Paulo, were used. The animals were assigned to 2 groups of nine animals each. The animals in both groups underwent resection of a 3.5 cm long by 0.5 cm wide fragment from the ventral portion of the penile urethra. The animals in Group I underwent interposition of a patch of decellularized heterologous matrices, of same size as the urethral segment withdrawn, seeded with autologous muscle cells; the animals in Group II underwent interposition of decellularized heterologous matrix patches, of same size as the segment withdrawn, however with no cells seeded. The matrix was sutured to the urethra using 6-0 polypropylene in a continuous fashion.

The collagen matrices were taken from porcine bladders submucosa obtained from animals undergoing different surgical procedures. They were kept in sterile tubes containing 50 ml PBS (Phosphate-Buffered Saline - Sigma Chemical Co., Saint Louis, USA), to which 10.000 UI/L penicillin and 50 mg/L streptomycin were added. They were continuously agitated for fourteen days in Triton X-100 1% detergent (Sigma Chemical CO, Saint Louis, USA) and 0.1% ammonia hydroxide, which solution was changed every two days. On day fourteen the bladders were kept for twenty-four hours in distilled water in continuous agitation. Then, they underwent ultraviolet radiation for one hour and, after that, they were cut into 3 x 5 cm fragments under a laminar flow chamber. The fragments were then kept in tubes containing sterile PBS and stored at -20° C Celsius until they were used. A fragment of the material obtained was put in a 10% tamponade formaldehyde solution and forwarded for anatomopathological assessment.

For the surgical procedures the animals were anesthetized with intramuscular ketamine (50 mg/kg) and xylazine (10 mg/kg). During the procedures a venous infusion of physiological solution was maintained. The animals in Group I underwent median laparotomy. The bladder was isolated and a 1 cm² segment was withdrawn from the anterior wall; the bladder was then sutured with 4-0 polyglactin and the abdominal wall was sutured with 3-0 polyglactin. The bladder fragment withdrawn was taken to the laminar flow chamber and cut into 2 mm² fragments after full removal of the mucosa. The fragments were placed on culture plates by using the “explant” technique, covered with Dulbecco’s modified Eagle’s medium (DMEM) and 10% fetal bovine serum, and incubated at 37° C in a 5% carbon dioxide atmosphere. Around day fourteen, when the culture plates showed cell confluence of approximately 80%, they were subjected to trypsin enzymatic action. The solution was centrifuged, the cells were counted in a Neubauer chamber and seeded in the collagen matrix, where they remained for another seven days in the same culture solution before being implanted.

The animals from both groups were sacrificed by anesthetic deepening; two animals on days seven, fourteen and twenty-eight and 3 animals in the third postoperative month. One animal in every sacrifice period had its bladder and urethra removed

in block for a cystourethrography. The penises of all animals were removed in block and fixed in 10% buffered formaldehyde for 24 hours. The pieces were placed in paraffin blocks, cut into 5 micrometer sections, stained with hematoxylin-eosin (H&E), Masson trichrome (MT), Picrosirius red and assessed with polarized light and immunohistochemistry using anti-Von Willebrand factor antibody and analyzed in optical microscopy with 150x augmentation. We observed the epithelial integrity, the inflammatory process and the amount of collagen, and scores 0 to 3 were created for every item assessed (Table-1).

At the end of the third postoperative month one animal in every group underwent urethroscopy with an Olympus Winter & Ibe GMBH, Hamburg, Germany, 17Fr sheath, 12 degree optics cystoscope and was assessed for the new urethra microcirculation through an SDF device, 1 mm² images from three different sites were captured, a 2 mm^{hh} deep surface was assessed in 320x image augmentation; the images were saved in 10 second videos (13).

RESULTS

The urethroscopies performed showed the urethra with normal caliber - the 17Fr cystoscope was passed with no technical difficulty and an adequate urinary flow was obtained after bladder compression.

The microcirculation assessed by the SDF showed low flow through venules, scarce capillaries, absence of arterioles, and low vascular density; these results were similar to the ones observed in normal rabbits urethras (Figure-1).

The cystourethrografies performed showed urethras with normal caliber and adequate urinary flow (Figure-2).

Histological analyses performed on postoperative day seven showed a disrupted area with a deposit of fibrin-leukocitary material and stroma rich in leukocytes, with predominating polymorphonuclears and presence of eosinophils in the animals of both groups, suggestive of an acute exudative inflammatory process and of a recent intervention. The matrix was already being eliminated into the lumen of the organ, and an epithelial layer of cells was being formed right below (Figure-3). On day fourteen, in both groups, the matrix was found to be in the ure-

thral lumen, the epithelium not fully formed yet, and the stroma with mixed inflammatory infiltrate with lymphocytes predominating, suggestive of an early chronification. The process was being resolved from bottom to top with good formation of the muscle layer (Figure-4). On day twenty-eight full reepithelization could already be observed in the animals in both groups with inflammatory process, with predominating lymphocytes, limited to the submucosa (Figure-5 a). A squamous metaplasia in the animals in the group without cells was observed (Figure-5 b). On day ninety the animals in both groups had urethral architecture similar to that of a normal urethra, however still with a less intense chronic inflammatory process, with predominating lymphocytes, showing the scarring not to be fully resolved on day ninety (Figure-6) The result of the assessment made by two pathologists in a blind study can be seen in Table-1.

Picrosirius red staining showed in all periods and in both groups, predominance of early type III collagen, even on day ninety (Figure-7).

Immunohistochemistry using anti-Von Willebrand antibody demonstrated intense neoangiogenesis in both groups, with no differences between them (Figure-8).

DISCUSSION

The need to partially or fully reconstruct the urethra is relatively usual given the high number of patients with congenital or acquired diseases, and trauma victims. Thus, a large number of techniques have been used in the attempt to restore the anatomy of the organ, aiming to achieve adequate micturition and, in the cases of male patients, to enable forward ejaculation with an adequate amount of spermatozooids (1-5,7). All techniques used to date have a high rate of complications and, when such patients undergo several procedures, which is quite usual, the use of flaps or grafts is almost always necessary (5-7,14,15).

The surgical interventions employing grafts for urethral reconstruction have already led to the use of segments from the saphenous vein, from the appendix, from partial skin grafts and even from tissues containing urothelium, such as the ureter and bladder mucosa (14-23). Synthetic materials such as dacron®, silicone and teflon®, have also been used, but, more recently, the use of buccal muco-

Figure 1 - A - Photomicrography of the urethra - group I (seeded matrix), SDF 320x. B - Photomicrography of the urethra - group II (non-seeded matrix). SDF 320x.

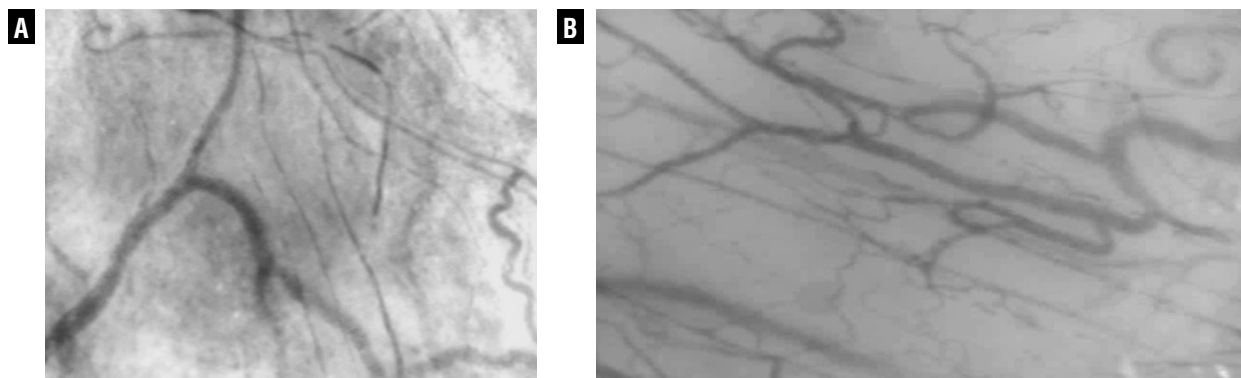


Figure 2 - A - Cystourethrography - Rabbit Group I (seeded heterologous matrix). Yellow line delimiting penile urethra subjected to matrix interposition. B - Cystourethrography Rabbit Group II (non-seeded heterologous matrix). Yellow line delimiting penile urethra subjected to matrix interposition.

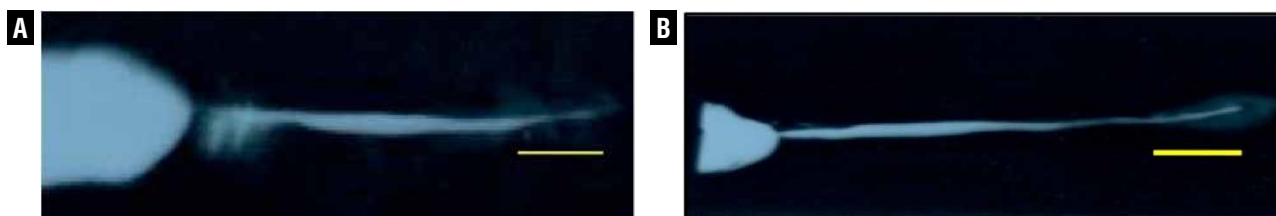


Figure 3 - A - Photomicrography of the urethra - Group I animal, postoperative day 7, Black arrow = Matrix. Green arrow = inflammatory process. Blue arrow = epithelium. H&E 25x. B - Photomicrography of the urethra - Group II animal, postoperative day 7, Black arrow = Matrix. Green arrow = inflammatory process. Blue arrow = epithelium H&E, 25x.

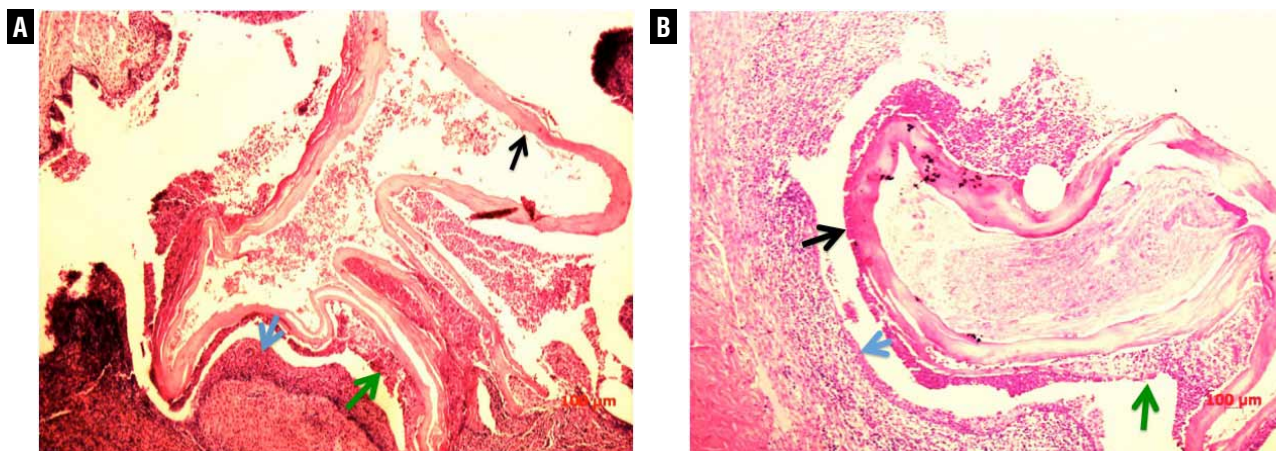


Table 1 - Anatomopathologic findings.

Groups	Inflammation intensity	Predominant Cells	Layers
7 days No cells	3	Lymphocytes Eosinophils Neutrophils	Submucosa (ulceration)
	3	Lymphocytes Eosinophils Neutrophils	Submucosa Muscular
7 days With cells	3	Lymphocytes Neutrophils	Mucosa Submucosa Muscular
	3	Lymphocytes Neutrophils	Submucosa Muscular
14 days No cells	3	Lymphocytes Eosinophils Neutrophils	Submucosa
	3	Lymphocytes Neutrophils	Submucosa Muscular
14 days With cells	2	Lymphocytes	Submucosa
	2	Lymphocytes	Submucosa
28 days No cells	2	Lymphocytes	Mucosa Submucosa
	3	Lymphocytes	Mucosa Submucosa
28 days With cells	1	Lymphocytes	Submucosa
	3	Neutrophils Lymphocytes	Mucosa Submucosa

continuation

3 months No cells	1	Lymphocytes Eosinophils Neutrophils	Submucosa
	2	Lymphocytes	Submucosa
	2	Lymphocytes	Submucosa
3 months With cells	2	Neutrophils Lymphocytes	Mucosa Submucosa
	2	Neutrophils Lymphocytes	Submucosa
	3	Neutrophils	Submucosa

0 - No inflammatory process

1 - Mild amount of inflammatory cells

2 - Moderate amount of inflammatory cells (more than 25% of the sample)

3 - Extended or severe inflammatory process (more than 50% of the sample)

Neutrophils - Acute Inflammation

Lymphocytes - Chronic Inflammation

Eosinophils - Exsudation

Figure 4 - A - Photomicrography of the urethra - group I animal, postoperative day 14, Blue arrow = epithelium Masson's trichrome, 25x. B - Photomicrography of the urethra - Group II animal, postoperative day 14, Blue arrow = epithelium. Masson's trichrome, 25x.

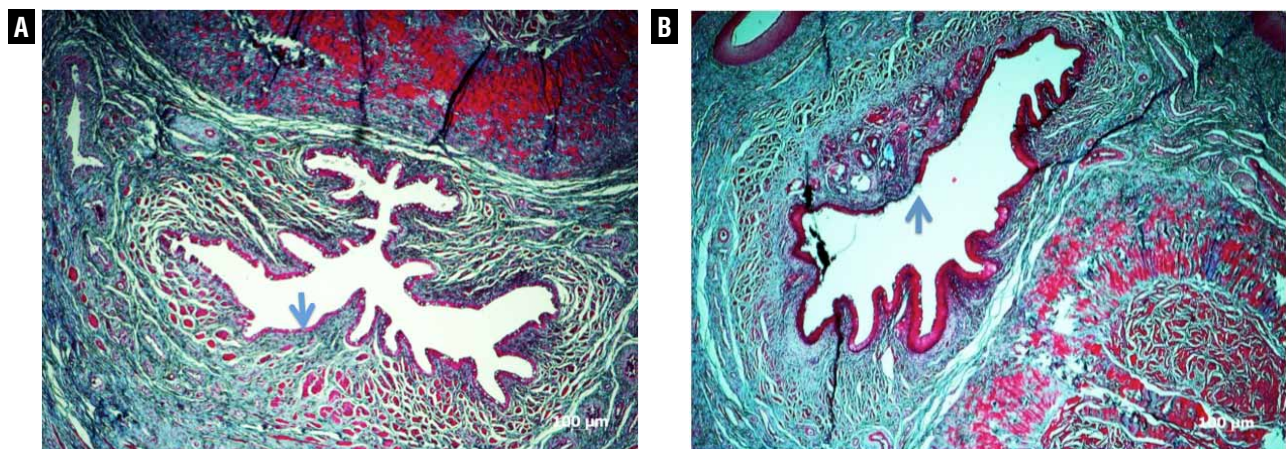


Figure 5 - A - Photomicrography of the urethra - Group I animal, postoperative day 28, H&E, 25x. B - Photomicrography of the urethra - Group II animal, postoperative day 28. Blue arrow = squamous metaplasia. Masson's trichrome, 25x.

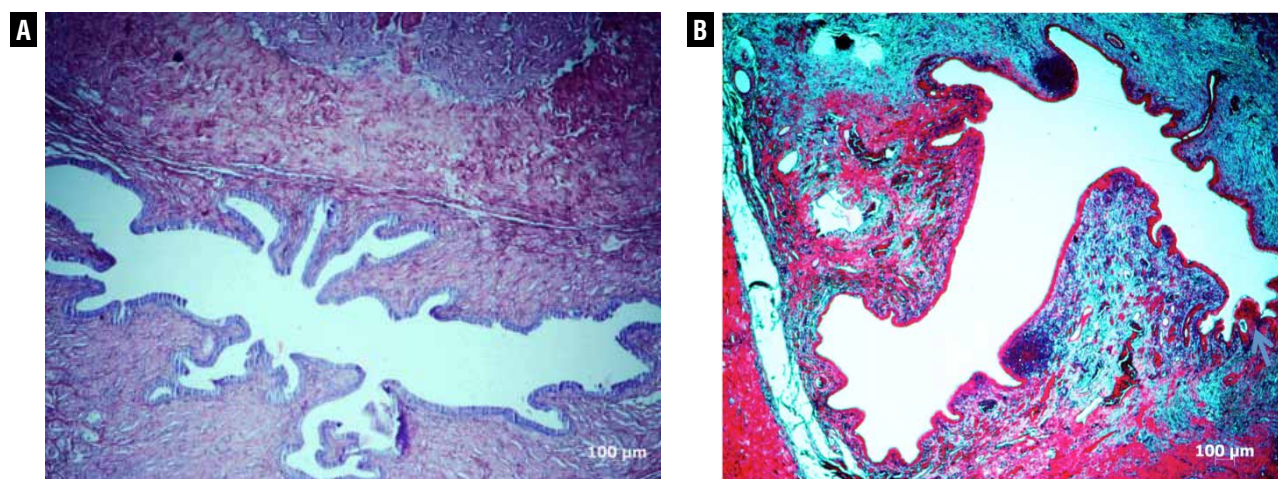
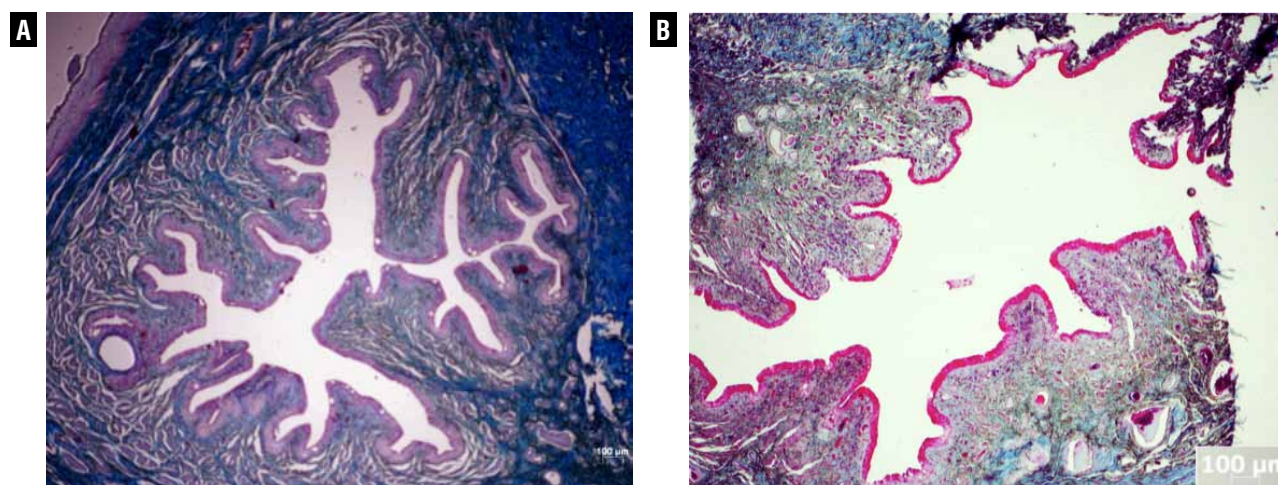


Figure 6 - A - Photomicrography of the urethra - Group I animal, postoperative day 90, Masson's trichrome, 25x. B - Photomicrography of the urethra - Group II animal, postoperative day 90, Masson's trichrome, 25x.



sa appears to have become the gold standard in the treatment of major reconstructions, presenting the smallest rate of stenosis and fistulas, in the medium and long term. The number of adverse events, however, does not allow one to say yet that this would be the ideal urethral substitute (12,23-26).

The recent advance of molecular biology, the large scale production of cell nutrients, and the possibility of cultivating cells “in vitro” and then implanting them in synthetic or natural ex-

tracellular matrices, thus reconstructing an organ, have led to the introduction of Tissue Engineering techniques. Combined with the employment of new materials these techniques may become important in the reconstruction or replacement of lost or insufficient organs or tissues, and a viable option in cases of lack or partial absence of tissues (5,27-29).

Tissue Engineering uses either natural or synthetic matrices that should favor cell adhesion, proliferation, differentiation, and growth, and

Figure 7 - A - Photomicrography of the urethra - Group I animal, postoperative day 90, Picrosirius Red staining, 25x. Green area collagen (type III). B - Photomicrography of the urethra - Group II animal, postoperative day 90, Picrosirius Red staining, 25x. Blue area collagen (type III)

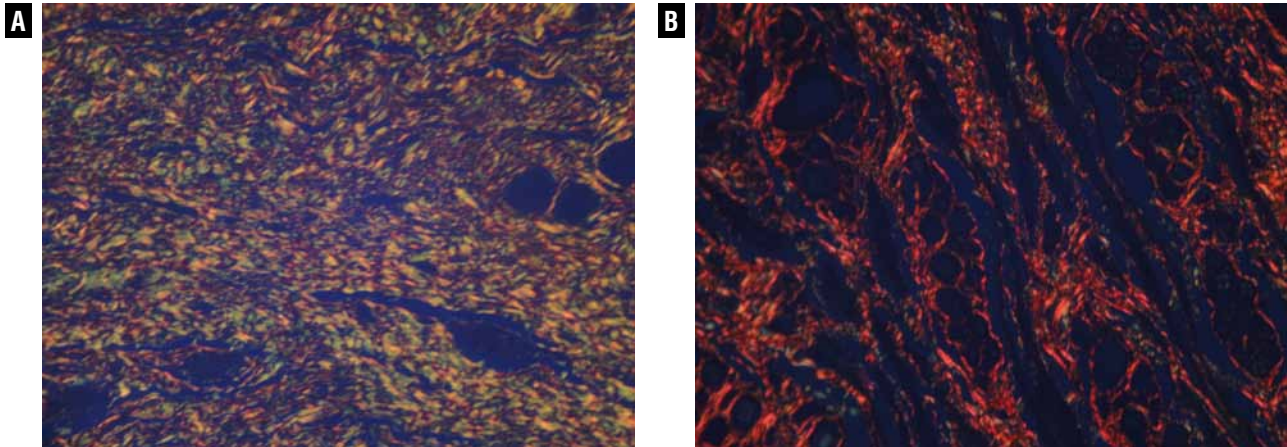
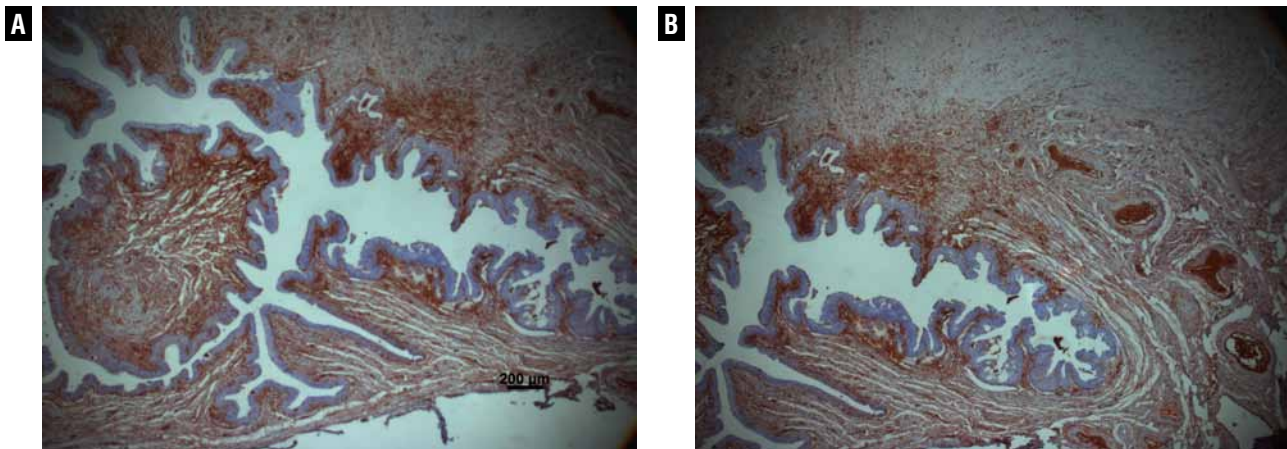


Figure 8 - A - Photomicrography of the urethra - Group I animal, postoperative day 90, immunohistochemistry with anti-Von Willebrand factor antibodies, 25x. B - Photomicrography of the urethra - Group II animal, postoperative day 90, immunohistochemistry with anti-Von Willebrand factor antibodies, 25x.



their slow degradation by lysosomal enzymes, in the case of synthetic matrices that gradually lead to their absorption, with minimal toxicity and inflammatory reaction (3,5,7,9,30,31). Tissue engineering techniques may allow for the creation of a reservoir of biocompatible tissues that can be used in extensive urethral reconstructions when there is a lack of autologous tissues for flaps or grafts on account of various prior surgeries.

In this study we sought to assess integration of a collagen matrix into the urethral wall. The collagen matrix was obtained of a porcine

bladder by employing tissue engineering techniques. Considering that in the different reconstruction techniques, urothelial growth frequently takes place starting from the lateral borders in the transition between the patient's urethra and the graft, we decided to test the hypothesis that implantation of the autologous smooth muscle cells alone with no need to use the urothelial layer could reconstruct the entire architecture of the organ. It was found, through endoscopy and radiographic exams, that the macroscopic aspect of the urethral caliber of the animals in both groups

studied resembled that of a non-operated urethra, fact that had previously been observed by Chen et al., Atala et al. and El-Kassaby et al. using similar techniques (1,2,5,7,8).

By using the SDF it was possible to assess the microcirculation that was established on the new urethra around postoperative day ninety; it also showed the vascular network to resemble that of a normal urethra, regardless of the matrix having or not been seeded with muscle cells.

In order to verify the process of incorporation of the matrices into the urethral bed, histological assessment with hematoxylin-eosin, Masson's trichrome, Picrosirius red and Von Willebrand staining were used. On day seven after implantation the animals in both groups had the same behavior in regard to epithelial integrity, to the inflammatory process and to the amount and type of collagen. The implanted matrix, instead of being incorporated and forming a portion of the urethral wall, was being eliminated into the organ's lumen while, at the same time, the anatomy of the urethra was being restored. On postoperative days twenty-eight and ninety both groups showed epithelial integrity; the only difference found was that on day twenty-eight most of the animals in which the cells were not seeded had squamous metaplasia, which means the existence of an intense inflammatory process that turns the original epithelium into a barrier that is more resistant to aggressions.

Decellularized collagen matrices of different origins are being used in urethral reconstructions. Chen et al observed cell infiltration and neo-angiogenesis after two weeks, with disorganized migration of muscle fibers after two months (2). De Filippo et al comparing decellularized matrices seeded or not with epithelial and muscle cells observed scarce vascular organization in the non-seeded matrices, contrasting with the good organization of the ones seeded (4). Our study was the first to make an early assessment of the heterologous matrices integration process. We demonstrated that the matrix was actually eliminated into the lumen instead of being reincorporated, and the reason for such may be a possible greater matrix permeability to the urine, which can be explained, perhaps, by an exaggerated inflammatory process.

Regardless of being incorporated, however, the entire layer was reconstructed, which may have occurred in other studies, considering that, apparently, what really matters seems to be the presence of the matrix at the beginning of scarring.

It can be said that the heterologous collagen matrix, although not having been integrated into the native tissue, was capable of stimulating urethral remodeling when the dorsal bed of the original urethra was maintained. In those cases muscle cells seeding was not necessary for the urethral remodeling to be completed, and autologous smooth muscle cells seeding, although having lessened the inflammatory process, was not found to be indispensable for regeneration of both the original muscle and the epithelial layer.

CONCLUSIONS

Natural heterologous matrices implanted in the urethra as onlay graft were not incorporated into the urethral walls but were capable of restoring its entire cell architecture whether or not seeded with autologous muscle cells.

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CONFLICT OF INTEREST

None declared.

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Correspondence address:

Dr. Luiz G. Freitas Filho
 Rua Batista Cepelos 87 / 61
 São Paulo, SP, 04109-120, Brazil
 Fax: + 55 11 5571-7965
 E-mail: luizfreitasepm@gmail.com



Sinusoidal Constriction and Vascular Hypertrophy in the Diabetes-Induced Rabbit Penis

Vivian Alves Pereira, Marcelo Abidu-Figueiredo, Marco Aurélio Pereira-Sampaio, Mauricio Alves Chagas, Waldemar Silva Costa, Francisco J. B. Sampaio

Urogenital Research Unit. State University of Rio de Janeiro (VAP, WSC, FJBS), Rio de Janeiro; Institute of Biology, Sector of Animal Biology, Rio de Janeiro Federal Rural University (MAF) Seropedica and Laboratory of Cellular and Extracellular Biomorphology, Department of Morphology, Federal Fluminense University (MAPS, MAC), Niterói, Rio de Janeiro, Brazil

ABSTRACT

Objective: To assess the morphological changes of penile vascular structures and the corpus cavernosum area in alloxan-induced diabetic rabbits.

Materials and Methods: Twenty male rabbits (2 months old) were divided into two groups with 10 rabbits each, the control group (CG) and the diabetic group (DG). The animals from DG received an intravenous injection of alloxan (100mg/kg) to induce the diabetes. Ten weeks after the induction of diabetes, all animals were euthanized. Two fragments of the penile shaft were harvested and samples were processed and paraffin embedded. Sections (5µm) were cut and stained for histological and immunohistochemical markers.

Results: Nuclear protrusion toward the lumen, and cytoplasmic vacuolization were observed in the tunica intima of the dorsal artery of the penis in DG. The thicknesses of the tunica media increased significantly in DG ($p = 0.0350$). It was also observed a significant increase in the area of the tunica media ($p = 0.0179$). There was no significant change in smooth muscle cell density in the tunica media of the dorsal artery of the penis ($p = 0.0855$). The collagen fiber pattern of the tunica adventitia of the dorsal artery of the penis was different between the control and diabetic groups. There was a significant decrease in the area occupied by the cavernous sinuses in DG ($p = 0.0013$).

Conclusion: Alloxan-induced diabetes mellitus in rabbits promotes important changes in penile vascular structures, thereby decreasing blood supply and affecting penile hemodynamics, leading to erectile dysfunction.

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INTRODUCTION

The penile erection results from blood pressure increases, relaxation of smooth muscle, and reduction of venous return (1). Smooth muscle cells, elastic system fibers, and collagen fibers are important penile structures involved in the erection process. These structures also give required rigidity to the penis when in a flaccid

state (2,3). Several studies have reported functional and morphological changes that take place during the erection process in different animal models (4,5) and also in humans (6,7).

Patients with diabetes mellitus (DM) have a high incidence of erectile dysfunction (ED). Several epidemiological studies have assessed the correlation between DM and ED (8). Evaluation of animal models demonstrated that neural (9)

and vascular (10) changes seen with DM may be associated with ED. Vascular diseases such as microangiopathy, atherosclerosis, and hypertension are often observed in patients with DM. A relationship between these vascular conditions and the occurrence of morphological changes in the mesenteric artery (11,12) and aorta in rats (13) has been demonstrated.

Although some morphological changes in penile elements have been described in rabbits with DM (3), there is no data regarding morphological changes in penile blood vessels associated with DM. The rabbit penis is, morphologically, classified as a vascular type and has a dorsal corpus cavernosum (CC) and a ventral corpus spongiosum (CS), which surrounds the penile urethra. Both CC and CS are covered by a dense connective layer, the tunica albuginea, which originates intra-cavernosum pillars or septa, mainly in the CC (14). There are pronounced resemblances between the rabbit and human penis in morphological, physiological, and neurological features (5,15). Therefore, the rabbit penis is often used as an experimental model to assess ED (5,14). The rabbit penis is a more suitable model than the rat penis, which is classified as a fibro-elastic type (4), with a penile bone inside and a penile protrusion during mating with little variation in its diameter (16).

The arterial vascularization of the penis in the New Zealand rabbit was shown to be supplied by the penile artery arising from the internal pudendal artery, and its branches, and the deep artery and dorsal artery of the penis (17). The dorsal artery of the penis (DAP) has a longitudinal path in the penis, with few variations, resulting in accurate transverse cuts by minimizing the bias in histomorphometric analysis. The DAP can be considered, from a histological perspective, as a representation of how DM and other disorders affect the penile arteries, as cited by Qiu et al. (18) and Kovanecz et al. (19). Additionally, the DAP is important for the homeostasis and function of penile tissues (19). Thus, the aim of this study was to assess the morphological changes in penile vascular structures and the area of the corpus cavernosum in diabetes-induced rabbits.

MATERIALS AND METHODS

Twenty New Zealand rabbits, 2 months old, weighing 1.5 to 2.0 Kg, were used in this study. The study was approved by the Ethics Committee in Animal Research at the State University of Rio de Janeiro (CEA 227/2008). All animals were housed in individual cages at room temperature. They were provided with a commercial rabbit feed (120 g/day) and water *ad libitum*. The animals were divided into two groups of 10 animals, the control group (CG) and the diabetic group (DG). All rabbits were anesthetized by using xylazine (5 mg/Kg IM) and ketamine (20 mg/Kg IM). Animals from the diabetic group received an intravenous injection of alloxan monohydrate (100 mg/Kg) for the induction of diabetes (20), while the control group received an intravenous injection of the same volume of saline.

Blood samples were drawn from the marginal ear vein from all animals for glucose evaluation after a fasting period of 10 hours, and at 24, 48, and 72 hours post-diabetes induction and weekly thereafter until the end of the experiment. The measurement of serum glucose levels was made by using the One Touch Ultra Glucometer (Johnson & Johnson Company, Rio de Janeiro, Brazil). Rabbits with serum glucose levels 126 mg/dL or above were considered diabetic. Ten weeks after the induction of diabetes, all animals were euthanized by an intravenous injection of high dose sodium thiopental.

Histological procedures

The penises were histomorphometrically assessed in both the control and diabetic groups. The area of the CC sinuses, thickness and area of the tunica media, and density of the smooth muscle cells of the DAP were measured and recorded. The tunica adventitia and the tunica intima of the DAP were qualitatively evaluated. Two fragments of the penile shaft were collected and fixed; one was placed in Bouin's fluid and another in 10% buffered formalin (pH 7.3). All samples were routinely processed and embedded in paraffin. Sections (5µm) were cut for use in immunohistochemistry and histological staining.

Hematoxylin and eosin stain and Masson's trichrome stain were used for preliminary evaluation of the histological preparations. Picrosirius red stain, with a polarizing kit adapted to the microscope, was used to assess collagen birefringence in the DAP. The periodic acid-Schiff (PAS) technique was used to evaluate the endothelium (21).

Immunohistochemistry

The immunostaining was used to estimate the smooth cell density and area of the DAP and CC sinuses. The avidin-biotin-peroxidase method was used to identify smooth muscle cells. Briefly, the sections were dewaxed in xylene, hydrated in a decreasing series of ethanol into water, and washed in phosphate buffered saline (PBS) for 5 minutes. Sections were then treated at room temperature with a 3% hydrogen peroxide solution in methanol to block endogenous peroxidase activity. Sections were then washed in PBS (3 x 5 minutes) and incubated with 1% goat serum in a moist chamber for 30 minutes at 37° C. Sections were then incubated with anti-alpha actin antibody (1:400, A-2547, Sigma-Aldrich Co, St Louis, MO, USA) in a moist chamber for 12 to 14 hours at 4° C. Negative controls were incubated with PBS instead of the primary antibody. Samples of a well-known tissue, with the antigen, were used for the positive controls, as previously described (4). Finally, the sections were washed in PBS (3 x 5 minutes) and incubated with the biotinylated secondary antibody (Sigma-Aldrich Co, St Louis, MO, USA) at 1:100 in a moist chamber for 30 minutes at room temperature, washed and incubated with the ABC complex (extravidin 1:100) for 30 minutes. The sections were then washed treated with a 393-diaminobenzidine tetrahydrochloride solution (Sigma-Aldrich Co, St Louis, MO, USA). The negative control was done by replacing the anti-smooth muscle α -actin antibody with PBS and no sign of staining was observed.

Histomorphometry

Selected images used for measurements and quantification were obtained using a light microscope (Olympus BX-41 coupled to a Sony CCD video camera), at magnifications of 4, 40, and 100 x. Three sections from each animal were analyzed

at different histological fields. ImageJ 1.44p (National Institute of Health, Bethesda, MD, USA) was used to quantify the area DAP wall. The cell_counter.jar plug-in was used to count the smooth muscle cells of the tunica media of DAP and of the trabeculae of the CC.

The percentual of area occupied by cavernous sinuses was obtained by the difference between the CC area and the cavernous sinus area (mm²). From these values we calculated the individual percentage of area occupied by the cavernous sinus, and then calculated the mean values for each group, in order to compare them.

Statistics

All results were expressed as mean \pm standard deviation (SD). Statistical analysis was performed using the Graphpad Instat software version 3.01 for Windows XP, (GraphPad Software Inc, San Diego, CA, USA). Student's t-test was used to test differences between the measurements from the control and diabetic groups, a value of $p < 0.05$ was considered statistically significant.

RESULTS

Serum glucose

After 72 hours from the intravenous injection of alloxan, serum glucose levels in rabbits reached 150 mg/dL, and rabbits were then considered diabetic. In subsequent samples, the serum glucose levels increased in the diabetes-induced animals. In contrast, the mean serum glucose concentration in the control group remained at 79 mg/dL throughout the experiment. At euthanasia, 10 weeks after the achievement of diabetes, the serum glucose levels of the diabetic group was approximately 350 mg/dL. Therefore, rabbits in the diabetic group remained in a hyperglycemic state throughout the 10-week experimental period.

Morphological changes in the DAP

Nuclear protrusions toward the lumen and vacuolization of the cellular cytoplasm were observed in cells of the epithelium of the tunica intima in the DAP (Figures 1 and 2). The tunica media of the DAP was observed at high magnification to assess thickness and area (Figures 3

Figure 1 - Tunica intima of the DAP of a control rabbit. Sections were stained with PAS stain, and images were taken at 1000x magnification. Scale bar: 10µm.

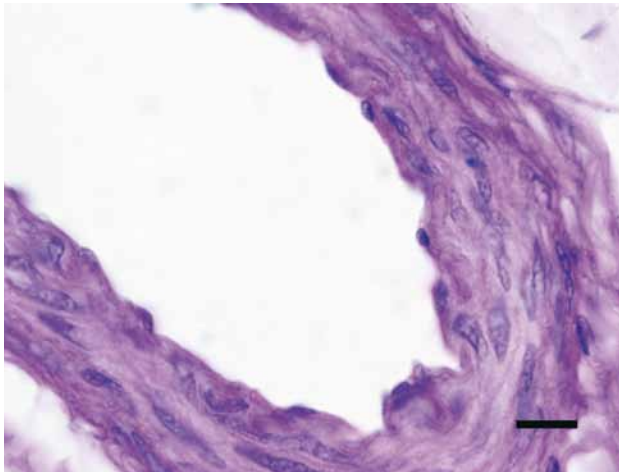


Figure 2 - Tunica intima of the DAP of a diabetic rabbit, showing cytoplasmic vacuolization (arrows) and nuclear protrusions (arrow heads) in the endothelium. Sections were stained with PAS stain, and images were taken at 1000x magnification. Scale bar: 10µm.

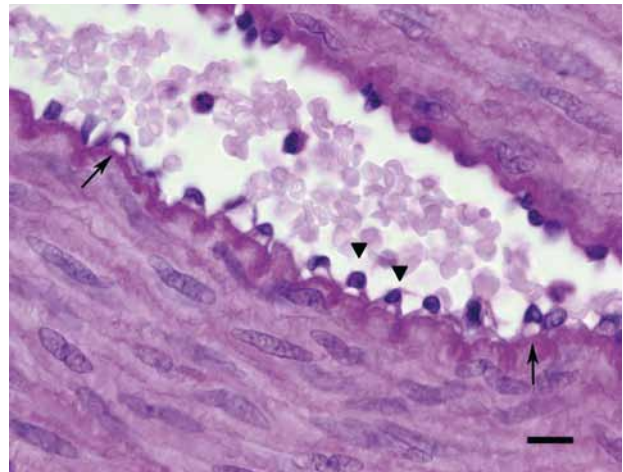


Figure 3 - Immunostaining of the smooth muscle in the tunica media of the DAP of rabbits from the control group. Sections were stained with the anti-alpha-actin antibody, and images were taken at 400x magnification. Scale bar: 50µm.

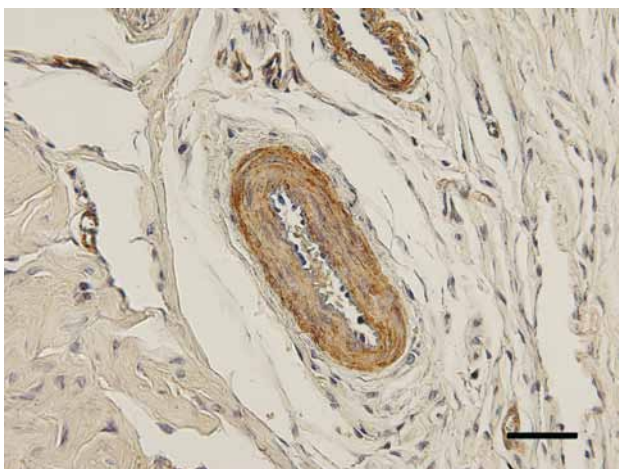
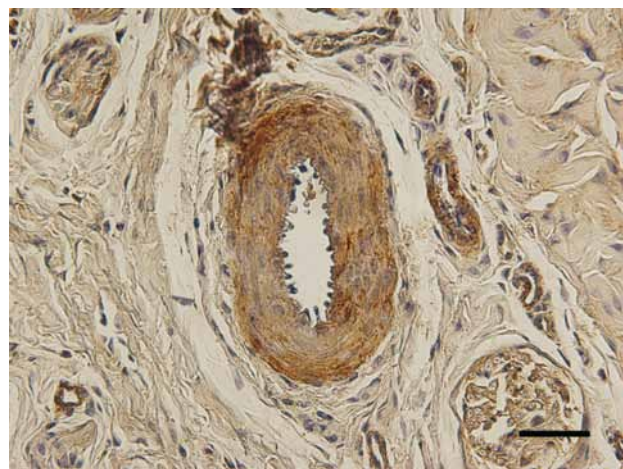


Figure 4 - Immunostaining of the smooth muscle in the tunica media of the DAP of rabbits from the diabetic group. Sections were stained with the anti-alpha-actin antibody, and images were taken at 400x magnification. Scale bar: 50µm.



and 4). There were significant changes in the thickness and area of DPA wall (Table-1). The values of DPA wall thickness were $35.012 \pm 3.177\mu\text{m}$ in CG and $44.330 \pm 8.434\mu\text{m}$ in DG ($P = 0.0350$). The mean DPA wall area was $12070.675 \pm 2938.2\mu\text{m}^2$ and $18221.298 \pm 6861.9\mu\text{m}^2$ in control and diabetic groups, respectively ($P = 0.0179$).

There was no significant change in smooth muscle cell density in the tunica media of the

DAP between the groups. The ratio of nucleus per unit area was 0.007154 ± 0.001954 nuclei/ μm^2 in CG and 0.004808 ± 0.002069 nuclei/ μm^2 in the DG ($P = 0.0855$).

The collagen fibers of the tunica adventitia of the DAP were different between the control and diabetic groups. An orange birefringence and increased fiber thickness was observed in the control group. In the diabetic group, the birefrin-

Table 1 - Morphological measurements of the DAP tunica media in control and diabetic rabbits. Data are presented as mean \pm SD.

Parameter	Control group	Diabetic group	P
Thickness	35.012 \pm 3.177 μ m	44.330 \pm 8.434 μ m*	0.0350
Area	12070.675 \pm 2938.2 μ m ²	18221.298 \pm 6861.9 μ m ² *	0.0179
Nuclear density	0.007154 \pm 0.001954 nuclei/ μ m ²	0.004808 \pm 0.002069 nuclei/ μ m ²	0.0855

* Statistically significant (p value < 0.05).

gence was greenish and the fibers were thinner (Figures 5 and 6).

Morphometric analysis of CC sinuses

There was a significant decrease in the mean area of the CC sinuses in the diabetic group compared to the control group (Figures 7 and 8). The values of the mean area in CG and DG were 60.76 \pm 7.883 % and 37.93 \pm 9.986 %, respectively (P = 0.0013).

However, there was no significant difference in the cellular density in the CC sinuses between the control and the diabetic groups.

DISCUSSION

The smooth muscle of both the CC and arterial wall of the DAP play an important role in penile erection (1). DM is associated with micro- and macrovascular diseases, which cause several morphological changes in the vascular wall (22). Wang et al. (23) demonstrated a high prevalence (> 75%) of penile arterial insufficiency in diabetic men with erectile dysfunction, using duplex ultrasound after intracavernous injection of prostaglandin E1. Hyperglycemia in diabetic New Zealand rabbits has been shown to be associated with a reduction in the number of smooth muscle cells and increased cellular density when compared to normal rabbits, suggesting that hyperglycemia can lead to permanent changes in the dynamics of smooth muscle cell proliferation (24).

Diabetes can cause arterial wall thickening, as reported in the mesenteric artery, renal artery, and aorta (11,12,25). However, this has not been previously described in penile arteries. The mechanism of arterial wall thickening associated

Figure 5 - Collagen arrangement in the tunica adventitia of the DAP of rabbits from the control group. Sections were stained with Picro Sirius red stain, and images were taken using polarized light at 400x magnification. Scale bar: 50 μ m.

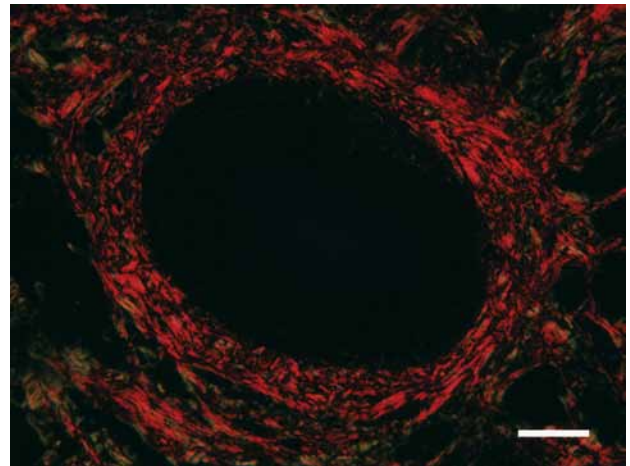


Figure 6 - Collagen arrangement in the tunica adventitia of the DAP of rabbits from the diabetic group. Sections were stained with Picro Sirius red stain, and images were taken using polarized light at 400x magnification. Scale bar: 50 μ m.

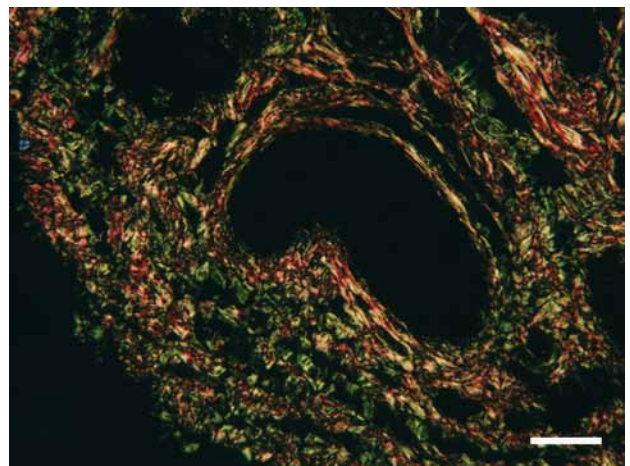


Figure 7 - Area of the CC sinuses of rabbits from the control group. Binarized image taken at 40x magnification. Scale bar: 200µm.

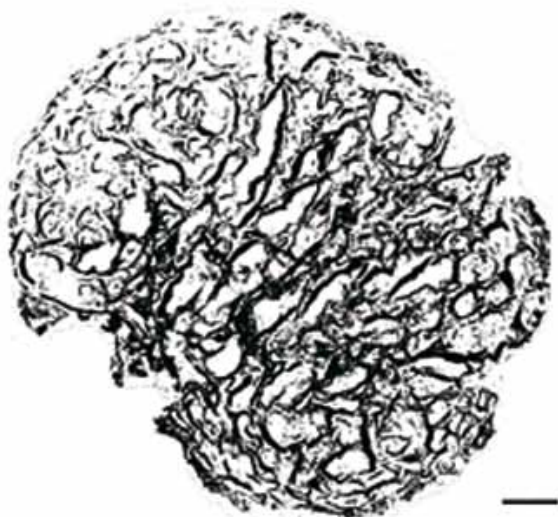


Figure 8 - Area of the corpus cavernosum sinuses of rabbits from the diabetic group. Binarized image taken at 40x magnification. Scale bar: 200µm.



with DM is not clear, because smooth muscle cells of the tunica media may increase in size as a result of hyperplasia or hypertrophy. Hyperplasia of the neointima associated with DM has been shown to result from smooth muscle cell proliferation in the tunica media and subsequent migration to the tunica intima, leading to vascular stenosis (26). This migration process did not occur in the current study, and there was no disruption of the internal elastic layer. However, we demonstrated increased smooth muscle area and thickening of the DAP wall in diabetic rabbits, but with no change in the cellular density, suggesting hypertrophy of the smooth muscle cells in the tunica media. This finding agrees with Vranes et al. (11), who reported hypertrophy of the smooth muscle cells in the tunica media of thickened mesenteric arteries in diabetic rats. The relationship between DM and smooth muscle hypertrophy has also been demonstrated in the urinary bladder (27). Hypertrophic remodeling in the DAP tunica media can increase vascular resistance and impair the myogenic response, which is a key component of autoregulation of blood flow and stabilization of capillary pressure (22).

In this study, vascular remodeling associated with DM reached the tunica adventitia, chan-

ging the pattern of collagen fibers and reducing their thickness from that seen in the control group (orange birefringence), to thinner fibers (greenish birefringence). Changes in the pattern of collagen fibers have been previously demonstrated in the mesenteric artery of streptozotocin-induced diabetic rats (11). Rearrangement of collagen fibers in the extracellular matrix, in both the wall of the DAP and periarterial region, suggests that it occurred simultaneously to vascular hypertrophy associated with DM. In this study, we also found nuclear protrusions toward the lumen and endothelial cytoplasmic vacuolization in the tunica intima. Hadcock et al. (25) have also reported cytoplasmic vacuolization in endothelial cells in the aorta of alloxan-induced diabetic rabbits. In cells from the umbilical cord, this vacuolization has been shown to be correlated with an increase in mitochondrial area (28). Mitochondrial and nuclear changes are indicators of the reversible injury mechanism, vacuolar degeneration (29). Chemically induced hyperglycemia may also cause oxidative stress leading to cellular vacuolization (25).

An increase in glucose promotes a small proliferative effect in the smooth muscle cells of coronary arteries. However, chronic hyperglycemia intensifies the response to growth factors

such as platelet derived growth factor (PDGF) and transforming growth factor- β 1 (TGF- β 1) (30). The hypertrophy associated with DM in smooth muscle cells seen in the wall of the mesenteric artery could be associated with Na/H equilibrium (31). The Na/H channels of smooth muscle cells are sensitive to intracellular pH reductions that occur due to the ketoacidosis DM-related (29), which leads to higher activation of the Na/H exchanger. This Na/H exchanger activation may be a response to higher amounts of glucose or to increased concentrations of the growth factors associated with DM, which can lead to hypertrophy (12).

The causes of vascular complications associated with DM are multifactorial, but glyco-oxidative stress has been shown to be a key factor among the several DM disorders (22). Advanced glycation endproducts (AGEs) play an important role in the decrease of vascular distensibility. The biochemical production of AGEs has been shown to be increased in DM, because of the chronic oxidative stress caused by hyperglycemia (32). In the current study, vascular impairment observed in the DAP associated with DM was not limited to the arterial wall. There was a decrease of 36% in the area of the CC sinuses, compared to control rabbits. This decrease in the sinus area can be explained by the increasing density of smooth muscle in the CC septa, thus enlarging the septal area (3).

In conclusion, experimentally induced DM by alloxan injection in rabbits resulting in 10 weeks of hyperglycemia causes important changes in the vascular structures of the penis, promoting alterations in the tunica intima, media, and adventitia, as well as significant decreases in the area of the CC sinuses. These changes decrease the blood supply and affect the hemodynamics of the penis, thus leading to ED.

ABBREVIATIONS

AGE: Advanced glycosilation end-product
CC: corpus cavernosum
CS: corpus spongiosum
DAP: dorsal artery of the penis
DM: diabetes mellitus
ED: erectile dysfunction
PBS: phosphate buffered saline

PDGF: platelet-derived growth factor
TGF- β : Transforming growth factor β

CONFLICT OF INTEREST

None declared.

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Correspondence address:

Prof. Waldemar Silva Costa
 Av. 28 de Setembro, 87, fundos
 Rio de Janeiro, RJ, 20562-030, Brazil
 Tel.: + 55 21 2868-8399
 Fax: + 55 21 2868-8399
 E-mail: wscosta@gmail.com



Leiomyoma of the urinary bladder: a case report

Margarita Ortiz, Daniel E. Henao, Walter Cardona Maya, Maurizio Massaro Ceballos

Department of Radiology (MO, MMC) and Reproduction Group (DEH, CM), University of Antioquia, Colombia

ABSTRACT

The case of a 71-year-old woman who presented with one year history of pelvic pain and occasional dysuria is reported. Computed tomography and Magnetic Resonance Imaging revealed a well defined intramural bladder mass. The histological findings of the surgical specimen confirmed a leiomyoma of the urinary bladder. The clinical presentation, imaging findings and management of this relatively rare benign tumor are discussed.

INTRODUCTION

Leiomyoma of the bladder is a rare benign tumor that occurs mainly in women between the fourth and fifth decades of life. Although no pathophysiological mechanisms have been described to explain the occurrence of this tumor, it might be related to an endocrine alteration. The most common clinical characteristics include urinary voiding symptoms (1).

We describe in this report the case of an elder woman who mainly presented with chronic pelvic pain. The mass detected within her bladder -by several imaging methods- was later histologically confirmed as leiomyoma.

CASE REPORT

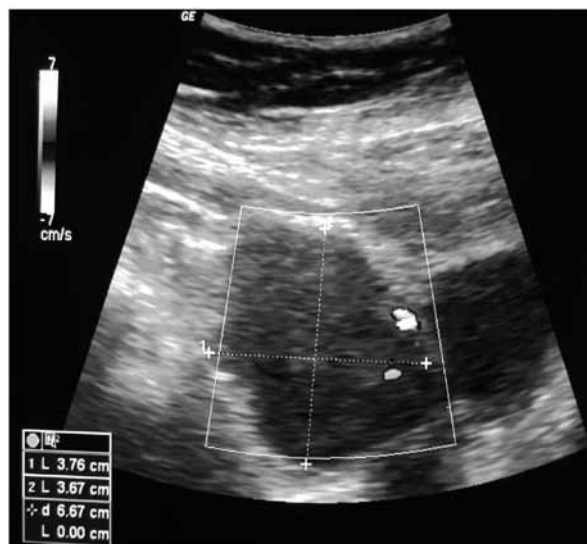
A 71-year-old female presented with a history of chronic pelvic pain radiating to the low back area associated with occasional dysuria. Poor symptoms relief after several antibiotic treatments for uri-

nary tract infections was documented. Initial abdominal ultrasound demonstrated normal kidney aspects and a mass of the bladder (Figure-1).

Subsequent CT of the pelvis confirmed a soft tissue mass at the right anterolateral aspect of the bladder's wall (Figure-2). MR images also demonstrated a mass in the same to location (Figure-3).

The patient underwent a laparoscopic partial cystectomy -after a biopsy of the mass acquired by cystoscopy reported a benign leiomyoma-obtaining a surgical specimen of 4 cm of diameter. Finally, histopathology confirms the diagnosis of leiomyoma (Figure-4).

Figure 1 - Abdominal Ultrasound.



Hypoechoic well-defined mass in the vesical wall measuring 3.67 cm and 3.76 cm in their transverse and antero-posterior diameters is shown. According to the Doppler it possesses no blood-flow.

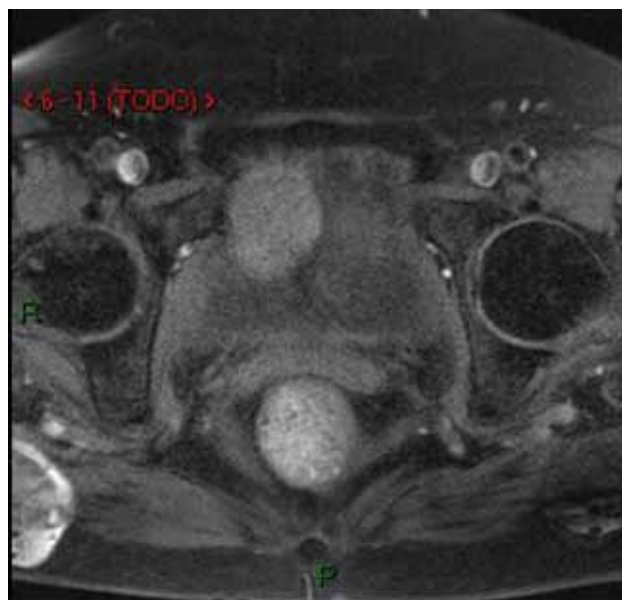
No recurrence was documented after a year follow-up. The patient experienced a relief of the pain and normal voiding function was retained after surgical excision.

Figure 2 - Computed Tomography (CT) of the pelvis.



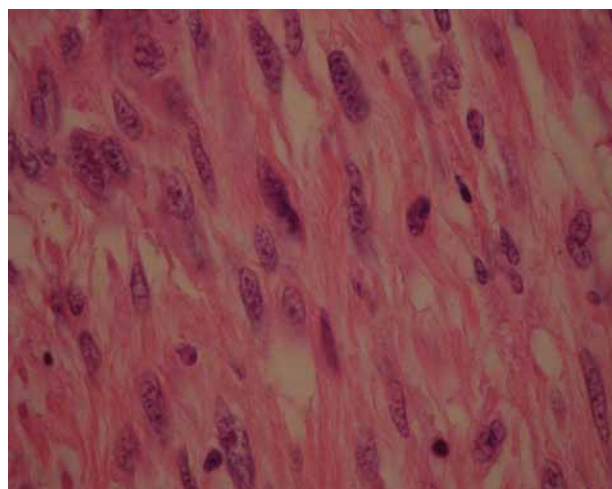
Coronal portal venous phase CT demonstrating a mass with soft tissue attenuation pattern on the right antero-lateral aspect of the bladder's wall is indicated. The mass possesses smooth boundaries with poor enhancement after contrast medium administration demonstrating a homogeneous lesion.

Figure 3 - Magnetic Resonance (MR) Imaging of the pelvis.



Axial fat-suppressed T1-weighted MR image pre-contrast revealed the mass previously described in the CT scan.

Figure 4 - Piece to specimen.



Histopathology of the removed specimen -composed of spindle-shaped cells without dysplasia, atypia or pleomorphism- confirms the diagnosis of leiomyoma.

DISCUSSION

Leiomyoma of the bladder is a relatively rare tumor: less than 1% of the tumors of this organ (1). In a systematic review of the literature carried out by Silva-Ramos et al. (2) the characteristics of patients affected by this tumor were: 75.6% were women, the media age of presentation was 45.3 years, 50% of patients presented urinary voiding symptoms.

Ultrasound, CT and MRI are regularly used to diagnose this tumor. It was once proposed that MRI by itself could confirm this diagnosis, nonetheless and considering it cannot differentiate mesenchymal tumors from the more common transitional cell tumors (TCT) the histopathology study is always necessary to confirm (3,4). Recent findings, nonetheless, indicate that the Apparent Diffusion Coefficient -obtained by diffusion-weighted magnetic resonance- values are significantly lower in malignant lesions (e.g. TCT) when compared to those obtained from benign tumors (i.e. mesenchymal tumors). The specificity claimed by this technique certainly deserves further empirical results and analysis.

The underlying cause of leiomyoma of the bladder is still unclear. No recurrences or ma-

lignat degenerations of leiomyomas have been reported. Treatment of leiomyoma of the urinary bladder is mainly surgical. Surgical excision has excellent prognosis after complete resection and should always be offered.

We reported the case of a leiomyoma of the bladder, successfully removed by laparoscopy without compromising the bladder capacity.

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Correspondence address:

Dr. Margarita Ortiz
Carrera 51D No. 62
Medellín, 29, Colombia
Fax: + 57 4 219-6476
E-mail: margaritamariaortizm@gmail.com



Unilateral renal cystic disease in the right kidney

Eun Hui Bae, Young-Hwan Hwang, Soo Wan Kim

*Departments of Internal Medicine, Chonnam National University Medical School, Gwangju, and
Department of Internal Medicine, Eulji General Hospital, Seoul, Korea*

A 51-year-old man was referred from oncology department with incidental detection of unilateral multiple renal cysts on computed tomography (CT) during evaluation of colon cancer (Figure-1). Abdominal CT revealed enlarged right kidney with variable-sized round, well-marginated multiple cysts without capsule formation or solid content, while the left kidney was normal. Family history was negative for kidney disease. Ultrasound imaging of the parents, five adult siblings, one son and two daughters showed normal kidneys. He received chemotherapy with 5-fluorouracil plus leucovorin after laparoscopic right hemicolectomy for colon cancer. The

relevant laboratory data were as follows: white blood cell count, 7000/mm³; hemoglobin level, 15.9 g/dL; platelet count, 174,000/mm³; blood urea nitrogen level, 15.5 mg/dL; creatinine level, 0.8 mg/dL and no proteinuria or hematuria. We did not introduce specific therapy for the unilateral cyst and follow-up was made with magnetic resonance image (MRI) six month after hemicolectomy. The exam showed numerous cysts in the right kidney with no changes according to previous CT (Figure-2).

This patient has unilateral renal cystic disease (URCD), a rare entity characterized by multiple cysts in one kidney or a portion of one

Figure 1 - Preoperative axial (A) and coronal scan (B, C) of abdominal CT showing multiple cysts in the right kidney.

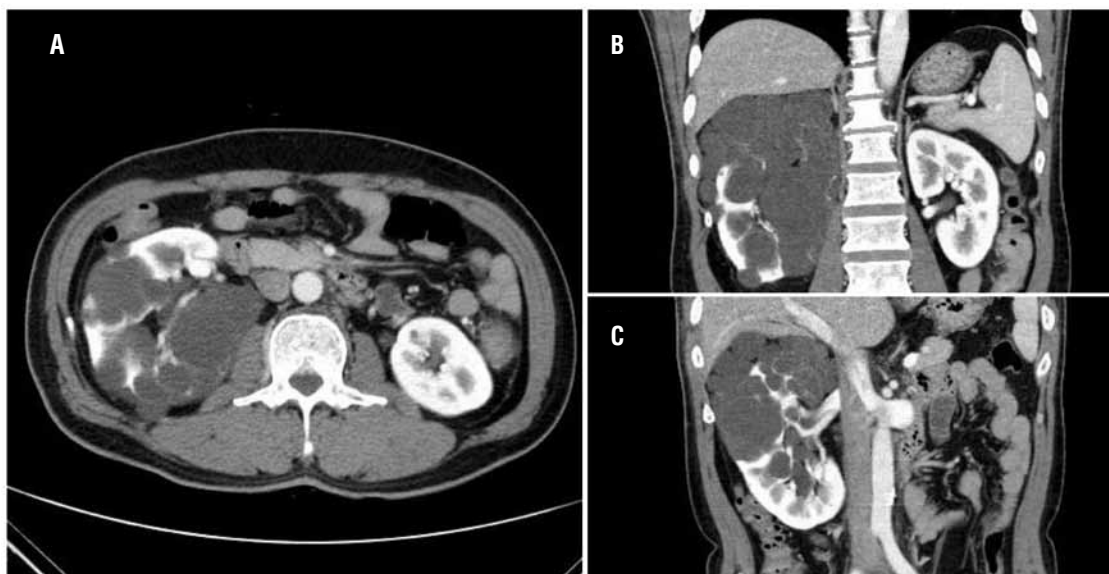
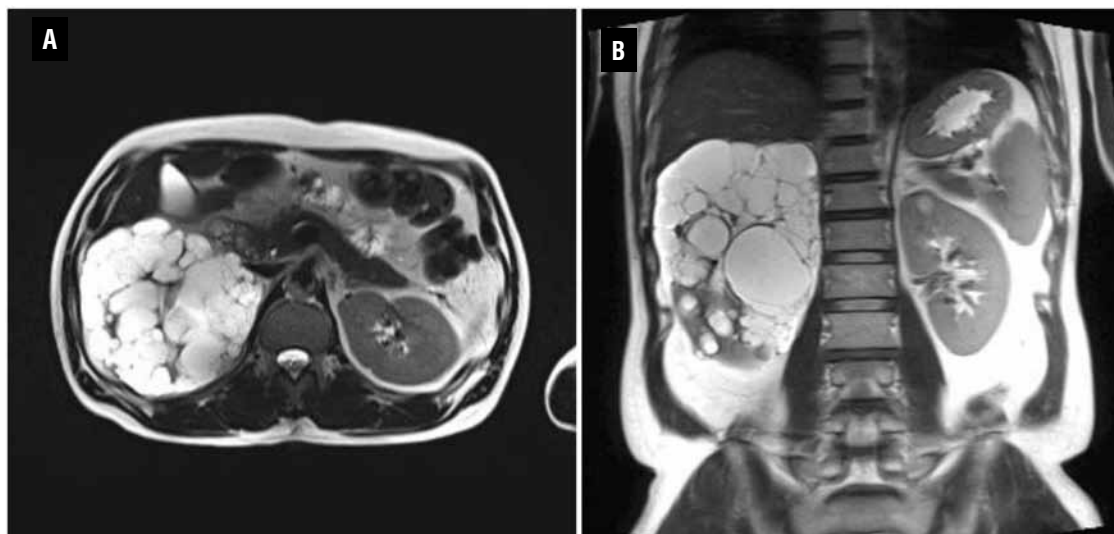


Figure 2 - Postoperative axial (A) and coronal scan (B) of the kidney MRI (T2 weighted image) showing multiple cysts in the right kidney.



kidney. The clinical importance of URCD is to make a differential diagnosis of such abnormalities including multilocular cystic nephroma, cystic partially differentiated Wilms' tumor, segmental cystic dysplasia, and atypical presentation of polycystic kidney disease such as asymmetric evolution and mosaicism (1,2). The pathogenesis of this cystic renal disease is unknown. Since there is a morphological similarity of this cystic change to autosomal dominant polycystic kidney disease (ADPKD), it is speculated that pathogenesis is similar (3). The gross and microscopic features are indistinguishable from ADPKD, and patients may present with hematuria, pain, or a flank mass (4). However, it can be differentiated from ADPKD by its unilateral localization, negative family history, no progression to chronic renal insufficiency, and no extrarenal manifestation. Cystic adenocarcinoma of the kidney can present in a very similar way and it can be differentiated by positive reaction to epithelial membrane antigen (EMA), vimentin and transducin-like enhancer protein 1 (TLE1), and CD99 (5). Most importantly, it must be differentiated against segmental cystic dysplastic disease. In case of URCD, collecting sys-

tem should be shown continuously though distorted collecting system. Otherwise it would lean to segmental cystic dysplastic disease (6,7). The collecting system of this patient was continuous (Figure-1C).

URCD is a stable disease and patients can be followed up by imaging techniques (8). In conclusion, unilateral cystic disease of kidney is a rare cystic disease of the kidney diagnosed by imaging techniques and requires nephrectomy only when suspicion of malignancy is strong.

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Correspondence address:

Dr. Soo Wan Kim
Department of Internal Medicine,
Chonnam National University Medical School
Hakdong 8, Dongku, Gwangju 501-757, Korea
Fax : + 82 62 225-8578
E-mail: skimw@chonnam.ac.kr



Laparoscopic approach to ureteropelvic junction obstruction in a bifid pelvis

Lessandro Curcio, Antonio Claudio Ahouagi, Juan Renteria, Igor Rui Araujo, Daniel Presto

Ipanema General Hospital, Rio de Janeiro, Brazil

Objective: About 10% of renal pelvis are bifids and not so there is a larger index of kidney disease over the normal pelves. The laparoscopy and minimally invasive techniques treat the ureteropelvic junction disease in a low aggressive manner. We showed a video of an atypical pyeloplasty of ureteropelvic junction obstruction of a lower unit. The patient is a 33 year-old woman with an intermittent lumbar pain for 3 years. Your image exams showed a bifid left pelvis with a stenosis of the lower unit. We chose to do the fix of this pathology laparoscopically.

Materials and Methods: We positioned the patient in a right lateral decubitus and 3 trocars was placed, we identify the obstructed junction and a terminolateral anastomosis was performed. Results: The procedure lasted 95 minutes, with little blood loss and the patient was discharged in 2 days. We withdraw the double J catheter after 1 month, a pyelography and a ureteroscopy was performed which showed a pervious anastomosis. After 2 months of follow-up the patient is doing well.

Conclusions: As far as we know, this is the first case of literature with correction by laparoscopy. The stenosis of ureteropelvic junction in the lower unit of a bifid pelvis can be corrected effectively by laparoscopic surgery.

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Correspondence address:

Dr. Lessandro Curcio Gonçalves
Av das Américas 13554- bl 02 / 805
Recreio dos Bandeirantes
Rio de Janeiro, RJ, 22790-702, Brazil
E-mail: lessandrocg@ig.com.br

EDITORIAL COMMENT

The video by Curcio et al. depicts a minimally invasive laparoscopic technique which may be utilized in patients with a bifid renal pelvis and obstruction.

Obstruction of the lower segment in a duplex kidney is not common (1).

Side-to-side or end-to-side pyeloureterotomy has been the usual open surgical solution, with the upper segment ureter lying close to the lower

segment pelvis serving as an alternative route of urinary drainage of the obstructed lower pole.

Minimally invasive approaches have evolved over the last several decades. Smith and Badlani first reported use of percutaneous endopyelotomy in these cases in 1985 (2). Fifteen years later Bruno et al. reported retrograde ureteroscopic holmium laser incision of the stenotic UPJ segment (3). As technology and surgeon experience improved, these types of delicate renal pelvic reconstructions that replicate traditional open surgeries have become possible (4).

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Dr. Hubert Swana

Pediatric Urology

Nemours Children's Hospital Orlando

Orlando, FL, USA

E-mail: hswana@nemours.org



Microdissection Testicular Sperm Extraction (micro-TESE) as a Sperm Acquisition Method for Men with Nonobstructive Azoospermia Seeking Fertility: Operative and Laboratory Aspects

Sandro C. Esteves

ANDROFERT, Center for Male Reproduction

Introduction: Rare foci of sperm production may be found in up to 60% of men with nonobstructive azoospermia (NOA). Sperm production, if present, is minimal for sperm appearance in the ejaculate. Given that there are no treatment options to restore fertility, sperm retrieval is the only alternative to find testicular sperm than then can be used for in vitro fertilization (IVF). Among sperm acquisition methods, micro-TESE has higher success rates at obtaining sperm compared with testicular sperm extraction and testicular sperm aspiration.

Materials and Methods: This video describes the operative aspects of micro-TESE, performed on an outpatient basis, in a man with NOA and history of cryptorchidism in whom orchidopexy was performed at age 6. The concept of micro-TESE is to identify areas of sperm production within the testes with the aid of optical magnification (15-25X) and based on the size and appearance of the seminiferous tubules (ST).

Results: Intraoperative findings revealed homogeneous pattern of collapsed STs in which an area containing dilated STs was clearly identified and extracted. Testicular tissue was then processed in the IVF laboratory to allow sperm search. In this case, micro-TESE was successful at obtaining testicular sperm for intracytoplasmic sperm injection (ICSI). Surplus retrieved testicular spermatozoa not used for ICSI was cryopreserved. The operative time was 120 minutes, and intraoperative blood loss was negligible. Postoperatively, the patient recovered to his normal activities within 5 days and no complications were recorded except for minor testicular pain and scrotal swelling.

Conclusion: Micro-TESE allowed the identification and extraction of sperm-containing STs with minimum tissue excision and marked reduction in time processing of testicular specimens for sperm injection.

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Correspondence address:

Dr. Sandro C. Esteves
ANDROFERT, Center for Male Reproduction
13075-460 - Avenida Dr. Heitor Penteado, 1464
Campinas, SP, Brazil
Phone: +55 19 3295-8877
Fax: +55 19 3294-6992
Email: s.esteves@androfert.com.br

EDITORIAL COMMENT

The authors from Sao Paulo, Brazil elegantly demonstrate the operative aspects of micro-Testicular Sperm Extraction (TESE), performed on a man with nonobstructive azoospermia (NOA). In men with NOA, retrieval of spermatozoa offers an opportunity for fertility despite limited sperm production. This is performed with the aid of optical magnification (25X) and is processed in an in-vitro fertilization laboratory. Micro-TESE is successful at obtaining testicular sperm for intracytoplasmic sperm injection (ICSI). This video

nicely portrays the surgical approach of micro-TESE and the importance of in-vitro fertilization in patients with NOA. While performing microdissection, one can identify seminiferous tubules with sperm and those with Sertoli cells only, which appear atretic. The difference between the larger and smaller tubules is not visible without optical magnification. The use of magnification and processing of the spermatozoa was accurately described by the authors. Since micro-TESE was described, microdissection has shown an improvement of sperm retrieval rate and decrease the amount of tissue excised (1).

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Dr. Rafael E. Carrion
University of South Florida
Program Chair
Department of Urology
Division of Andrology
Tampa, FL, 33602, USA
E-mail: rcarrion@health.usf.edu



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