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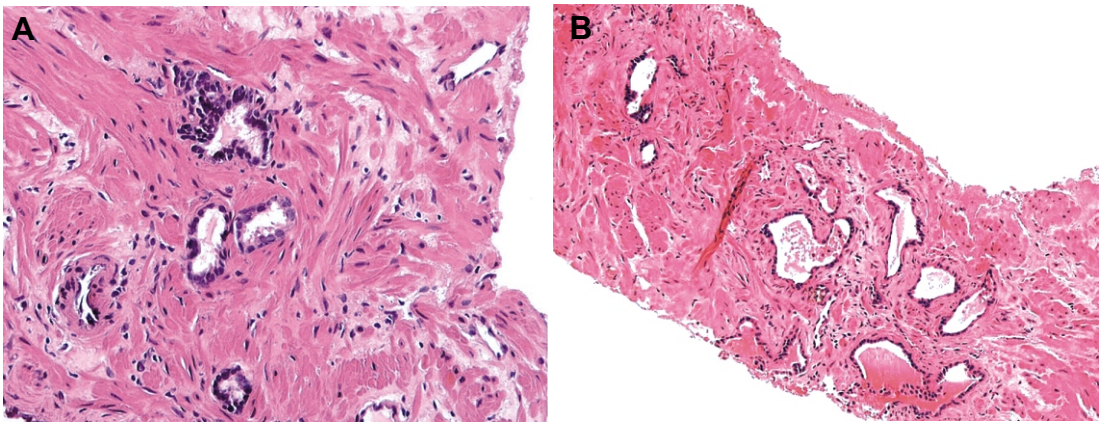

Simple prostatic atrophy. A) Small acini with crowded nuclei, scant cytoplasm and low epithelial height. B) Atrophy with cystic dilatation.(Page 401)

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EDITOR'S COMMENT

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Prostatic Atrophy

The July - August 2010 issue of the International Braz J Urol presents original contributions and editorials from many different countries, such as USA, Germany, France, Brazil, Italy, Taiwan, England, India, Portugal, Venezuela, etc., and as usual, the editor's comment highlights some papers.

Doctor Billis, from Department Pathology, University of Campinas, Sao Paulo, Brazil, presented on page 401 an interesting review article on Prostatic Atrophy, which is a benign lesion that may mimic adenocarcinoma histologically and on imaging. It is more frequent in the peripheral zone and has gained importance with the increasing use of needle biopsies. Diffuse atrophy occurs secondarily to radiotherapy and/or endocrine therapy. Inflammation and/or chronic local ischemia may cause focal atrophy with an increasing frequency in age. Atrophy may be classified morphologically into diffuse and focal. Chronic inflammation associated to focal atrophy (proliferative inflammatory atrophy) has been linked to high-grade prostatic intraepithelial neoplasia and/or carcinoma. This link, however, remains controversial in the literature. The question whether inflammation directly produces tissue damage and atrophy or some other insult induces atrophy directly, with inflammation occurring secondarily, is still unresolved. An intriguing finding that needs further studies is a possible association of extent of atrophy to serum PSA elevation.

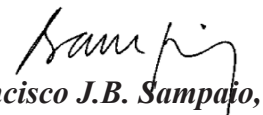
Doctor Goo and colleagues, from University of Washington, Seattle, USA, performed on page 464 a study to profile the urinary proteome of women with IC/PBS to identify possible specific proteins and networks associated with interstitial cystitis/painful bladder syndrome (IC/PBS). Urine samples from 10 female IC/PBS patients and 10 controls were analyzed in quadruplicate by liquid chromatography-tandem mass spectrometry on a hybrid linear ion trap-orbitrap mass spectrometer. Alpha-1B-glycoprotein and orosomucoid-1 were detected in all IC/PBS patients, and $\geq 60\%$ of these patients had elevated expression of these two proteins compared to control subjects. The authors concluded that there are qualitative and quantitative differences between the urinary proteomes of women with and without IC/PBS. They also identified a number of proteins as well as pathways/networks that might contribute to the pathology of IC/PBS or result from perturbations induced by this condition. Dr. Tseng, from Chang-Gung University College of Medicine, Tao-Yuan, Taiwan and Dr. Amaro, from Botucatu School of Medicine, Sao Paulo, Brazil, provided editorials on this paper.

Doctor Botelho and collaborators, from University of Porto Medical School, Portugal, compared on page 430 the serum vascular endothelial growth factor (VEGF) circulating levels across

different prostate pathologies (including benign prostatic hyperplasia, prostatitis, high grade prostate intraepithelial neoplasia and prostate cancer) in patients at high risk of prostate cancer. It was consecutively enrolled 186 subjects with abnormal digital rectal examination and/or total PSA $\geq$ 2.5 ng/mL. The prostate biopsy main diagnoses were normal or benign prostatic hyperplasia (27.3%), prostatitis (16.6%), and prostatic cancer (55.0%). The median VEGF levels (ng/mL) in these groups were 178.2, 261.3 and 266.4 ($p = 0.029$), respectively, but no significant differences were observed for benign vs. malignant pathologies The authors concluded that in patients at high risk of prostate cancer, circulating VEGF levels have no clinical role in deciding which patients should be submitted to prostate biopsy. Prostatitis patients, often with higher PSA levels, also present high serum levels of VEGF, and their inclusion in control groups might explain the heterogeneous results in previous studies. Dr. Katia Leite, from University of Sao Paulo, Brazil provided an editorial on this paper.

Doctor Aggarwal and collaborators, from Jaypee University of Information Technology, Solan, India, evaluated on page 480 the antilithiatic properties of Tribulus terrestris by investigating nucleation and the growth of the calcium oxalate (CaOx) crystals as well as oxalate induced cell injury of NRK 52E renal epithelial cells. The authors found that Tribulus terrestris extract exhibited a concentration dependent inhibition of nucleation and the growth of CaOx crystals. When NRK-52E cells were injured by exposure to oxalate for 72 h, Tribulus terrestris extract prevented the injury in a dose-dependent manner. The current data suggests that Tribulus terrestris extract not only has a potential to inhibit nucleation and the growth of the CaOx crystals but also has a cytoprotective role and therefore, it could be a potential candidate for phytotherapy against urolithiasis. Dr. Miyaoka, from University of Minnesota, Minneapolis, MN, USA, and Dr. Boim from Federal University of Sao Paulo, provided interesting editorials on this paper.

Doctor Regadas and collaborators, from Federal University of Ceara, Fortaleza, Brazil, described on page 490 a technique for en bloc harvesting of the corpus cavernosum, cavernous artery and urethra from transplant organ donors and contraction-relaxation experiments with corpus cavernosum smooth muscle. The harvesting technique and the smooth muscle contraction-relaxation model described in this study were shown to be useful instruments in the search for new drugs for the treatment of human erectile dysfunction. Drs. Iacono, Taglialatela and Ruffo, from University "Federico II", Naples, Italy, presented an important editorial on his study.



Francisco J.B. Sampaio, M.D.
Editor-in-Chief

Review Article

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Current Status of Natural Orifice Trans-endoscopic Surgery (NOTES) and Laparoendoscopic Single Site Surgery (LESS) in Urologic Surgery

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ABSTRACT

Laparoendoscopic single site surgery (LESS) and natural orifice transluminal endoscopic surgery (NOTES) represent novel approaches in urological surgery. To perform a review of the literature in order describe the current status of LESS and NOTES in Urology. References for this manuscript were obtained by performing a review of the available literature in PubMed from 01-01-02 to 15-05-09. Search terms included single port, single site, NOTES, LESS and single incision. A total of 412 manuscripts were initially identified. Out of these, 64 manuscripts were selected based in their urological content.
The manuscript features subheadings for experimental and clinical studies, as NOTES-LESS is a new surgical technique and its future evolution will probably rely in initial verified feasibility. A subheading for reviews presents information regarding common language and consensus for the techniques. The issue of complications published in clinical series and the future needs of NOTES-LESS, are also presented.

Key words: *laparoscopy; urology; robotic surgery; minimally invasive procedures; NOTES; LESS*
Int Braz J Urol. 2010; 36: 385-400

INTRODUCTION

Natural orifice transluminal endoscopic surgery (NOTES) involves the intentional penetration of hollow viscera with an endoscope in order to access the abdominal cavity and perform an intraabdominal operation (1). In 2002, Gettman et al. reported the first experience with NOTES, performing transvaginal nephrectomies in pigs (2). The initial clinical experience in NOTES was performed by Antony Kalloo in transgastric surgery in 2004 (3).

Closely related to NOTES, laparo-endoscopic single-site surgery (LESS) describes minimally access surgical procedures that are performed through a single incision/location (4). Rane et al. published the first true LESS experience in abstract form in 2007, performing a transumbilical laparoscopic nephrectomy (5).

Currently, the application of NOTES-LESS has been expanding in the clinical setting and several experiences have been reported. The preliminary experience with NOTES has confronted several ques-

tions, such as the safety entrance into a healthy hollow organ lumen and adequate closure methods (6). Single site surgery has likewise faced questions regarding the added difficulty from lack of triangulation of instruments. Nonetheless, Urology has significantly participated in the development of minimal access surgical techniques, and NOTES-LESS is steadily gaining momentum in our field.

RATIONALE FOR NOTES AND LESS

NOTES-LESS are attractive surgical approaches due to several potential benefits and advantages. With NOTES there are no abdominal incisions and, therefore, abdominal wound infections and incisional hernias could be potentially avoided. This could also translate into less pain and improved cosmesis. In NOTES, the translumenal access to the peritoneal cavity may have definite advantages in situations whereby the transcutaneous path into the peritoneal cavity is not optimal, as in obese patients (7). Of further interest, consistently reduced levels of TNF-alpha have been observed in experimental experiences with NOTES in animal models in the late postoperative period. This suggests an immunomodulatory effect of the NOTES not present in laparoscopy or laparotomy (8).

LESS likewise offers the potential advantages of a more rapid recovery, fewer adhesions, fewer opportunities for hernias, and less postoperative ileum. Esthetically, use of a single incision minimizes the visible evidence of surgical intervention. This is most notable when the natural scar of the umbilicus is used for the port site. Furthermore, the risk related to the use of ports has been reported in 0.003-0.3% for both vascular and visceral injuries (9).

THE CHALLENGE OF NOTES AND LESS

Both NOTES-LESS drastically limit the surgeon's ability to choose the site of entry for operative instruments. Therefore, the advantages NOTES-LESS are gained with the caveat of difficult surgical performance due to the lack of space.

NOTES present the additional challenges of access to the peritoneal cavity through a hollow viscus. The safety of this strategy is of utmost importance, and studies are needed to evaluate the long-term consequences of the breach of a hollow viscus of the gastrointestinal or urinary tract. Flexible or small-bore instruments are required to utilize natural orifices, and this can present a challenge in maintaining orientation within the surgical space. Adequate transmission of force through flexible instruments for dissection and retraction may also present added complexity. Overcoming these limitations in NOTES and LESS require a great deal of laparoscopic experience and skill, and the application of novel instruments and techniques.

THE STEPWISE PROGRESS OF MINIMAL ACCESS SURGERY

The purpose of the laparoscopic approach has been clear: to provide improved tolerability and decreased morbidity while delivering the same or better clinical outcomes as open surgery (10). Laparoscopic surgery was not generated de novo; rather, it was developed by applying the techniques and standards developed in open surgery to the laparoscopic environment.

A similar evolutionary process has contributed to the progress of NOTES-LESS. Early laboratory research has been focused on feasibility. Animal and cadaveric models have been used to demonstrate the possible applications of NOTES and LESS, including transgastric peritoneoscopy, tubal ligation, gastrojejunostomy, partial hysterectomy, oophorectomy, and transcolonic exploration, liver biopsy and cholecystectomy (11). Investigation has also been brought to the operative suite - ablative and reconstructive procedures using NOTES-LESS have been reported by many surgical teams, utilizing a variety of techniques, devices and approaches.

NOTES-LESS IN UROLOGY

Laboratory (Table-1)

The initial experience in urologic minimal access surgery came in 2002, when Gettman et al.

performed transvaginal laparoscopic dissection and nephrectomy in 6 porcine models (2). They acknowledged the limitations imposed by both the porcine anatomy and available laparoscopic instruments at the time.

Lima et al. have presented several experiences in pig models were they have described transvesical access (12), and transvesical transdiaphragmatic endoscopic thoracoscopy (13). Lima et al. have also presented a combined approach in the experimental setting, in which they installed a transvesical tube into the peritoneal cavity under cystoscopic guidance; and a flexible gastroscope was passed orally, then into the peritoneal cavity via a gastrotomy. They performed 6 nephrectomies with instruments introduced by both approaches (14). Crouzet et al. described transgastric and transvaginal renal cryoablations in a porcine model, accomplished without complications (15). Several approaches have been described for NOTES-LESS nephrectomy, including transvaginal hybrid NOTES nephrectomy in a porcine model (16,17), pure NOTES transvaginal technique (18) and NOTES nephrectomy using magnetic anchoring and guidance system instrumentation (19).

Robotic assistance has been introduced to facilitate NOTES procedures using the da Vinci robotic interface. Box et al. performed robotic-assisted hybrid NOTES renal surgery, completing a right nephrectomy. They utilized transvaginal and transcolonic ports for the robotic arms, and a transumbilical port for the camera. While this approach avoided camera/instrument conflict, the authors noted significant instrument/instrument conflict due to inability to separate the ports by greater than 7.5 cm (20). Haber et al. reported on their experience with robot-assisted transvaginal hybrid NOTES, which enabled them to completed pyeloplasties, nephrectomies and partial nephrectomies in porcine models. They acknowledged success with both ablative and reconstructive procedures, noting that the wristed robotic instruments and 3D vision made intracorporeal suturing possible, and that the robotic platform allowed the surgeon to operate through ports that are too widely separated to allow a single surgeon to manipulate them simultaneously (21).

While the majority of laboratory research in NOTES-LESS has focused on NOTES applications, significant experimental experience has also been gained in LESS. A research group was formed to build a prototype system of magnetically anchored instruments for trocar-free laparoscopy. The system was then evaluated in vivo in a porcine laparoscopic nephrectomy model with promising results (22). A novel approach to the robot-assisted LESS radical prostatectomy was completed by Desai et al. in 2 human cadavers, utilizing a transvesical approach. Again, articulated robotic instruments facilitated the complex motions need for vesico-urethral anastomosis and bladder closure (23). An interesting work of radical prostatectomy performed transvesically has been presented in a cadaver model. The entire resection was performed with the laser and a rigid offset 27F nephroscope was used to perform the vesicourethral anastomosis using a laparoscopic suture device and knot pusher in an interrupted fashion (24).

Bridging Studies

Surgeons have taken their experimentally gained experience in LESS, and then applied it clinically, reporting both experiences simultaneously. Raman and colleagues presented single keyhole nephrectomy in a porcine model, followed by the same procedure in three human patients (25). Barret et al. described a robot-assisted LESS radical prostatectomy completed in a cadaveric model, and the technique was then transitioned to a human patient. The team used standard laparoscopic ports gathered at a single umbilical incision. Despite some instrument clashing that made exchanging robotic instruments difficult, they reported good results with an operative time of 150 minutes (26).

Clinical (Table-2)

Clinically, NOTES experience has been much more limited than LESS. Gettman et al. performed transvesical peritoneoscopy using a flexible ureteroscope in a patient who subsequently underwent standard robotic-assisted laparoscopic prostatectomy. The cystotomy was performed via a cystoscope, but with guidance from the previously

Table 1 *– Experimental data.*

Authors	Number/ procedures	Approach	Comments
Gettman et al. (2002) 2	6 Nephrectomies.	1 pure transvaginal and 5 combined transvaginal and transabdominal approaches.	First publication on NOTES and first pure transvaginal nephrectomy in porcine model.
Lima et al. (2006) 12	8 Transvesical peritoneoscopies.	Transvesical placement of peritoneal Endo-Eye™, and ureteroscope.	Porcine model, performed liver biopsy and falciform liver section.
Lima et al. (2007) 13	6 Transvesical thorascopies.	Transvesical entry of peritoneum, then into plural cavity with an ureteroscope.	Porcine model, lung biopsies performed without complications.
Lima et al. (2007) 14	6 Nephrectomies	Combined transvesical and transvaginal approach.	Porcine model, completed without complications.
Zeltser et al. (2007) 22	2 Nephrectomies.	15 mm Transumbilical trocar.	Prototype magnetic anchoring and guidance system camera in porcine model.
Clayman et al. (2007) 16	1 Nephrectomy.	Transvaginal single port NOTES nephrectomy.	Use of the multiport TransPort platform in porcine model.
Raman et al. (2007) 25	8 Nephrectomies.	Transumbilical 25mm single-port in 3 cases and 10mm + 2 5mm ports in 5 cases.	Experience from porcine models was used in human nephrectomies (see Clinical Data).
Box et al. (2008) 20	1 Nephrectomy.	Combined transvaginal and transcolonic, single port, robot-assisted nephrectomy.	First robotic NOTES experience in porcine model.
Haber et al. (2008) 21	10 Pyeloplasties; 10 Partial nephrectomies; 10 Nephrectomies.	R-NOTES combined transumbilical and transvaginal.	Robot allows suturing using both umbilical and vaginal access simultaneously in porcine model.
Isariyawongse et al. (2008) 17	1 Bilateral nephrectomy.	NOTES access, transgastric and transvaginal.	To prove the technical feasibility of NOTES nephrectomy with standard laparoscopic and endoscopic instruments in porcine model.

Table 1 *– continued.*

Crouzet et al. (2008)15	4 Renal cryoablations.	Transvaginal and transgastric approach.	Porcine model, no complications.
Desai et al. (2008)23	2 Radical prostatectomies.	Robotic transvesical approach.	Clashing da Vinci arms is worse with single-port than in a multi-port cadaveric procedure, but closure easier.
Barret et al. (2009) 26	1 Radical prostatectomy.	Robotic transumbilical extraperitoneal approach.	LESS radical prostatectomy experience in cadaver was transitioned in human radical prostatectomy procedure (see Clinical Data).
Haber et al. (2009) 18	5 Total nephrectomies.	Transvaginal approach with single and dual channel gastroscope.	Pure NOTES transvaginal nephrectomy was verified as feasible in the porcine model. Authors state the need for further development of instrumentation.
Humphreys et al. (2009) 24	4 NOTES radical prostatectomies.	Transvesical approach.	The entire resection was performed with the laser in a cadaver model. A rigid offset 27F nephroscope was used to perform the vesicourethral anastomosis using a laparoscopic suture device and knot pusher in an interrupted fashion.
Raman et al. (2009) 19	2 Nephrectomies.	Magnetic anchoring and guidance system (MAGS). 40-cm dual-lumen rigid access port inserted into the peritoneal cavity. A MAGS camera and cauterizer were deployed through the port and manipulated across the peritoneal surface by way of magnetic coupling via an external magnet. A prototype 70-cm articulating laparoscopic grasper introduced through the vaginal access port facilitated dissection after deployment of the MAGS instruments.	NOTES nephrectomy using MAGS instrumentation was verified feasible in the porcine model. Authors stated that the approach might improve shortcomings of previously reported NOTES nephrectomies regarding triangulation.

Table 2 – Clinical data.

Authors	Number/procedures	Approach	Comments
Milliken et al. (2006) 30	22 Insertions of peritoneal dialysis catheters.	Supraumbilical port.	Partial omentectomy and the catheter placement in the pelvis under vision.
Gettman et al. (2007) 27	1 Transvesical peritoneoscopy.	Endoscopic transurethro-transvesical.	First publication on the feasibility of transvesical peritoneoscopy.
Desai et al. (2008) 39	1 Nephrectomy; 1 Pyeloplasty.	Transumbilical single port.	The procedures were performed with the help of R-Port and curved at the shaft instruments.
Kaouk et al. (2008) 33	4 Renal cryotherapy procedures; 1 Kidney biopsy; 1 Radical nephrectomy; 4 Abdominal sacrocolpopexies.	Transumbilical or retroperitoneal.	Using the Uni-X Single Port Access Laparoscopic System, a single port, multichannel cannula with specially designed curved laparoscopic instrumentation.
Raman et al. (2007) 25	3 Radical nephrectomies.	Transumbilical.	The experimental experience on porcine models was transited into human procedures.
Kaouk et al. (2007) 34	3 Varicocelectomies.	Transumbilical.	First report on use of multicannula single laparoscopic port in children.
Kaouk et al. (2008) 52	4 Radical prostatectomies.	Transumbilical.	Use of multichannel port, urethrovesical anastomosis with a free-hand interrupted suturing and extracorporeal knot tying.
Ponsky et al. (2008) 35	1 Nephrectomy.	SAS transperitonealy.	Use of GelPort requires 7cm incision.
Desai et al. (2009) 41	4 Pyeloplasties; 1 Ileal ureter; 1 Ureteroneocystostomy.	Transumbilical.	The use of R-port allows to exteriorize the bowel for isolating the ileum segment and restoring the continuity.
Desai et al. (2008) 43	3 Simple prostatectomies.	Transvesical.	Report on initial experience with single port transvesical enucleation of the prostate.
Kaouk et al. (2008) 53	1 Radical prostatectomy; 1 Pyeloplasty; 1 Radical nephrectomy.	Transumbilical.	Robotic single port surgeries via multichannel port.
Branco et al. (2008) 29	1 Nephrectomy.	Transvaginal Hybrid NOTES.	Transumbilical camera, transvaginal instruments, with an additional subxyphoid trocar for a right nephrectomy.
Goel et al. (2008) 32	6 Renal cryoablations.	Single port access.	4 retroperitoneal through lumbar incision, 2 transperitoneal through umbilical site.

Table 2 – continued.

Rane et al. (2008) 31	2 Simple nephrectomies; 1 Orchiopexy; 1 Orchiectomy; 1 Ureterolithotomy.	Single port access.	Nephrectomies performed through port at mid-axillary line, other procedures through umbilicus. Use of R-Port described.
Castellucci et al. (2008) 37	1 Adrenalectomy.	Transperitoneal supraumbilical.	Single port adrenalectomy via triangular port arrangement.
Kaouk et al. (2008) 46	7 Partial nephrectomies.	Transumbilical.	5 cases of single-port laparoscopic and 2 cases of single-port robotic partial nephrectomies. Single port laparoscopic and single port robotic partial nephrectomy is feasible for select exophytic tumors. Robotics may improve surgical capabilities during single-port surgery.
Gill et al. (2008) 38	4 Live donor nephrectomy.	Transumbilical.	Initial experience with E-NOTES donor nephrectomy.
Aron et al. (2008) 39	5 Partial nephrectomies.	Single transumbilical port.	4 completed U-LESS; 1 required an additional port.
Barret et al. (2009) 26	1 Radical prostatectomy	Transumbilical extraperitoneal.	The experimental experience on cadaver model was transited robotic radical prostatectomy into human patient.
White et al. (2009) 36	5 Cryotherapy; 1 partial nephrectomy; 1 metastectomy; 1 renal cyst decortications.	Retroperitoneal single port.	Single port retroperitoneal surgery is feasible and offers comparable surgical outcomes and superior cosmesis and pain control compared with traditional retroperitoneoscopy.
Sotelo et al. (2009) 29	4 Transvaginal nephrectomies. 1 totally accomplished with the technique.	Multichannel access port in both the vagina and the umbilicus.	Transvaginal nephrectomy is feasible in the highly selected patient with favorable intraoperative circumstances, considerable refinements in technique and technology are necessary.
Sotelo et al. (2009) 42	1 Transumbilical simple prostatectomy; 20 Transvesical simple prostatectomies; 1 Simple nephrectomy (NS); 1 Enterocystoplasty augmentation; 5 Simple hysterectomies.	Multi-channel access.	LESS is a feasible and reproducible surgical option in urogynecologic surgical treatment.

established laparoscopic instruments. This experience represents the first published clinical experience in urologic NOTES (27).

Branco et al. published a report of a hybrid NOTES simple nephrectomy, in which an endoscope was placed through the posterior vaginal cul-de-sac, along with 5 mm ports at the umbilicus and below the xiphod. The specimen was extracted through the vaginal incision. The authors noted difficulty grasping intra-abdominal organs with endoscopic instruments; as well as problems with lateral viewing using the endoscope, necessitating the use of a 5 mm 30^{o} scope during portions of the case (28). Recently, transvaginal nephrectomy was reassessed by Sotelo et al. with a hybrid approach (29).

In contrast to NOTES, clinical experience with LESS is more extensively published. There are reports on peritoneal dialysis catheter placement using a single umbilical port in pediatric patients (30), simple nephrectomy, orchiopexy, orchiectomy and ureterolithotomy (31). The Cleveland Clinic (formally known as the Cleveland Clinic Foundation) has extensively presented their experience with LESS-NOTES. Kaouk et al. presented a clinical series of ten patients operated by single-port technique for different urological procedures (32). Their early results show feasibility along with good outcomes. This institution has also reported on LESS renal cryotherapies (33), and varicocelectomy (34).

The radical nephrectomy technique with single port surgery has also been assessed by Ponsky and coworkers (35). This experience was undertaken in a patient with an enhancing renal tumor. The technique featured three trocars through a GelPort device and the use of only standard laparoscopic instruments. Renal surgery for different procedures has also been evaluated in retroperitoneal single port access (36).

Additional extirpative surgeries have been accomplished using LESS. Castellucci et al. have reported details of a LESS adrenalectomy (37). Gill et al. performed LESS left donor nephrectomies (38).

Reconstructive procedures add new dimensions of complexity to LESS procedures. Aron et al. reported five single-port partial nephrectomies; of which only one required an additional 5 mm port (39). Desai et al. presented transumbilical nephrectomy and pyeloplasty using the R-Port; 2 mm needle-ports were used in these cases as well. Procedures were successfully accomplished with no extra-umbilical skin incisions and adequate results (40). Desai et al. used LESS to complete bilateral pyeloplasties, ileal ureteral interposition, and psoas hitch ureteroneocystostomy (41). Sotelo et al. have presented a series of NOTES-LESS procedures including enterocystoplasty augmentation (42).

Pelvic surgery involving reconstruction has also been accomplished. Desai et al. completed transvesical LESS simple prostatectomies utilizing an R-port placed through the bladder dome (43). Kaouk et al. completed LESS radical prostatectomies using a Uni-X port at the umbilicus, and flexible shaft laparoscopic instruments. After dividing the bladder neck, the surgical team found it difficult to maintain adequate traction to dissect the seminal vesicles; therefore they proceeded to the apical dissection and completed the surgery in a retrograde manner. Anastomotic sutures were tied extracorporeally. There were no intra operative complications; however, one patient developed a recto-urethral fistula 2 months postoperatively (44).

Similar to the experimental experience, robotic technology has been used to augment clinical LESS procedures. Kaouk et al. completed a radical prostatectomy, a dismembered pyeloplasty, and a radical nephrectomy via LESS placement of da Vinci robotic instruments (45). The procedures were completed without additional ports or instruments, and no complications were reported. There is also a report of a series of robotic LESS partial nephrectomies (46).

Comparative Studies

Raman et al. performed a retrospective case-controlled study comparing the outcomes of 11 LESS nephrectomies to 22 matched, conventional laparoscopic nephrectomies. LESS nephrectomies were shown to be feasible, with comparable interoperative times, blood loss and complication rates; the study failed to demonstrate any significant improvement in analgesic use or convalescence (47). While this study is retrospective, it represents an important step in validating LESS procedures in comparison to standard approaches.

NOTES-LESS Reviews, Nomenclature, Consensus Papers (Table-3)

Outside of the realm of original research, the increasing enthusiasm for NOTES-LESS has been reflected in a growing body of articles, which summarize and review the details of this topic. This has included a number of review articles, often written by individuals actively participating in NOTES-LESS research. Our search revealed 20 review articles addressing current status and future development of NOTES-LESS techniques (48-65). Additionally, a Urology Working Group on NOTES was formed in November 2007, and subsequently generated a consensus statement on NOTES-LESS. In this statement, they expressed enthusiasm for the future of NOTES and LESS, but recognized that NOTES remains a research topic in need of further development before widespread clinical application (66). The Urologic Working Group on NOTES also observed the plethora of names and acronyms relating to NOTES-LESS; in order to clarify and standardize the language describing these techniques, they published and article defining NOTES-LESS, and provided a framework for standard language to describe these procedures (67).

FUTURE ENDEAVORS

When Gettman et al. performed the first NOTES procedure, he noted the difficulty of the procedure given the limits of the available instruments (2). This has been a recurring theme in the NOTES-LESS literature that has followed: lack of mobility demands highly developed laparoscopic skills to overcome. In order to broadly apply these techniques, new instruments specially suited to these procedures will likely need to be developed. Curved or articulating instruments, streamlined or flexible optics, and robotic assistance have all been applied toward this end. On the horizon, novel devices such as flexible robotics may bring us closer to the wide application of NOTES-LESS.

Creating these new devices, and the new approaches to utilize them, will require open, inquisitive minds to constantly rethink the problems and imagine solutions. Urologists are uniquely suited to this task. Urologists are already well versed in laparoscopic, endoscopic and radiologic modalities that will likely be needed to perform minimal access procedures. The specialty also has a "pioneering spirit" that readily embraces new and innovative techniques (66). Along with open minds, there is also the need for healthy scientific skepticism. NOTES- LESS cannot be embraced simply because they are new. They must be carefully validated, both experimentally and clinically, focusing on patient safety and an evidence-based assessment of the benefits of these approaches. The future needs of NOTES-LESS are specific studies to evaluate the technique. At this point, we probably do not need more feasibility studies and the work should be focused primarily in improving and refining techniques, in order to proceed with comparison. The prospect of a randomized prospective study seems far away, but the idea should be kept in mind, even if it seems unlikely to ever happened, as we all have seen in the fields of laparoscopic adrenalectomy and laparoscopic radical prostatectomy. Meantime, solid reports of complications should be incorporated in the clinical work as limited information is available at this point (Table-4); in order to clearly define limitations and potential improvement of outcomes and also very important to consider the need for surgical certification to perform this approaches.

CONCLUSIONS

NOTES-LESS are novel techniques that hold tremendous promise for delivering safe, effective treatment of urologic disease but they also pose great obstacles. Investigators have applied a great deal of innovation and skill to begin to overcome these challenges. The big part of the NOTES-LESS experience has been performed in renal surgery and this field is probably the most fitted for future development. Further progress will likely rely on novel technologies, as well as innovative minds to imagine and apply them. Finally, NOTES-LESS will need careful validation in comparison to the current standard of care to ensure that they deliver the improved outcomes of which they seem capable.

Table 3 – Reviews/Related Publications.

Authors	Reviewed Period	Number of Publications Reviewed or Searching Strategy	Comments
Raman et al. (2007)	2002-2007	23	Mini review on the status of SILS (single incision laparoscopic surgery) and NOTES, describing future directions and concluding on the future exploration of the technique.
Gettman et al. (2008)	2002-2008	32	A review on current NOTES technique status since first publication on transvaginal nephrectomy by the authors in 2002, concluding the need of new technologies to appear to make NOTES the next clinical frontier.
Canes et al. (2008)	1969-2008	31	A review on history of E-NOTES across surgical disciplines for the past 40 years. The review covers questions of nomenclature, surgical technique, instrumentation and perioperative outcomes.
Tracy et al. (2008)	1960-2008	39	A review on LESS, covering instrumentation descriptions, surgical techniques, analysis of the comparative studies on benefits of LESS, concluding on the need of the future research to unveil the benefits over standard laparoscopy.
Swain (2008)	1993 - 2008	39	Review of current status of NOTES nephrectomy, with review of history of NOTES surgery and current experimental approaches.
Lima et al. (2008)	2002-2008	33	Review on NOTES history, development, applications, experimental and human studies, current limitations and urological role in the technique, that states the urologists are welcomed to contribute into the development of the novel approach.
Cindolo et al. (2008)	2002-2008	16	Short review on NOTES in urology, covering history, current status, techniques, future potentials, concluding that NOTES are still at the development phase.
Gettman et al. (2008)	----------	----------	Consensus statement concerning NOTES and LESS, and detailing the formation of the Urology Working Group on NOTES. Highlights the potential of NOTES and LESS, as well as the challenges and need for scientific confirmation of benefits.
Box et al. (2008)	----------	----------	Nomenclature of NOTES and LESS surgical procedure; defining terms to describe procedures in order to provide standardized terminology for NOTES and LESS operations.
Lima et al. (2009)	2002-2008	Manuscripts and abstracts of annual meetings of the American Urological Association, the European Association of Urology, and the World Congress of Endourology from 2007.	NOTES procedures remain highly complex operations in the urology. In an attempt to overcome these limitations, the hybrid approach (adding a single abdominal port access) or the pure NOTES combined approach (joining multiple natural orifice ports) as proposed in the literature.

Table 3 – continued.

Kommu et al. (2009)	1965-2008	Reports in major urological meeting abstracts, Embase and Medline.	Advances in instrumentation have been essential to achieve adequate results in LESS-NOTES urological surgery. Testing survival in animals is also necessary to further expand these techniques. Improved instrumentation and technology together with increasing experience in LESS-NOTES approaches have prompted the transition from porcine models to human patients.
Irwin et al. (2009)	---------- ------	Comprehensive review on LESS.	Success of LESS surgery is associated with familiarity of current practitioners with advanced laparoscopic techniques and technology. Advancements in instrumentation and future robotics platforms will expand the technique. Prospective studies comparing safety and effectiveness of NOTES-LESS with the laparoscopic and robotic approaches will objectively determine its role in surgical practice.
Box et al. (2009)	----------	Transvaginal access.	Transvaginal approach may be the best suited NOTES portal in selected patients.
Xavier et al. (2009)	----------	Transgastric access.	Application of transgastric NOTES in urology has been limited to case reports in the porcine model, and no urologic procedure has been performed solely via a transgastric route.
Ponsky et al. (2009)	----------	Feasibility and future of NOTES in Urology.	Experimental experience has showed the feasibility of NOTES and provided insight into the technical innovations necessary to improve the technique. NOTES is starting to be deployed in clinical experiences in both hybrid laparoscopic-endoscopic cases and pure procedures. Critical evaluation of outcomes is mandatory.
Canes et al. (2009)	----------	Deployment of flexible robotics and in vivo mini-robots for NOTES.	NOTES present specific instrumentation challenges (lack of stable platforms, loss of spatial orientation, and limited instrument tip maneuverability) that might be overcome with incorporation of robotic technology. Development of such technology is a challenge that has already been approached in Urology.
Granberg et al. (2009)	----------	Transvesical NOTES in Urology.	Initial studies have demonstrated the feasibility of transvesical NOTES. This technique has the potential to develop into a viable technology in the clinical setting, but it remains in phase of development.
Shin & Kalloo (2009)	----------	Transcolonic NOTES in Urology.	NOTES represent the symbiosis of the concepts of both minimally invasive surgery and interventional endoscopy. The transcolonic approach to the peritoneal cavity is presented.
Gamboa et al. (2009)	----------	Education and training for NOTES.	Development of guidelines for training will be necessary for the safe adoption of NOTES. Educational experience coined in laparoscopy (animal and cadaveric laboratories) will have an impact in the development of knowledge and surgical skills for NOTES.
Rasswei-ler et al. (2009)	----------	Imaging and navigation for NOTES.	Image-guided soft-tissue navigation represents a logic option to diminish the hazards of the technically challenging procedures of NOTES in Urology.

Table 4 *– Report on complications in NOTES-LESS clinical series.*

Authors	Number/procedures	Approach	Complications reported
Garg et al. (2005)	26 Nephrectomies	Extraperitoneal single port	1 Pleura perforation – managed intraoperatively 1 Vena cava hemorrhage – managed intraoperatively
Kiyokowa et al. (2006)	21 Prostatectomies	Retropubic extraperitoneal	No complications reported
Milliken et al. (2006)	22 Insertion of peritoneal dialysis catheters	Supraumbilical port	1 rectal injury
Gettman et al. (2007)	1 Transvesical peritoneoscopy	Endoscopic transurethro-transvesical	1 leak – resolved within 24 hours 1 exit-site infection – resolved via oral flucloxacillin 1 blockage due to fibrin clot – resolved with urokinase 1 blockage 2 weeks later - reoperation
Desai et al. (2007)	1 Nephrectomy; 1 Pyeloplasty	Transumbilical single port	No complications reported
Kaouk et al. (2007)	4 Renal cryotherapy procedures; 1 Kidney biopsy; 1 Radical nephrectomy; 4 Abdominal sacrocolpopexies.	Transumbilical or retroperitoneal	1 Postop complication in cryo procedure resolved via ventilation mask and blood transfusions (CT scan revealed small perinephric hematoma).
Raman et al. (2007)	3 Radical nephrectomies	Transumbilical	No complications reported

Table 4– continued.

Kaouk et al. (2007)	3 Varicocelectomies	Transumbilical	No complications reported
Kaouk et al. (2008)	4 Radical prostatectomies	Transumbilical	No intraoperative or postoperative complication 1 delayed complication – rectourethral fistula managed with mucosal advancement flap.
Ponsky et al. (2008)	1 Nephrectomy	SAS Transperitonealy	No complications reported
Desai et al. (2008)	4 Pyeloplasties 1 Ileal ureter 1 Ureteroneocystostomy	Transumbilical	No complications reported
Desai et al. (2008)	3 Simple prostatectomies	Transvesical	1 inadvertent enterotomy – fixed intraoperatively.
Kaouk et al. (2008)	1 Radical prostatectomy 1 Pyeloplasty 1 Radical nephrectomy	Transumbilical	No complications reported.
Castellucci et al. (2008)	1 Adrenalectomy	Transperitoneal supraumbilical	No complications reported
Kauok et al. (2008)	7 Partial nephrectomies	Transumbilical	1 intraoperative bleeding from tumor bed – managed intraoperatively with conversion to standard laparoscopy and blood transfusions.
Gill et al. (2008)	4 Live donor nephrectomy	Transumbilical	No complications reported
Barret et al. (2009)	1 Radical nephrectomy	Transumbilical extraperitoneal.	No complications reported

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CONFLICT OF INTEREST

None declared.

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Review Article

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Prostatic Atrophy. Clinicopathological Significance

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ABSTRACT

Prostatic atrophy is a benign lesion that may mimic adenocarcinoma histologically and on imaging. It is more frequent in the peripheral zone and has gained importance with the increasing use of needle biopsies. Diffuse atrophy occurs secondarily to radiotherapy and/or endocrine therapy. Inflammation and/or chronic local ischemia may cause focal atrophy with an increasing frequency in age. Atrophy may be classified morphologically into diffuse and focal. The latter may be partial, complete or combined. Partial focal atrophy is the most frequent mimicker of adenocarcinoma on needle biopsies. Complete focal atrophy may be subtyped into simple, sclerotic and hyperplastic (or postatrophic hyperplasia). Combined lesions are frequent and partial atrophy may precede complete atrophy. The several morphologic types of focal atrophy may represent a morphologic continuum and the hyperplastic (or postatrophic hyperplasia) subtype seems to be at the extreme end of this continuum. Chronic inflammation associated to focal atrophy (proliferative inflammatory atrophy) has been linked to high-grade prostatic intraepithelial neoplasia and/or carcinoma. This link, however, remains controversial in the literature. The question whether inflammation directly produces tissue damage and atrophy or some other insult induces atrophy directly, with inflammation occurring secondarily, is still unresolved. An intriguing finding that needs further studies is a possible association of extent of atrophy to serum PSA elevation.

Key words: *prostate; atrophy; prostatic neoplasms; carcinoma; biopsy; needle*
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INTRODUCTION

Prostatic atrophy (PA) is one of the most frequent histologic mimics of prostatic adenocarcinoma (1). On conventional and color Doppler transrectal ultrasound and on magnetic resonance spectroscopic imaging studies, PA may also simulate prostate cancer (2,3). Thus, PA should be considered together with prostatitis as an important cause of false-positive results on imaging of the prostate (2). Of further interest as regards this lesion relates to a possible link to high-grade intraepithelial neoplasia (HGPIN) and/or carcinoma (4). PA occurs most frequently in the peripheral zone (5-7) and gained importance with the increasing use of needle biopsies for the detection of prostatic carcinoma (8). It is a very frequent lesion: 83.7% on needle biopsies (9) and 85% in autopsies (10).

HISTORICAL BACKGROUND AND NOMENCLATURE

The nomenclature of PA is cumbersome and confusing. Moore (5), in 1936, was one of the first authors to describe prostatic atrophy in a systematic

autopsy study. He found that there was a strong correlation with age and, according to his study, prostatic atrophy is initiated during the 5th decade and continues as a progressive process into the 8th decade. Moore classified PA into simple acinar atrophy and sclerotic atrophy.

In 1954, Franks (6) added to simple acinar atrophy and sclerotic atrophy a lesion that he called postatrophic hyperplasia. When hyperplasia followed simple acinar atrophy, it was called lobular hyperplasia. Sclerotic atrophy with hyperplasia was called postsclerotic hyperplasia. According to the author, lobular hyperplasia and postsclerotic hyperplasia are closely related existing conditions in which it is impossible to decide to which group a particular area of hyperplasia belongs.

Since these first studies, authors have described similar morphologic features with different names. For example, despite not using the term hyperplasia, Moore's Figure-14 called by the author as simple acinar atrophy (5) is identical to the lesion shown in Figure-8 and called lobular hyperplasia in Franks' article (6).

PATHOGENESIS

Radiotherapy and/or endocrine therapy are established causes of diffuse atrophy. Inactive or active inflammation is a frequent cause of focal atrophy. Based on a study of autopsies there is evidence that chronic local ischemia may be a cause of focal atrophy, which was clearly more frequent in advanced age (10). A further evidence for a possible role of chronic local ischemia in the pathogenesis of prostatic atrophy was the finding that blood flow, assessed by color Doppler transrectal ultrasound, was absent in 60% of hypoechoic nodules due to prostatic atrophy (3). However, many examples of atrophy are still considered idiopathic in nature (11).

MORPHOLOGIC CLASSIFICATION

A morphologic classification of prostatic atrophy is shown in Table-1. In diffuse atrophy, all acini of the gland are atrophic. This occurs secondarily to radiotherapy and/or hormonal therapy. Focal atrophy is the usual lesion seen by the pathologist on routine practice. It occurs in patches and preserves the lobular architecture of the gland. The latter feature helps in the histological differential diagnosis of adenocarcinoma.

Partial atrophy. This is the most common benign variant of focal atrophy that causes difficulty in the differential diagnosis of adenocarcinoma (1,12). The lesion was reported in the literature in 1998 (13). Architecturally, partial atrophy consists of crowded glands often with a disorganized growth pattern. In contrast to complete atrophy, which can typically be diagnosed at scanning magnification owing to the presence of well-formed glands with a very basophilic appearance, partial atrophy has a pale cytoplasm lateral to the nuclei giving rise to pale staining glands that more closely mimic cancer (Figure-1). An additional difficulty in distinguishing cancer from partial atrophy is the positivity for alpha-methylacyl coenzyme A racemase (AMACR) in some acini (1,12,14).

Complete atrophy. The glands show a basophilic appearance due to the scant cytoplasm and crowding of nuclei. The hyperplastic (or postatrophic hyperplasia) subtype most frequently mimics adenocarcinoma. In a working group classification (15), complete focal atrophy was subtyped into simple atrophy, simple atrophy with cyst formation, and postatrophic hyperplasia. Based on an autopsy study, complete focal atrophy was subtyped into simple atrophy, sclerotic atrophy, and hyperplastic atrophy (or postatrophic hyperplasia) (10). Sclerotic atrophy in the morphologic classification shown in Table-1 corresponds to simple atrophy with cyst formation in the working group classification.

Simple atrophy. Usually involves an entire lobule, although isolated acini may be affected. The acini are small showing scant cytoplasm and decrease in the height of the epithelial cells (Figure-2A). They may be cystic with flattened epithelium (Figure-2B). The surrounding stroma may or may not show any fibrosis.

Sclerotic atrophy. This is a very peculiar and distinctive lesion described in detail by Moore in 1936 (5). There is simultaneous atrophy of the epithelium and a proliferation of the fibroblasts around the acinus. Continued proliferation results in a fibrohyaline

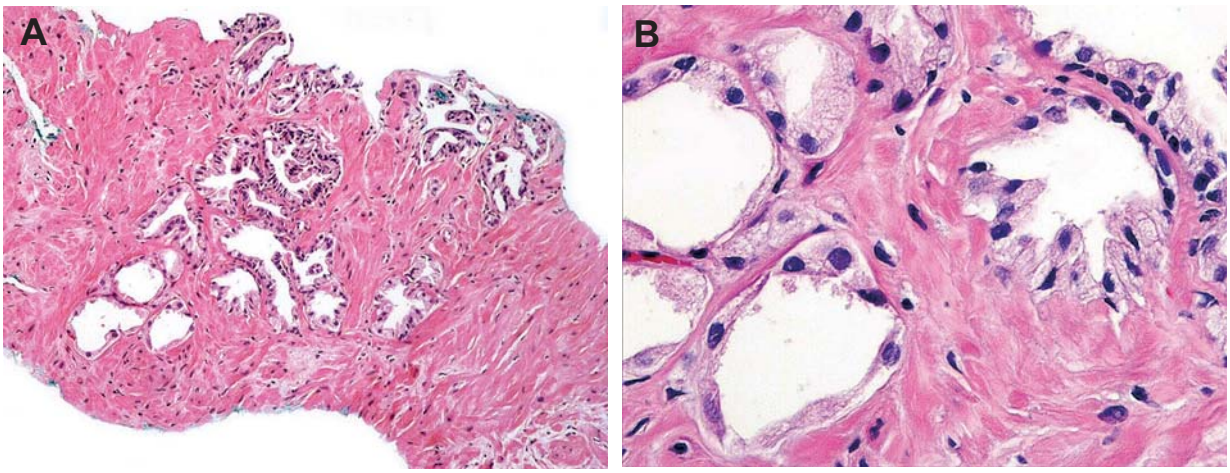

Figure 1 *– Partial atrophy. A) Crowded acini with pale cytoplasm lateral to the nuclei giving rise to pale staining glands that more closely mimic cancer. There is scant apical cytoplasm with nuclei extending to the full cell height (HE, X165); B) Higher power view*

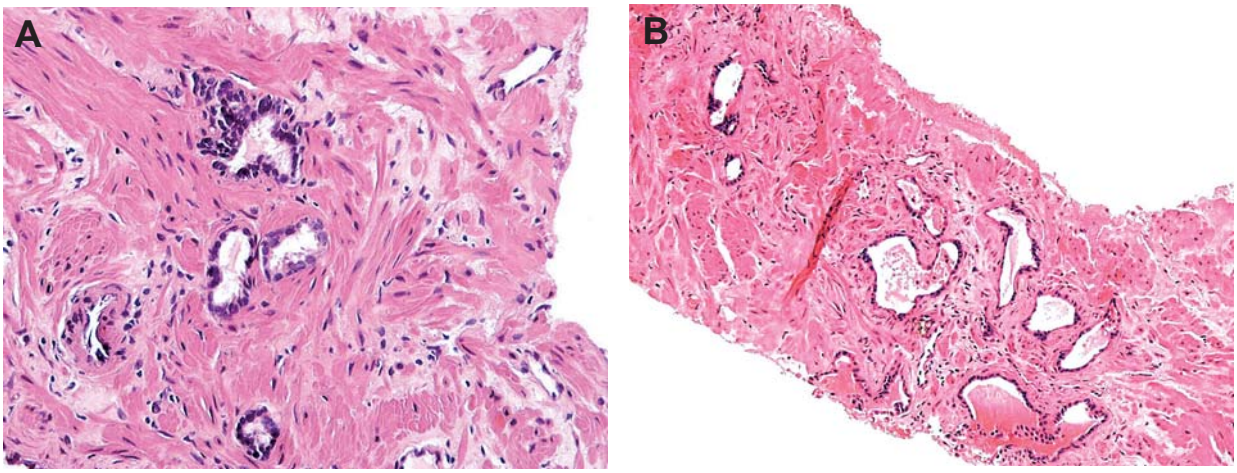

Figure 2 *– Simple atrophy. A) Isolated small acini showing crowded nuclei, scant cytoplasm and decrease in the height of the epithelial cells (HE, X220); B) Simple atrophy with cystic dilatation (HE, X165).*

Table 1 *– Morphologic classification of prostatic atrophy.*

A - Diffuse
B - Focal
1. Partial
2. Complete
a) Simple
b) Sclerotic
c) Hyperplastic (or postatrophic hyperplasia)
3. Combined

collar frequently associated to elastosis that involves the acinus. With higher degrees of hyalinization the acini dilate, sometimes prominently, and the epithelium becomes extremely flattened and eventually no longer can be identified (Figure-3A). In advanced stages, the lumen is replaced by loose mesenchyme-like tissue with scattered inflammatory round-cells (Figure-3B).

Hyperplastic atrophy (or postatrophic hyperplasia). Shows small acini closely packed together and lined by atrophic epithelium (Figure-4A). The

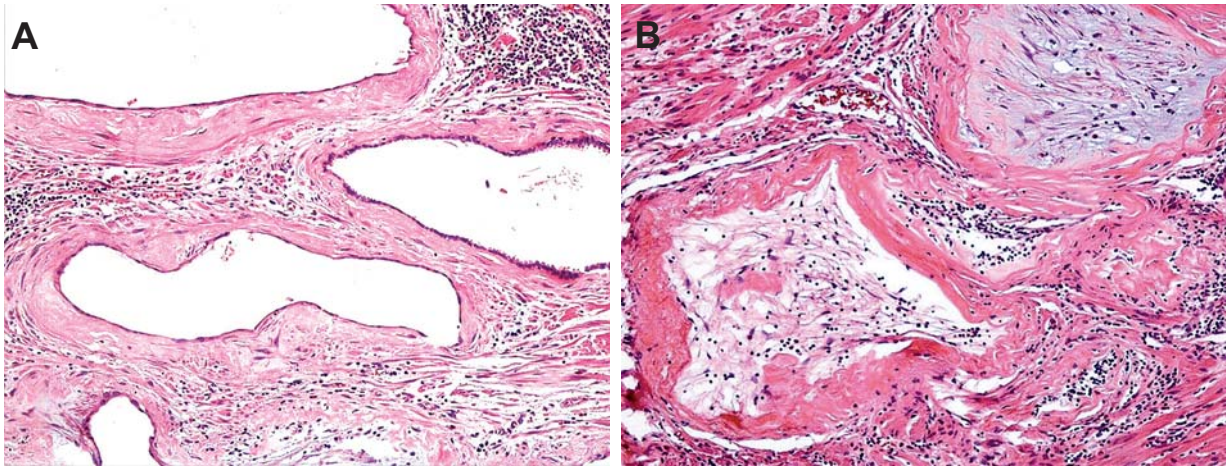

Figure 3 – *Sclerotic atrophy. A) Dilated acini, atrophy of the epithelium and a thick fibrohyaline collar involving the acinus (HE, X165); B) Advanced stage of sclerotic atrophy. The epithelium disappeared and the lumen is replaced by loose mesenchyme-like tissue with scattered inflammatory round-cells (HE, X165).*

acini may also show cystic dilatation (Figure-4B). In contrast to partial atrophy, the cytoplasm is very scant and the nuclei are crowded conferring a basophilic tinge to the lesion. When fibrosis is present in the stroma, the proliferation is irregular and can result in distortion of the acini mimicking infiltrative adenocarcinoma. Elastosis of the stroma (Figure-4A) may be seen in all subtypes of complete atrophy and is a microscopic feature useful for the differential diagnosis of adenocarcinoma (16). The cysts of simple or hyperplastic atrophy must be differentiated from other simple or multiple parenchymal cysts due to retention of prostatic secretions, difficult drainage of secretions due to benign prostatic hyperplasia (BPH) nodule, inflammation of ducts that leads to obstruction, and cystic degeneration of BPH (17).

Combined. Subtypes of focal complete atrophy are frequently combined (10). They may occur in adjacent but separate foci or merging in the same focus. Hyperplastic atrophy with a central duct or acinus showing sclerotic atrophy is a frequent combined lesion in the same focus and very characteristic of

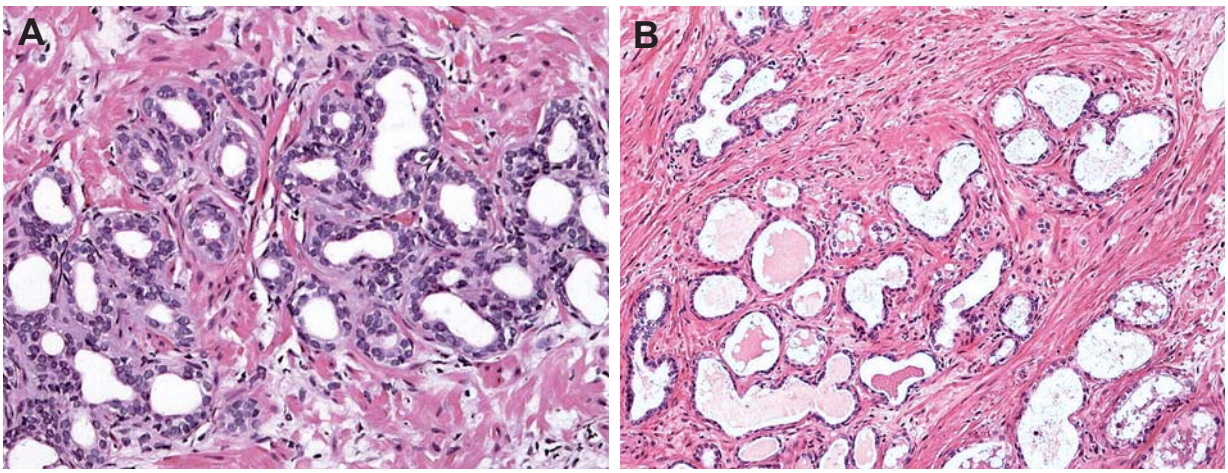

Figure 4 – *Hyperplastic atrophy (or postatrophic hyperplasia). A) Small acini closely packed together and lined by atrophic epithelium. In contrast to partial atrophy, the cytoplasm is very scant and the nuclei are crowded. The stroma shows fibrosis and elastosis (HE, X220); B) Hyperplastic atrophy with cystic dilatation (HE, X165).*

prostatic atrophy being a feature useful for the differential diagnosis with adenocarcinoma (Figure-5). The existence of combined subtypes supports the hypothesis that complete prostatic atrophy is a morphologic continuum and that hyperplastic type (or postatrophic hyperplasia) seems to be at the extreme end of this morphologic continuum (8). The hyperplastic small acini seem to represent a regenerative process. A high cell proliferation using immunohistochemistry seems to support this hypothesis (18).

Partial and complete atrophy may be also combined. Studying needle prostatic biopsies we found that partial atrophy and complete atrophy were present concomitantly in 47/75 (63%) biopsies. In 20/75 (27%) biopsies, we found topographical mergence of partial and complete atrophy. In these areas, transitions between partial and complete atrophy could be appreciated in the same gland (Figure-6). Based on the aberrant phenotypic expression of the secretory compartment in complete atrophy (19) but not in partial atrophy, immunohistochemistry may highlight these transitions in the same gland (Figure-7).

In a study by Oppenheimer et al. (13) partial atrophy was present simultaneously with more fully developed atrophy (complete atrophy) in 35.35% of biopsies. In the study by Wang et al. (12), 48 of 278 (17.3%) partial atrophy cases were mixed with postatrophic hyperplasia. Przybycin et al. (20) found a much higher frequency. The authors described that

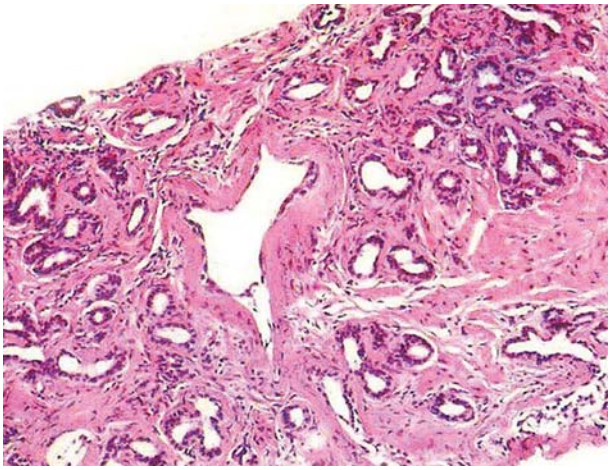

Figure 5 *– Combined atrophy. Hyperplastic atrophy with a dilated central duct or acinus with sclerotic atrophy (HE, X165).*

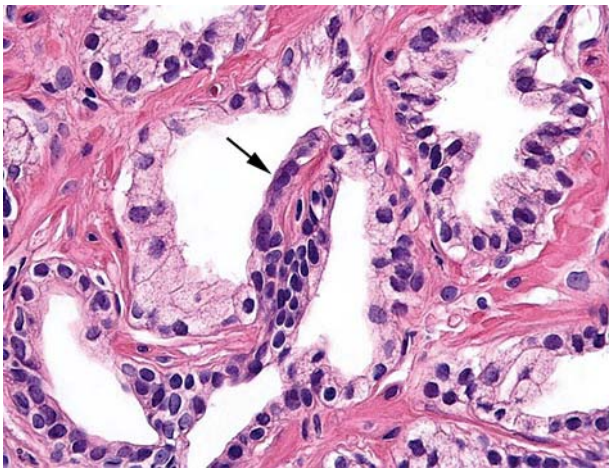

Figure 6 *– Morphologic transition between partial and complete atrophy in the same gland (arrow shows segment of complete atrophy) (HE, X220).*

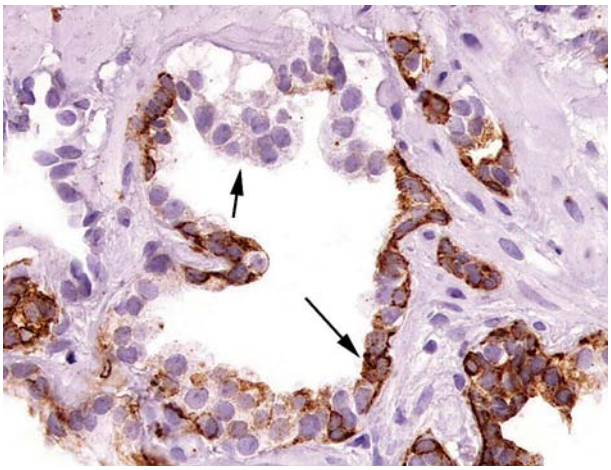

Figure 7 *– Immunohistochemistry for 34βE12. Negative in cells of the secretory compartment in segment of partial atrophy (short arrow) and aberrant positivity in the segment of complete atrophy (long arrow) (HE, X220).*

complete atrophy was present but distinct from partial atrophy foci in 41/45 (91%) needle biopsies.

What is the significance of the mergence of partial atrophy and complete atrophy and the transitions in the same gland? According to Oppenheimer et al. (13) the transition from partial to more established atrophy, is accompanied by a parallel increase in the nuclear/cytoplasmic ratio, suggesting gradual evolution from the partial form of atrophy to the more complete variety. This suggests that partial atrophy may be also part of a morphologic continuum in focal prostatic atrophy.

An intriguing finding in partial atrophy foci is the very rare presence of chronic unspecific inflammation. Przybycin et al. (20), found inflammation in an insignificant 1% of partial atrophy foci. In a study on 75 biopsies, we did not find chronic unspecific inflammation in partial atrophy foci as well as in areas of topographic mergence between these lesions. On the other hand, inflammation was frequently seen in complete atrophy foci: 56.2%, 48%, and 54.3%, in simple, sclerotic, and hyperplastic atrophy, respectively.

PRECANCEROUS LESION?

The term "proliferative inflammatory atrophy" (PIA) was proposed by De Marzo et al. (4) to designate focal simple or postatrophic hyperplasia occurring in association with inflammation. Several studies have postulated that PIA may represent a precursor lesion to high-grade prostatic intraepithelial neoplasia (HGPIN) and, therefore, prostatic carcinoma (4,19,21-23). Chronic inflammation of longstanding duration has been linked to the development of carcinoma in several organ systems and HGPIN is considered the most likely precursor of prostate carcinoma (4,24).

Several separate findings provide supportive evidence for this novel hypothesis:

1) There is a shift in the topographic fidelity of proliferation in PIA similar to HGPIN and carcinoma (4). Most cell division in the normal human prostate epithelium occurs in the basal cell compartment, yet HGPIN and adenocarcinoma cells possess phenotypic and morphologic features of secretory cells. Thus, cell proliferation has been shifted up from the basal into the secretory compartment in HGPIN and carcinoma;

2) The phenotype of many of the cells in PIA is most consistent with that of an immature secretory-type cell similar to that for the cells of HGPIN and carcinoma (4,19,22). Atrophic luminal cells show an intermediate phenotype in that many cells express bcl-2 (normally a basal cell marker in the prostate), and virtually all of the cells express high levels of keratins 8/18. Intermediate cell population has been suggested to represent amplifying cells modulating the expansion and development of the prostate epithelium. Increased proliferation has been observed in atrophic glandular epithelium (18);

3) PIA, HGPIN, and carcinoma all occur with high prevalence in the peripheral zone and low prevalence in the central zone of the human prostate (21);

4) Topographic relation of PIA to HGPIN, i.e. areas of atrophy merging directly with areas of HGPIN within the same glands. In radical prostatectomy specimens, Putzi and De Marzo (21) identified morphologic merging between PIA and HGPIN in 34% of the PIA lesions. They also found frequent occurrences of small carcinoma lesions in the vicinity of focal atrophy. In a study by Wang et al. (22), a total of 1,188 HGPIN lesions were identified, of which 17% (198) were in the morphological process of merging with PIA. Thirty-six PIA-merging prostatic carcinoma lesions were also detected. The atrophic epithelial cells in such merging lesions had increased Ki-67 proliferative index and an intermediate phenotype: increased expression for cytokeratin 5, GSTP1, c-MET, and C/EBPβ.

The link of PIA to prostatic carcinogenesis and the morphological transition of PIA, HGPI, and invasive carcinoma, however, are not favored in other studies. In autopsies, prostates with atrophy showed no association with histologic carcinoma and/or HGPIN (10). The only significant association found was with arteriosclerosis. In this study ischemia caused by local intense arteriosclerosis seemed to be a potential factor for the pathogenesis of atrophy. In a subsequent study, the same lack of association was found to HGPIN and/or histologic carcinoma comparing atrophy with and without inflammation (9).

In 272 radical prostatectomies, Anton et al. (25) analyzed the presence, location, and number of foci of postatrophic hyperplasia. They found the lesion in 32% of radical prostatectomy specimens and in 12% of cystoprostatectomy specimens, and concluded that is a relatively common lesion but without any topographical association with prostatic carcinoma. In 172 needle prostatic biopsies, from a total of 481 cores with cancer, 184/481 (38.25%) cores showed no atrophy; 166/481 (34.51%) cores showed atrophy and no inflammation; 111/481 (23.08%) cores showed both PIA and atrophy without inflammation; and 20/481 (4.16%) cores showed only PIA (26).

Postma et al. (27) evaluated whether the incidence of atrophy reported on sextant biopsies was associated with subsequent prostate cancer detection. The authors concluded that atrophy, especially in its simple form, is a very common lesion in prostate biopsy cores (94%). Atrophy in an asymptomatic population undergoing screening was not associated with a greater prostate cancer or HGPIN incidence during subsequent screening rounds.

A question to be raised is whether atrophy by itself is implicated in carcinogenesis or the key event is chronic inflammation leading to atrophy, HGPIN and cancer. This question is still unresolved (28,29). Inflammation directly may produce tissue damage and atrophy or, alternatively, some other insult like ischemia induces the atrophy directly, with inflammation occurring secondarily. Hypothesizing that partial atrophy precedes complete atrophy, the absence of inflammation in the former as well as in areas of mergence between these two lesions seems to favor that chronic inflammation in complete focal atrophy may be a secondary phenomenon.

PROSTATIC ATROPHY AND PSA

An intriguing finding was a positive and significant association between extent of atrophy and total or free serum prostate-specific antigen (PSA) elevation (30). The study was based on 131 needle prostatic biopsies corresponding to 107 patients. The only diagnosis in all biopsies was focal prostatic atrophy without presence of cancer, HGPIN or suspicious for cancer (ASAP). In a subsequent study it was shown that this association is not related to the type of atrophy (31).

What would be a possible pathogenesis for the serum PSA elevation associated with focal prostatic atrophy? It is intriguing that cells of the secretory compartment of atrophic acini may produce higher levels of PSA. The authors hypothesize that injurious stimuli causing focal prostatic atrophy may interfere in the physiologic barrier that prevents the escape of any significant amounts of PSA to the general circulation.

PSA is a single chain glycoprotein with proteolytic enzyme activity mainly directed against the major gel-forming protein of the ejaculate (semenogelin). PSA induces liquefaction of semen with release of progressively motile spermatozoa (32). There are several efficient physiologic barriers to prevent the escape of any significant amounts of PSA from the prostatic ductal system: basement membrane of the acini, basal cells lining the acini, prostatic stroma, basement membrane of capillary endothelial cells, and endothelial cells. These barriers normally prevent PSA from entering the general circulation at concentrations of more than 3 ng/mL (32).

Focal prostatic atrophy represents a form of adaptive response to injury most commonly to inflammation and/or local ischemia. Inflammation and/or ischemia are injurious stimuli resulting in diminished oxidative phosphorilation, membrane damage, influx of intracellular calcium, and accumulation of oxygen-derived free radicals (oxidative stress) (4). Studies showing elevated levels of glutathione S-transferase P1, glutathione S-transferase A1, and Cox-2 in prostatic atrophic epithelial cells suggest a stress-induced response (33-35). We do not know which mechanisms are involved in the physiologic barrier that prevents the escape of any significant amounts of PSA to the general circulation, however, all these stress-induced responses may affect this barrier. Inflammation and particularly ischemia may have also a field effect affecting the physiologic barrier of normal acini close to atrophic acini.

CONCLUSION

Prostatic atrophy is a benign lesion frequently found on needle biopsies, which may mimic histologically and on imaging adenocarcinoma. Diffuse atrophy may be secondary to radiation and/or endocrine therapy. Inflammation and chronic local ischemia are the main causes involved in the pathogenesis of focal atrophy, which increases in frequency with age. Atrophy may be morphologically classified into diffuse and focal. The latter may be partial, complete and combined. Partial atrophy is the most frequent mimicker of adenocarcinoma on needle biopsies. Probably the several morphologic variants of focal atrophy represent a morphologic continuum and the hyperplastic (or postatrophic hyperplasia) subtype

seem to be at the extreme end of this continuum. A possible link of prostatic atrophy to HGPIN and/or carcinoma remains controversial in the literature. The possible association of extent of prostatic atrophy to serum PSA elevation needs further studies.

CONFLICT OF INTEREST

None declared.

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Gene Expression Profile of Renal Cell Carcinoma Clear Cell Type

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ABSTRACT

Purpose: The determination of prognosis in patients with renal cell carcinoma (RCC) is based, classically, on stage and histopathological aspects. The metastatic disease develops in one third of patients after surgery, even in localized tumors. There are few options for treating those patients, and even the new target designed drugs have shown low rates of success in controlling disease progression. Few studies used high throughput genomic analysis in renal cell carcinoma for determination of prognosis. This study is focused on the identification of gene expression signatures in tissues of low-risk, high-risk and metastatic RCC clear cell type (RCC-CCT).

Materials and Methods: We analyzed the expression of approximately 55,000 distinct transcripts using the Whole Genome microarray platform hybridized with RNA extracted from 19 patients submitted to surgery to treat RCC-CCT with different clinical outcomes. They were divided into three groups (1) low risk, characterized by pT1, Fuhrman grade 1 or 2, no microvascular invasion RCC; (2) high risk, pT2-3, Fuhrman grade 3 or 4 with, necrosis and microvascular invasion present and (3) metastatic RCC-CCT. Normal renal tissue was used as control.

Results: After comparison of differentially expressed genes among low-risk, high-risk and metastatic groups, we identified a group of common genes characterizing metastatic disease. Among them Interleukin-8 and Heat shock protein 70 were over-expressed in metastasis and validated by real-time polymerase chain reaction.

Conclusion: These findings can be used as a starting point to generate molecular markers of RCC-CCT as well as a target for the development of innovative therapies.

Key words: *carcinoma, renal cell; microarray analysis; neoplasm metastasis; oncogenes; Interleukin 8, heat-shock protein; gene expression profiling*

Int Braz J Urol. 2010; 36: 410-9

INTRODUCTION

Renal cell carcinoma (RCC) accounts for approximately 5% of all malignancies and is considered the most lethal urological cancer (1,2). At early stages, it can be curable by surgical resection, but no effective treatment option is available for patients at advanced stage. Up to 30% of the cases have metastasis at initial diagnosis and 30% of initially organ-confined cases will develop metastases during follow-up (3,4).

Treatment options available for patients with metastatic disease are very limited and currently tar-

get therapy has been developed based on molecular peculiarities of RCC. Transcriptional profiling has also emerged as a powerful approach to identify the molecular mechanism underlying renal carcinogenesis and in predicting clinical outcomes (5). Gene expression profile may help to identify new biomarkers of aggressiveness and prognosis, selecting patients who could benefit from ancillary therapy. Microarray-based expression profiles have become a standard methodology in any high-throughput analysis.

There are few reported studies of gene expression in RCC clear cell type (RCC-CCT) which have assessed prognosis. Most of these studies used different subtypes of RCC, which is inappropriate since they have different carcinogenesis pathways and clinical behavior (6-9).

This study is focused on the identification of gene expression signatures in tissues of low-risk, high-risk and metastatic RCC-CCT. It was carried out using the Whole Genome Microarray platform, which simultaneously evaluates the mRNA level of 55.000 transcripts ESTs (Expressed Sequences Tags). The resulting expression panel is a statistical representation of physiological responses occurring in the finely tuned transcriptional regulation.

MATERIALS AND METHODS

Patients and Tumor Samples

Tissue samples of RCC-CCT obtained from the surgical specimens extracted from open nephrectomy of 19 patients were evaluated. The patients were divided in three groups: 1) Low risk RCC-CCT (Fuhrman nuclear grade 1 or 2, pT1 and no microvascular neoplastic invasion or tumor necrosis); 2) High risk RCC-CCT (Fuhrman nuclear grade 3 or 4 all staged pT3, all tumors had necrosis and microvascular neoplastic invasion), and 3) Metastatic RCC-CCT. Group 1 was composed of five men and two women submitted to tumor resection or partial nephrectomy; mean age 53.3 years-old (median 53, range 48-56), pT1, mean tumor size of 3.7 cm (median 3, range 1.8-6.5), Fuhrman grade 1 or 2, no microvascular invasion or necrosis. Group 2 was constituted of four males and one female submitted to radical nephrectomy, mean age 60 years-old (median 65, range 39-73), T2-3 mean tumor size of 8.2 cm (median 9, range 3.9-11), Fuhrman grade 3 or 4 with necrosis and microvascular invasion present. Group 3 was characterized by seven patients with metastatic RCC, (six males and one female) mean age 57.7 years-old (median 60, range 39-69) extracted from metastatic specimens of the primary tumor. The control group was a pool of normal cortical renal tissue from 4 patients with chronic kidney infections.

Surgical specimens were immediately sent to surgical pathology laboratory, and frozen at -170°C in liquid nitrogen maximum after 15 minutes. Institutional Review Board approved the protocol and informed consent was obtained from all patients.

Microarray Experiment

Frozen tissue samples were mechanically disrupted in liquid nitrogen and total RNA was extracted with Trizol reagent according to a pre-established protocol (Invitrogen Life Technologies, Carlsbad, CA). For each of the three group described above, 10 μg of total RNA from each tissue sample was distributed between three pools. Double-stranded cDNAs were synthesized from 10 μg of total RNA using SuperScript Choice double-stranded cDNA synthesis kit from Invitrogen following the manufacturer's protocol. cDNAs were purified by phenol/chloroform extraction and ethanol precipitation. Biotin-labeled cRNAs were synthesized by an in vitro transcription reaction using the BioArray HighYield RNA Transcript Labeling Kit (Enzo Diagnostics, Farmingdale, NY). cRNAs were purified from the in vitro transcription reaction using RNeasy Mini kit (Qiagen, Valencia, CA). Biotin-labeled cRNA was generated from each sample following the manufacturer's protocol. cRNA was hybridized onto CodeLink® whole genome microarray slides, washed and hybridized cRNA species were detected using Cy5-Streptavidin (Amersham, UK). Slides were scanned using GenePix Personal 4100A Microarray Scanner (Axon Instruments) and analyzed with CodeLink® Expression Analysis software.

Microarray Statistical Analysis

Statistical analysis of the CodeLink® microarray slides was performed using the publicly available R statistical environment (http://www.r-project.org). The normalization and background correction were performed using the LIMMA package (Linear Models for Microarray Analysis) (10); a part of the Bioconductor Microarray Suite (www.bioconductor.org).The background noise was corrected using the normEXP algorithm and the values were normalized by a cyclic LOESS smooth function with a hundred of interactions using the a adjusting parameter of 1.0.

The normalized data were organized locally and using a Perl (http://www.perl.org) script we determined the minimum variation (fold change) threshold accepted as been significant. Datasets of each histological group were compared in a pairwise fashion. For each comparison performed, the fold change for a given spot was calculated. These values were distributed and the mean and standard deviation (SD) values of expression variation of all genes were determined. A gene was accepted as differently expressed if its expression variation was greater than the mean plus one SD or lower than the mean minus one SD. Finally, only genes accepted as significant on all comparisons were selected. This group of candidate genes were identified and organized locally. The gene lists were numerically sorted and the top UP and DOWN regulated genes were determined for each comparison. Functional classification of these genes was performed using Gene Ontology Consortium 2000.

Quantitative Real-Time PCR and Gene Expression

For qRT-PCR gene expression validation we evaluated 7 patients from group 1 (Low risk RCC-CCT), 5 patients from group 2 (High risk RCC-CCT) and 7 patients from group 3 (Metastatic RCC-CCT).

Total RNA extraction was performed using Trizol (Invitrogen Life Technologies, Carlsbad, CA) as mentioned previously. Pureness and concentration of RNA were measured in a spectrophotometer (260/280 nM), and integrity was verified in an Agilent 2100 bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Synthesis of cDNA was performed from at least 5µg of total RNA with the enzyme M-MLV reverse transcriptase and random primers (Invitrogen Life Technologies, Carlsbad, CA, USA). The reactions were incubated at 65°C for 5 min followed by 37°C for 1h and finally 95°C for 5 min. The cDNA reactions were diluted to 100 µL in nuclease-free water (Invitrogen Life Technologies, Carlsbad, CA, USA) and stored at -20°C until further use.

The expression of two genes was analyzed from cDNA through the qRT-PCR technology in the Abi7500 platform using the TaqMan® protocol (Applied Biosystems). TaqMan® Endogenous Control Assay ID is Hs99999907-m1 (B2M) and Gene Expression Assay IDs are Hs00359147-s1 (HSPA1A and HSPA1B) and Hs00174103-m1 Interleukin 8 (IL-8). cDNA (2µL) from each tumor sample was added to a PCR reaction mix containing 1X TaqMan® Universal PCR Master Mix, AmpErase® UNG and 1 µL Endogenous Control Assay or Gene Expression Assay (Applied Biosystems) in a 20 µL reaction volume. The cycling conditions were 50ºC for 2 min, 95ºC for 10 min and 40 cycles of 95ºC for 15 sec and 60ºC for 1 min. The ΔΔCT method was used to calculate the relative expression of the two target genes and the fold change in gene expression in tumor relative to normal tissues determined by 2-ΔΔCT (11).

RESULTS

Several analyses were performed to identify the differentially expressed genes among the three groups of patients and controls. A significant proportion of differently expressed genes were identified in each comparison tested, the microarray plots are shown in Figure-1. As regards to low risk RCC-CCT (Figure-1A), there was little dispersion of the features referring to the differences in genetic expression, which tends to be distributed in a straight line, next to zero. Therefore, low-risk tumors showed insignificant alterations in their genetic expression when compared to normal tissues. High-risk and metastatic tumors (Figures 1B and C) have shown a significant increase in the proportion of differently expression

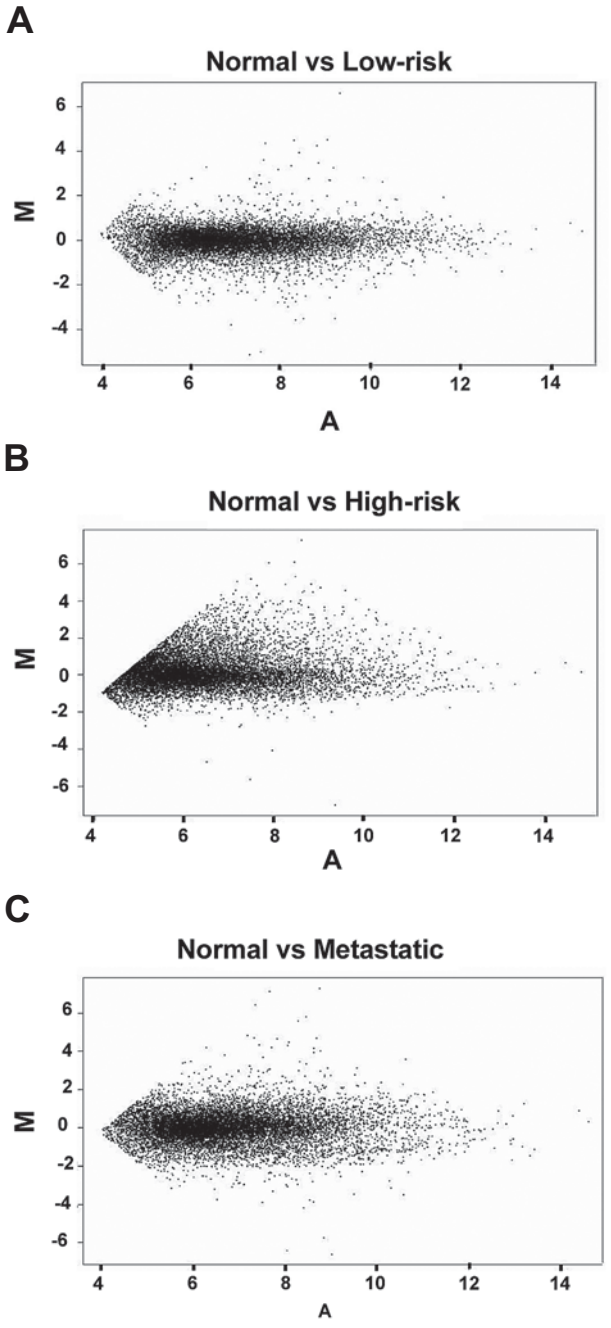

Figure 1 *– Representation of gene expression variation in different ranges of intensity (MA-plot) between (A) normal vs. low-risk, (B) normal vs. high-risk, and (C) normal vs. metastatic genes.*

genes. This could reflect the expected disequilibrium in gene regulation of metastatic or prone to be tissues. The MA plots were used as quality control of the microarray experiments, since it is expected that variations in global gene expression tend to be subtle and any variation of linearity can reflect physiological/pathological adaptations.

To identify the most important genes in the progression of RCC-CCT we selected the 50 most differentially expressed genes in each comparative group. After comparison among the differentially expressed genes in the low-risk, high-risk and metastatic groups, we identified a group of common genes, which presented either increase or reduction in their expression, from the low risk to the metastatic state. These genes are shown in Tables 1 and 2. When compared to the low-risk and high-risk groups, nine over-expressed and eleven under-expressed genes were found in the metastatic group. The differentially expressed genes in each comparison were functionally classified using the GO (Gene Ontology) database (Figure-2).

Two genes, IL-8 and HSP70, which had presented greater expression differences, were chosen to be validated by qRT-PCR. The validation was performed in the three groups mentioned as low-risk (LR), high-risk (HR) and metastatic (M) renal cell carcinomas. As seen in Figure-3, over expression of HSP70 and IL-8 was present in 100% (13/13) and 77% (10/13) respectively of metastatic carcinoma cases tested. The graph shows quantitative expression of genes in RCC tissue relative to normal cells. Fold change in gene expression was calculated using the ΔΔCT method (QRel = 2-ΔΔCT). Kruskal-Wallis test showed significant difference between metastatic and the other two groups ($p = 0.0002$). This pattern was significantly different from high-risk and low-risk carcinomas.

COMMENTS

Description of thousands of genomic sequences along with the technological development to identify the gene expression profile on a large scale has provided a remarkable improvement in the analysis of carcinogenesis process. This improved knowledge has had an impact on the latest advances regarding classification of neoplasias, identification of new diagnostic and prognostic markers, and finding of possible therapeutic targets. Until recently, the studies that evaluated genetic expression through the

Table 1 – *Genes with lower expression from Low-risk to metastatic stage.*

Abbreviation Official/GI*	Identification CodeLink®	Gene Identification
gi40578461	12878	EST- atypical PKC isotype-specific interacting protein long variant mRNA
C7	16412	complement component 7 (C7)
FCGBP	16152	Fc fragment of IgG binding protein
PRAP1	46920	proline-rich acidic protein 1
PCP4	19612	Purkinje cell protein 4 (PCP4)
gi52793583	24985	EST-QV4-BN0090-210400-182-h09 BN0090 Homo sapiens cDNA
gi3870266	38798	EST-qz33c05x1 NCI_CGAP_Kid11 cDNA clone IMAGE:2028680 3' similar to gb:X02747 FRUCTOSE-BISPHOSPHATE ALDOLASE B
WFDCD2	20765	WAP four-disulfide core domain 2 transcript variant 1
gi1401452	23196	EST-zh66h10s1 Soares_fetal_liver_spleen_1NFLS_S1 cDNA clone IMAGE:417091 3'

**GI = genomic identification according to National Institutes of Health data bank. (http://www.ncbi.nlm.nih.gov)*

microarray technique in RCC had focused particularly on the description of genes for diagnostic molecular classification (6). The purpose of our research was the identification of gene expression profiles related to known anatomopathological parameters that are correlated to the prognosis (12). These genetic expression profiles can help to describe a comprehensible pattern via RCC progression and metastatization. Among the genes identified in our study the most important ones are IL-8, and the heat shock protein (HSP-70) genes, which are closely linked to the known carcinogenesis way of the clear cell carcinoma.

Table 2 – *Genes with higher expression from low-risk to metastatic stage.*

Abbreviation Official/GI*	Identification CodeLink®	Gene Identification
gi22685430	34169	AGENCOURT_7932449 NIH_MGC_72 cDNA clone IMAGE:6156942 5'
R39367	11050	clone 24734 mRNA sequence
DUSP2	16648	dual specificity phosphatase 2
HSPA1A	39816	heat shock 70kDa protein 1A
HSPA1B	50162	heat shock 70kDa protein 1B
gi66255625	49424	EST-hypothetical protein LOC90637
IL8	24261	interleukin 8
-	36561	EST-mRNA; cDNA DKFZp667A182 (from clone DKFZp667A182)
UBC	6469	ubiquitin C
gi5863270	45082	EST-UI-H-BI0-aai-f-11-0-UIs1 NCI_CGAP_Sub1 cDNA clone IMAGE:2709644 3'
gi2077388	29444	EST-zu07b03.r1 Soares_testis_NHT cDNA clone IMAGE:731117 5' similar to contains MER30.t2 MER30 repetitive element

**GI = genomic identification according to National Institutes of Health data bank. (http://www.ncbi.nlm.nih.gov)*

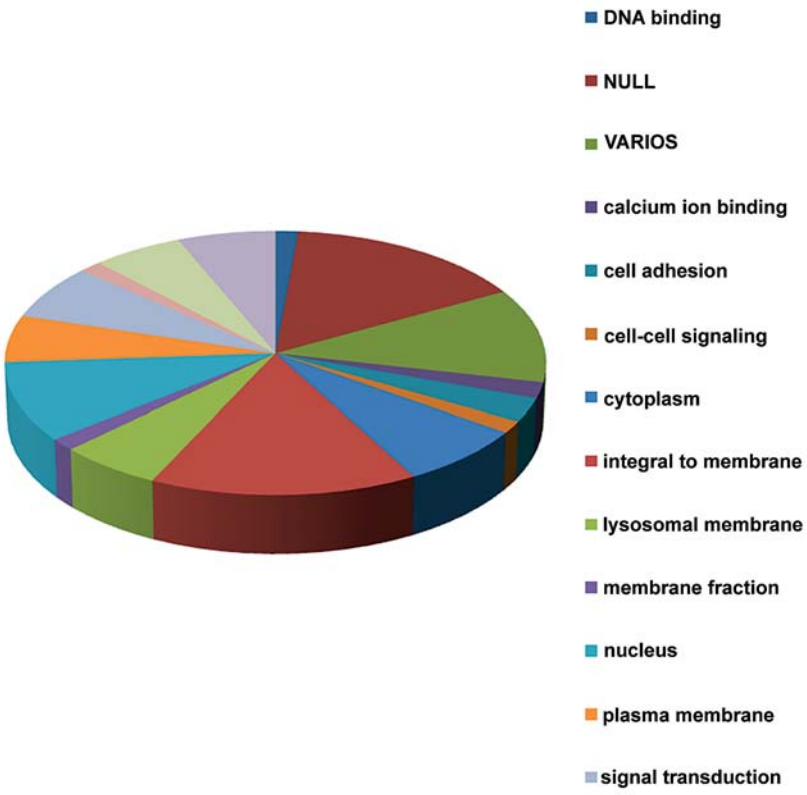

Figure 2 *– Functional classes of overexpressed genes when comparing high-risk tumors to low-risk tumors.*

The gene profiles of the high-risk and metastatic disease are quite similar, and this was described by Jones et al. (7) These authors, studying clear cell type RCC, identified a similar profile of genetic expression among both locally advanced and metastatic tumors, which was named metastatic signature. Kosari et al. (9) in a study quite similar to ours, also identified genes expressed in both aggressive and metastatic carcinomas.

In recent years, the analysis of the gene expression profile on a large scale has been widely used to define genetic expression patterns that can be related to neoplasia aggressiveness (13). Current studies on RCC have sought the identification of new prognostic markers. By studying 16 RCCs in a platform comprising 21,632 genes, (6) a correlation between the histological and genetic classifications in 14 renal tumors. Jones et al. was identified. (7), studying 65 RCC (23 CCC, 13 papillary, 7 chromophobes, 12 oncocytomas) and 24 normal renal tissues defined a genetic profile associated with the development of metastasis, based on a platform of 22,283 genes (Affymetrix). Liou et al. (8) studied six RCCs and compared the differentiated genic expression with six normal renal tissues. By using a platform of 7,129 genes (Affymetrics), they were able to demonstrate that 25% of genes are differentially expressed and among them, an over expression of adhesion molecules (laminin A and fibronectin) which would act in the progression of the neoplasia.

The membrane receptors, like the epidermal growth factor receptor (EGFR), were poorly expressed in the initial RCC, as pT1a tumors; however, its overexpression was correlated with an increase in the tumor stage. These transmembrane glycoproteins interact with tyrosine kinase and promote invasion, metastasis and expression of biomarkers (14). Many target drugs have been studied in the attempt to inhibit cellular events acting directly on these receptors. Presently, tyrosine kinase inhibitory drugs have shown

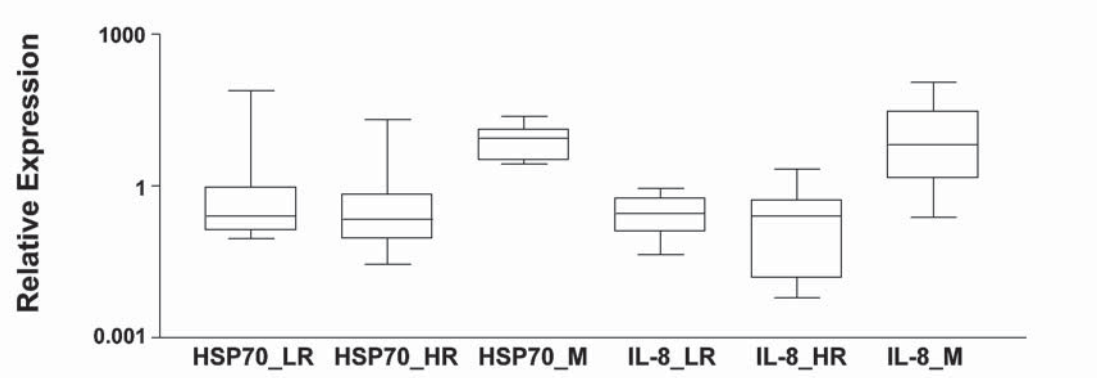

Figure 3 – *Box-and-whisker plots of HSP70 and IL-8 expression in low-risk (LR), high-risk (HR) and metastatic (M) renal cell carcinoma.*

promising results in cases of metastatic RCC, thus pointing to more reasonable expectations of disease control than isolated immunotherapy (15).

The VHL gene is responsible for the codification of a protein, which is part of the elongin B and C complex, whose function is the degradation of the hypoxia-induced factor (HIF-1). This factor is an upregulator of the tyrosine kinase VEGFR receptor, that is overexpressed in RCCs. VEGFR regulates the hypervascular characteristic of RCC (16,17), which has already been the target for the development of inhibitory molecules and antibodies for therapeutic use. Both familiar and sporadic RCCs are related to mutation and/or loss of VHL gene, resulting in nonformation of the elongin B and C complex and HIF-1 accumulation. HIF-1 induces translation of genes related to angiogenesis, favoring the carcinogenesis. The VHL suppressor gene mutations are responsible for the VHL syndrome. The mutated gene is found in 75% of sporadic RCC cases (18). It is believed that the tumor necrosis factor alpha (TNF-α) contributes to the VHL gene suppressive function (19); in our study, this gene was overexpressed in the low-risk cases in relation to the normal ones, validating Caldwell's theory.

Clear renal cell carcinoma occurs in approximately 80% of RCC cases, and a great deal of research shows different gene groups - either underexpressed or overexpressed - without significant intersections among the various studies. These discrepancies probably occur due to different criteria in the selection of the altered genes and the use of different microarray platforms with distinct anchored markers. Another aspect of criticism in any microarray analysis is the improper collection of neoplastic tissue and loss of cellular lineage in tumors, which are mostly heterogeneous (14).

Using qRT-PCR we were able to validate the overexpression of IL-8 and HSP-70 in metastatic RCC-CCT. HSP is expressed by cells under pathological and physiological conditions; its most important functions include homeostasis, apoptosis, and also a relevant role in antigenicity mediated by T cells (20). HSP27 and 72 overexpression are clinically relevant (21), particularly HSP27 overexpression in CCR when compared to normal renal cells (22). Conversely, in our study HSP70 1A and 1B were overexpressed in metastatic cases in relation to low-risk and high-risk cases. The HSP 70 is considered one of the most powerful stimulants to human immune response and the structure and function of these proteins and their relation with immunity have been extensively investigated (23). HSP is an integral component of HIF and this interaction induces HSP overexpression. Drug-oriented actions inhibiting the HIF-HSP complex might exert an inhibitory potential over this important carcinogenesis mechanism; it is necessary, however, to distinguish the effects of the HSP family members (23).

IL-8 is over expressed by tumors and has been related to angiogenesis, mitotic activity and metastatization (24). Yoshida et al. (25) showed IL-8 expression in breast, ovary, pancreas and prostate carcinoma related to higher stage and tumor progression.

It regulates metalloproteinases 2 and 9 promoting stromal infiltration and angiogenesis facilitating the metastatic progression.

Based on carefully analyzed molecular events in RCC, the benefits of individualized therapies will become prominent in the near future. However, considering that genetic alterations in cancer progression are complex and frequently imply multiple paths, the combination of new target-drugs for particular genes involved in each RCC histological subtype will be necessary.

CONCLUSIONS

Distinct gene expression profiles of low-risk, high-risk and metastatic RCCs were demonstrated, with emphasis on the progressive higher expression of the HSP 70 and IL-8 genes from Low-risk to metastatic stage. Based on our findings, it is possible to suggest these genes as starting points for prognostic molecular markers and/or targets for specific therapies.

CONFLICT OF INTEREST

None declared.

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EDITORIAL COMMENT

In this nicely written paper by Dall'Oglio et al., the gene expression profile of clear cell type renal cell carcinoma (RCC) was conducted to identify the functional genes selectively expressed in low-, high-, and metastatic RCC patients. Although several groups have conducted tissue microarray studies focusing on RCC (1-4), the present study has several merits; firstly, the authors have evaluated the gene expression profiles of a uniform patient cohort (i.e. those with clear cell histology) removing study population heterogeneity as a confounding variable, secondly, the authors have stratified their study population according to risk of progression whereby allowing to better define the gene expression profiles of these prognostic subsets. It is clear to me that although major strides have been made in the systemic therapy of metastatic RCC (i.e. tyrosine kinase inhibitors, mTOR inhibitors), it remains clinically disappointing that a partial response or disease stability for a typical period of several months is noted in responders to these systemic agents. The treatment panacea for metastatic RCC (i.e. complete response rendering patients disease-free) will only likely come with a better understanding of the genetic and mechanistic pathways underlying this heterogeneous malignancy. Studies such as this will likely lead to a more personalized therapeutic approach to patients in which the genetic alterations specific to the various subtypes of RCC will be targeted. It is likely that in the not too distant future, a patient with

metastatic RCC will undergo a pre-treatment percutaneous renal biopsy enabling us to not only identify the histologic tumor type but rather develop a tissue microarray identifying the specific genetic alterations in an individual patient's tumor which can then be targeted using a selective treatment combination and enabling a more personalized and highly effective therapeutic approach to be initiated.

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Endourological Management of Forgotten Encrusted Ureteral Stents

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ABSTRACT

Purpose: To present our experience and discuss the various endourological approaches for treating forgotten encrusted ureteral stents associated with stone formation.
Materials and Methods: From July 2006 to December 2008, 14 patients (11 men and 3 women) with encrusted ureteral stents were analyzed. The average indwelling time of the stent was 4.9 years (range 1 to 12). Plain-film radiography was used to evaluate encrustation, stone burden, and fragmentation of the stents. Intravenous urogram and a Tc99m diethylene triamine penta acetic-acid renogram was used to assess renal function.
Results: In seven patients, the entire stent was encrusted, in three patients the encrustation was confined to the ureteral and lower coil part of the stent, two patients had encrustation of the lower coil, and minimal encrustation was observed in two patients. Percutaneous nephrolithotomy was performed in 5 cases and retrograde ureteroscopy with intra-corporeal lithotripsy in 9 patients. Cystolithotripsy was used to manage the distal coil of the encrusted stent in eight patients. Simple cystoscopic removal of the stents with minimal encrustation was carried-out in two cases. Looposcopy and removal of the stent was performed in one patient with an ileal conduit and retained stent. Only one patient required open surgical removal of the stent. Thirteen out of 14 patients were rendered stone and stent free in one session. All except two stents were removed intact and stone analysis of encrustation and calcification revealed calcium oxalate and calcium phosphate in the majority of the cases.
Conclusion: Endourological management of forgotten encrusted stents is highly successful and often avoids the need for open surgical techniques.

Key words: *stents; ureteral; forgotten; lithiasis; management; endourology*
Int Braz J Urol. 2010; 36: 420-9

INTRODUCTION

Ureteral stents are widely used in urological practice. They are mainly indicated after any ureteral surgery and for managing ureteral obstruction due to intrinsic or extrinsic causes like stones, strictures, uretero-pelvic junction obstruction, retroperitoneal fibrosis, malignancies, and congenital anomalies (1-5). They are also placed after iatrogenic injuries to the ureter and before any complex abdominal procedure for identification and protection of the ureters (6). Because of their wide spread usage, complications due to these stents have also increased like, stent encrustation, stent fragmentation, stone formation and recurrent urinary tract infection (7,8). Retention of ureteral stents, often due to poor compliance of the patient is not uncommonly seen (9). If left untreated, these retained stents result in significant morbidity and mortality. Various methods of treatment combinations of extracorporeal shock wave lithotripsy

(SWL), cystolithotripsy (CLT) retrograde ureteroscopy with intracorporeal lithotripsy, percutaneous nephrolithotomy (PCNL) and open surgery have been used for retrieval of these encrusted stents (10-14). We present our experience with the management of these forgotten stents, associated with significant encrustation and stone burden in 14 patients.

MATERIALS AND METHODS

Fourteen patients (11 men and three women) with forgotten ureteral stents with severe encrustation, who presented at our department between July 2006 and December 2008, were treated. Information was obtained through a retrospective review of patients records. The mean patient age was 42.4 years (range 27-55 years) and the average indwelling time of the stent was 4.9 years (range 1-12 years).All the stents were placed elsewhere. Poor compliance and inability of the treating surgeon to counsel the patients were the reasons for retention of these stents. All the patients were evaluated for stent encrustation and associated stone burden by plain-film radiography and intravenous urogram. In patients with non visualized kidneys on intravenous urogram, Tc99m diethylene triamine penta acetic-acid (DTPA) renogram was done to estimate the renal function. Treatment decision was made on clinical and radiological findings. Before intervention, all patients had negative urine cultures, and antibiotic prophylaxis was given for all cases.

Combined endourological procedures PCNL, Cystolithotripsy (CLT), retrograde ureteroscopy with intracorporeal lithotripsy were performed in one session. Retrograde ureteroscopy was performed using 8/9.8F and 6/7.5F semi rigid ureteroscope, under fluoroscopic guidance. Intracorporeal lithotripsy was performed with a pneumatic lithotripter. PCNL was carried-out using a rigid 24F nephroscope. In stents with minimal encrustation on plain-film radiography, a gentle attempt is made for removal with the help of grasping forceps passed through the cystoscope under local anesthesia and fluoroscopic guidance. For patients with encrustation and stone burden involving the lower coil, ureteric (body) or whole of the stent, initially, cystolithotripsy, retrograde ureteroscopy and intra corporeal lithotripsy was performed in the dorsal lithotomy position. Following this, a gentle attempt was made to retrieve the stent with the help of an ureteroscopic grasper. If the stent failed to uncoil, a ureteric catheter was placed adjacent to the encrusted stents for injection of radio-contrast material to delineate the renal pelvis and the calyces. Then the patient was placed in the prone position and PCNL of the upper coil of the encrusted stent along with calculus was done. The approach to the collecting system was through the lower calyx and middle posterior calyx and no patient required upper pole or supra costal access. A 14F nephrostomy tube was kept indwelling for 48 hours, in patients who required PCNL. Stone analysis and encrustation analysis was done in all cases. Post operatively, plain-film radiography was done to confirm the stone free and stent free status.

RESULTS

The patient characteristics, initial indications for stenting, indwelling time, site of encrustation, type of procedure performed and length of hospital stay are shown in Table-1. The entire stent was encrusted in 7 patients; three patients had predominantly ureteric (body) and lower coil encrustation (Figure-1) and in 2 patients, there was minimal encrustation. In 11 out of 14 patients, the initial indication for stent placement was for urinary stone disease. General anesthesia was required for removal of the encrusted stents in eleven patients, and three patients were managed as outpatients under local anesthesia. The mean hospital stay in 11 patients was 4.9 days. As shown in Table-1, CLT was required to treat the distal end of the stent in seven cases. PCNL was done in 5 cases. Retrograde ureteroscopy and intra corporeal lithotripsy was performed in eight cases. Simple cystoscopic removal of the stent under fluoroscopic guidance was done in two cases.

A 54-year-old male patient who underwent radical cystectomy and ileal conduit 4 years previously, presented with left loin pain and dysuria for 3 months. Plain-film radiography of the abdomen revealed a retained stent on the left side with minimal encrustation. Looposcopy with 24F nephroscope and removal of the retained stent was done with the help of a grasping forceps under fluoroscopic guidance.

Table 1 *– Patient characteristics, initial indications for stenting, indwelling time, site of encrustation and procedure details of the encrusted stents.*

N	Age/Sex	Initial Indications for Stenting	Indwelling Time (years)	Site of Encrustation			Procedure	Length of Hospital Stay (days)
				Kidney	Ureter	Bladder		
1	50/M	Left Pyelolithotomy	4	+++	++	+++	CLT, retrograde ureteroscopy, intracorporeal lithotripsy and PCNL	4
2	29/M	Left Pyeloplasty	4	+++	++	+++	Retrograde ureteroscopy, intracorporeal lithotripsy and PCNL	4
3	39/M	Left Pyelolithotomy	9	+++	+++	+++	CLT, retrograde ureteroscopy, intracorporeal lithotripsy and PCNL	5
4	45/M	Right URSL	4	-	++	++	Retrograde ureteroscopy, intracorporeal lithotripsy and PCNL	2
5	35/F	Left Pyelolithotomy	9	++++	++++	++++	Pyelolithotomy, ureterotomy and cystolithototmy	8
6	40/F	Left URSL	1	-	-	+	Cystoscopic removal of the stent	Out patient
7	55/M	Left Pyelolithotomy	12	+++	++	+++	CLT, retrograde ureteroscopy, intracorporeal lithotripsy and PCNL	14
8	45/F	Ovary Ca	1	-	-	-	Cystoscopic removal of the stent	Out patient
9	54/M	Bladder Ca, post cystectomy, ileal conduit	4	-	-	-	Looposcopy and removal of the stent	Out patient
10	54/M	Right URSL	3		++	++	Retrograde ureteroscopy, intracorporeal lithotripsy	2
11	44/M	Right PCNL	5	+++	++	+++	CLT, retrograde ureteroscopy, intracorporeal lithotripsy and PCNL	3
12	42/M	Right Pyelolithotomy	6	++	++	+++	CLT, retrograde ureteroscopy, intracorporeal lithotripsy and PCNL	3
13	27/M	Post ESWL	2	-	++	+	Retrograde ureteroscopy, intracorporeal lithotripsy	2
14	35/M	Left Pyelolithotomy	5	-	-	+++	CLT and stent removal	2

M = male; F = female; URSL = ureterorenoscopic lithotripsy; SWL = extracorporeal shock wave lithotripsy; PCNL = percutaneous nephrolithotomy; CLT = cystolithotripsy.

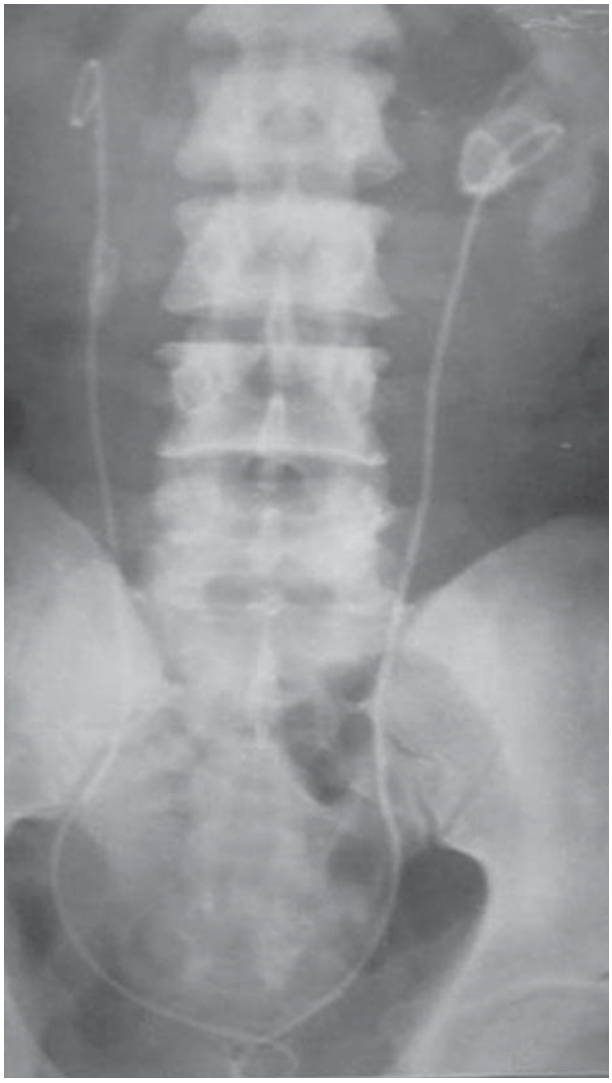

Figure 1 *– Plain-film radiography of a patient who presented with renal failure. Left side retained ureteral stent with extensive calcification at the proximal end of the stent. On the right side, a ureteric calculus was seen and a stent was placed preoperatively to normalize serum creatinine level.*

One patient presented with a left retained ureteral stent for 9 years and right upper ureteric calculus, and a serum creatinine level of 5.4 mg%. Initially, a right ureteral stent was placed to normalize the renal parameters, and subsequently, a push back PCNL was done for the right ureteric calculus to achieve complete stone clearance. On the left side, a retrograde ureteroscopy with intracorporeal lithotripsy and PCNL was required to fragment and retrieve the encrusted stent (Figure-2).

Another patient presented with retained stent for 9 years following pyelolithotomy on the left kidney. Plain-film radiography showed extensive calcification of the entire stent. Intravenous urogram and DTPA renogram showed good renal function. CLT of the lower coil of the encrusted stent was attempted, but the pneumatic lithotripter failed to fragment the stone. Since we do not have the facility of laser lithotripsy open surgical removal by cystolithotomy, ureterotomy and pyelolithotomy was done to retrieve the stent (Figure-3).

Percutaneous nephrostomy was carried-out in one patient who presented with acute pyelonephritis. The dwelling time of the encrusted stent in this patient was 12 years. After negative bacterial culture of the blood and urine, the patient underwent surgery. CLT, retrograde ureteroscopy with intracorporeal lithotripsy was required for removal of the stent. On the second postoperative day, he developed urosepsis, requiring broad spectrum antibiotics and intensive care management. This patient had a prolonged hospital stay of 14 days. Thirteen out of 14 patients (93%) were rendered stone free and stent free after a single anesthetic session. Re-stenting was done in one patient requiring open surgery and subsequently, his stent was removed after four weeks. No intra operative complications occurred in any patient. All the stents were removed intact except in two patients, who had fragmented stents at presentation. Stone analysis showed calcium oxalate and phosphate in the majority of cases. Struvite stones were seen in two cases.

COMMENTS

Forgotten ureteral stents are observed in urologic practice because of poor compliance of the patient or failure of the physician to adequately counsel the patient. These forgotten stents can produce considerable morbidity and mortality, due to extensive encrustation with significant stone burden, knot formation, upward migration and fragmentation (7,15). Encrustation of forgotten stents associated with large stone burden is a serious problem, due to complications like recurrent urinary tract infection, hematuria, obstruction and renal failure (16). The deposition of encrusted material on retained ureteral

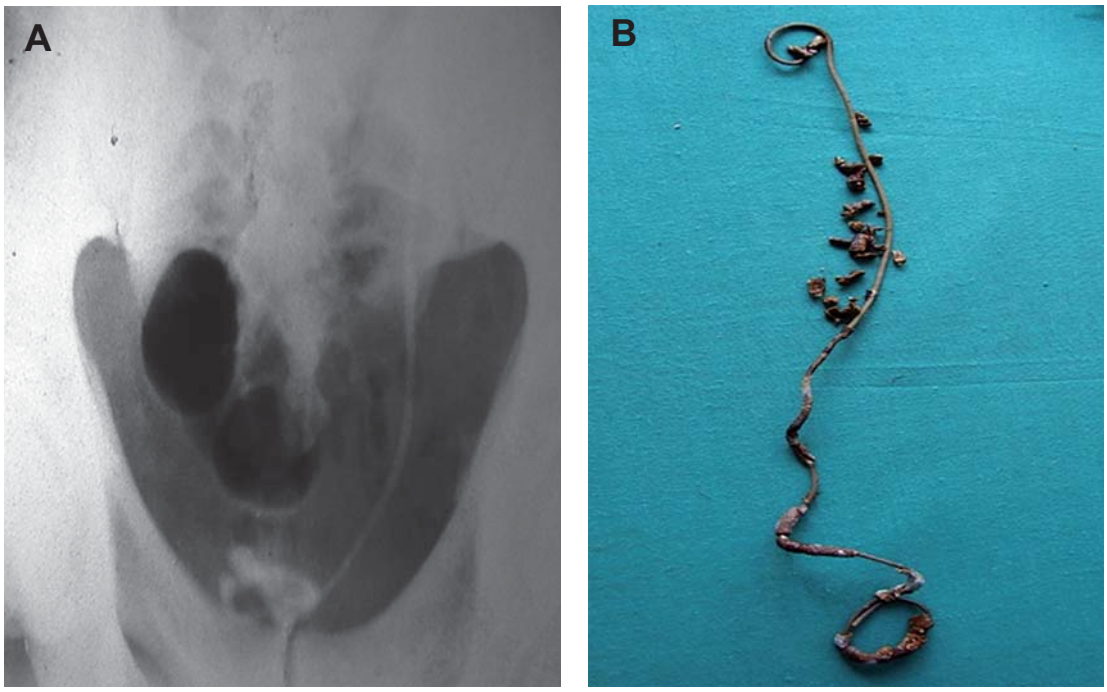

Figure 2 – *A) Plain-film radiography of a 54 yr old male showing encrusted stent with calculus formation at the lower part and bladder coil of the stent. B) Retrieved stent showing extensive encrustation and stone formation.*

stents can occur in both infected and sterile urine. The mechanism of encrustation in infected urine is a result of organic components in the urine crystallizing out onto the surface of biomaterial and becoming incorporated into a bacterial biofilm layer. Urease produced by the adhered bacteria hydrolyses the urea to produce ammonia. This elevates urinary pH, favoring the precipitation of magnesium and calcium as struvite and hydroxyl apatite (17,18). Although the exact mechanism of encrustation in sterile urine is unclear, it appears to be dependent on the pH, ionic strength and biomaterial hydrophobic properties (19). The degree of encrustation is dependant on the dwelling time. El faqih et al. found that encrustation increased from 9.2% at < 6 weeks to 47.5% at six to 12 weeks to 76.3% at > 12 weeks of dwelling time (20). Other factors implicated in the increased incidence of encrustations are chronic recurrent stone formers, metabolic predisposition to stone disease, congenital renal anomalies, malignant urinary obstruction and pregnancy (21).

Fragmentation is another important complication of the forgotten stents. It is the result of loss

Figure 3 – *Retrieved stent with extensive calcification. This patient had a retained stent for 9 years. Open surgery was required to remove the stent.*

of tensile strength, which is due to hardening and degeneration of the stent polymers (22). The risk of encrustation and fragmentation is dependant on the type of material of the stent. Silicone was found to be least prone to encrustation, followed by polyurethane, silitek, percuflex and hydro gel coated polyurethane (23). Fragmentations of polyurethane stents are four times as frequent as the silicone stents (9). In our series, fragmentation of the lower coil of the stent is seen in two cases at the time of presentation. The indwelling time in both the cases was five years. All the retrieved encrusted stents in our series were made of polyurethane.

Retained ureteral stents with encrustation is a challenging problem for endourologists. Very often, multiple endourological approaches are needed because of encrustations and the associated stone burden that may involve bladder, ureter and kidney. This may require single or multiple endourological sessions or rarely open surgical removal of the encrusted stents. Singh et al. described multiple accesses and approaches including open surgery to treat the retained stents (24). Borboroglu et al. also reported the endourological treatment of four patients with severely encrusted ureteral stents with a large stone burden. All patients required two to six endourological approaches (average 4.2) performed at one or multiple sessions, to achieve stone-free and stent-free status. These authors concluded that percutaneous nephrolithotomy and ureteroscopy are often necessary for treating a severely encrusted stent and associated stone burden (13). One stage removal of 12 encrusted retained ureteral stents has been reported by Bukkapatnam et al., in ten patients. Of these, 11 were managed by ureteroscopy alone and in one patient; the stone was treated through a percutaneous approach. They concluded that, these stents can be removed in one sitting with minimal morbidity and short hospital stay (25). Using a combination of SWL, PCNL, CLT, ureteroscopy with intra corporeal lithotripsy, clearance rates ranging from 75 to 100% have been reported (10,12,22).

The site of encrustation, associated stone burden and the function of the affected kidney often dictate the method of access and treatment (Figure-4). Our approach towards management of these difficult stents is based on the findings on plain-film radiography. The proximal, distal coils and the body of the stent are examined for encrustation, calcification and fragmentation. We did not find any additional benefit in the management plan with the use of non contrast computed tomography. Intravenous urogram and DTPA renogram is obtained to determine the function of the kidney. Nephrectomy is done for non salvageable function of the kidney. Nephrostomy or placement of second stent is done, if the patient presented with pyelonephritis and sepsis. It is possible to put a second stent adjacent to the encrusted stent because the ureter is dilated in majority of these cases.

Extracorporeal shock wave lithotripsy (SWL) is the initial treatment with stents with minimal encrustation. However, in our series, no patient required SWL because of extensive stone burden in majority of cases. If there are no encrustations visible on plain-film radiography, our approach is cystoscopic removal using a grasping forceps under local anesthesia with fluoroscopic guidance. Gentle traction on the stent is applied, if patient complains of pain and if the stent does not uncoil, the procedure is abandoned. An important precaution during the procedure is to avoid using excessive force, which can result in breakage of the stent along with ureteral injury or ureteral avulsion.

The next stage is CLT with the help of pneumatic lithotripter on stents with minimal encrustation and those with lower coil encrustation. This followed by gentle pull under fluoroscopic guidance. If the cystoscopic approach fails, and in patients with encrustation involving the ureteric portion of the stent, the next approach is under anesthesia, a safety guide wire is passed along the retained stent and ureteroscope is passed retrograde. Calcifications over the stent can be fragmented with a pneumatic lithotripter or laser energy, while carefully advancing the ureteroscope into the renal pelvis. After all the encrustations and calcification have been fragmented, the stent is gently removed with the help of grasping forceps passed through the ureteroscope under fluoroscopic guidance. Following removal of the stent, it is mandatory to do a retrograde uretrogram and check ureteroscopy to rule out a ureteric injury. If any signs of ureteric injury or contrast extravasation present, the patient should be re-stented.

For stents with large stone burden and those stents which fail to be retrieved by the above-men-

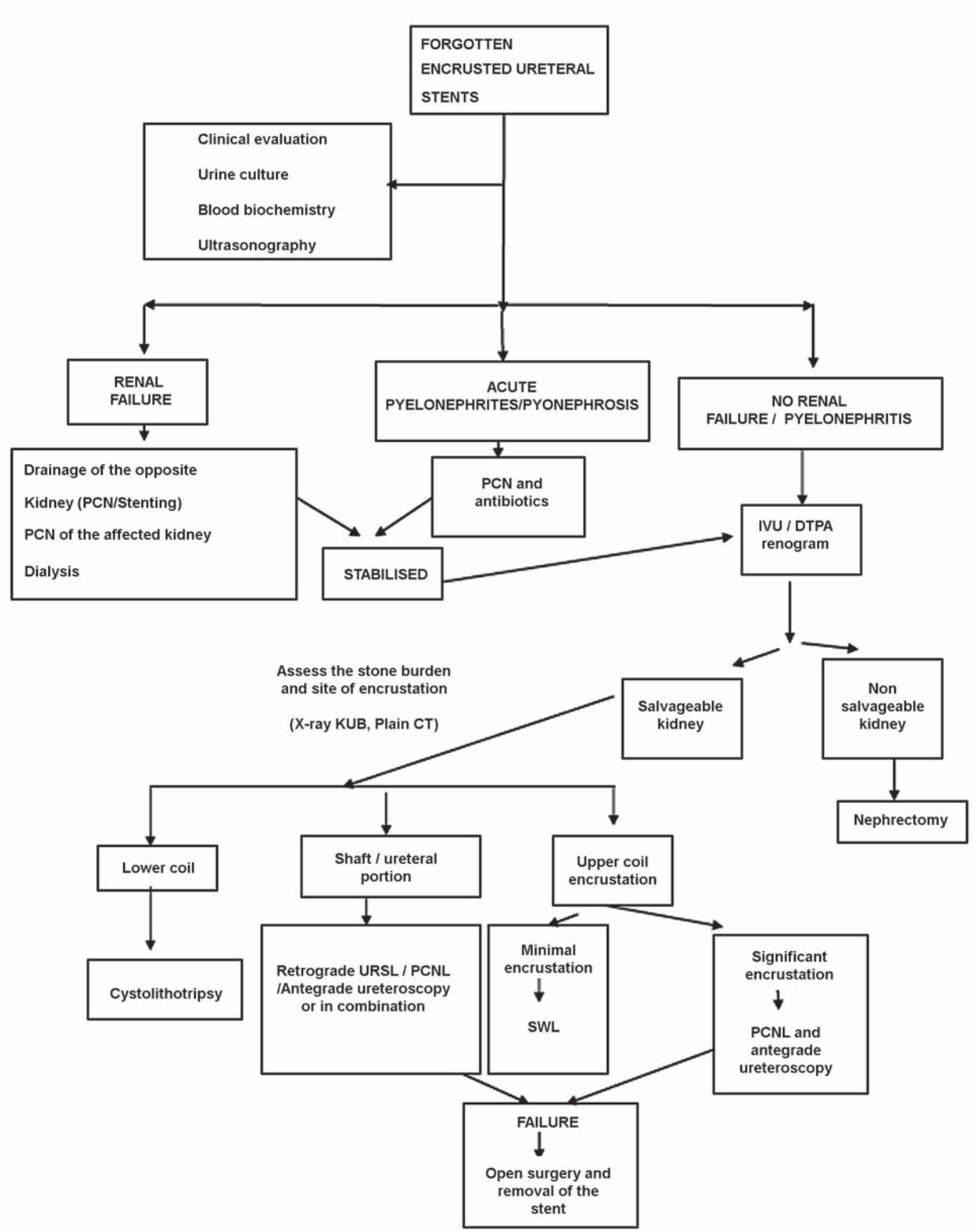

Figure 4 *– Algorithm for the management of forgotten encrusted stents. CT = computer tomography; DTPA = diethylenetriamine penta-acetic acid; SWL = extracorporeal shock wave lithotripsy; IVU = Intravenous Urogram; KUB = kidneys, ureters, and bladder; PCNL = percutaneous nephrolithotomy; URSL = ureterorenoscopic lithotripsy.*

tioned techniques, a 5F ureteric catheter is placed to enable the injection of radio contrast material into the renal pelvis and calyces as an aid to subsequent percutaneous access and the patient is placed in the prone position. Percutaneous access is established by a lower calyceal or middle calyceal puncture and the proximal coil of the stent along with stone is fragmented. The stent is gently removed under fluoroscopic guidance through the percutaneous nephrostomy tract.

Using the above-mentioned approach, it was possible to remove all stents in 13 out of 14 patients, using the endourological approach alone under a single anesthesia. Open surgery was done in one case because of the extensive stone burden and failure of the pneumatic lithotripter to fragment the stone. Based on our method of approach, an algorithm has been proposed for the management of these stents (Figure-4).

Although, endourological management of these stents achieves success in majority of the cases with minimal complications, the best treatment that remains is prevention of this complication. The treating physician should be very selective in placing the stents and they must be tracked very closely by documenting insertion and removal of the stents. All patients should be counseled with respect to the complications of long term use and advised when their stent should be changed. As mentioned earlier, the degree of encrustation is dependant on the indwelling time, so, it is necessary to keep the indwelling time to as short as possible. Various authors have reported that indwelling time between 2-4 months is safe (9-12,20). For patients requiring stents beyond this period, they should be kept on prophylactic antibiotics and have their stents frequently changed.

It is interesting to note that, two of three patients who did not have stents for stone disease, were able to have their stents simply removed by cystoscopy, while this was successful in none of the stone formers. The reason for this could be due to increase risk of encrustation and stone formation in patients who have a history of stone disease. This underscores the importance of frequent monitoring in these groups of patients to avoid life threatening complications.

It is also important to maintain a proper record of all stents inserted and keep a track of their due date of removal. Some authors have proposed a computerized tracking program for removal stents (26). Coatings such as hydrophilic polymers, heparin, pentosan polysulfate, or oxalate -degrading enzymes have been used in an attempt to reduce encrustation (27-30). The use of bio-degradable compound of poly-L-lactic and glycolic acids which are designed to disintegrate can eliminate the problem of retention and encrustation of the stents in the near future (31).

CONCLUSION

Encrustation and stone formation in forgotten stents often lead to life threatening complications and pose a challenging management task for the treating surgeon.

The use of various combinations of endourological techniques can achieve effective stent and stone treatment after a single anesthesia session with minimal morbidity and short hospital stay. Imaging and assessment of the degree of stone burden is important, before making any attempt to remove these stents. Maintenance of efficient log book under direct supervision of treating surgeon and proper patient counseling is required to prevent this complication.

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CONFLICT OF INTEREST

None declared.

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Vascular Endothelial Growth Factor (VEGF) and Prostate Pathology

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ABSTRACT

Purpose: Previous studies suggest that vascular endothelial growth factor (VEGF) circulating levels might improve identification of patients with prostate cancer but results are conflicting. Our aim was to compare serum VEGF levels across different prostate pathologies (including benign prostatic hyperplasia, prostatitis, high grade prostate intraepithelial neoplasia and prostate cancer) in patients at high risk of prostate cancer.
Materials and Methods: We consecutively enrolled 186 subjects with abnormal digital rectal examination and/or total PSA (tPSA) ≥ 2.5 ng/mL. Blood was collected before diagnostic ultrasound guided trans-rectal prostate biopsy, or any prostate oncology treatment, to measure PSA isoforms and VEGF. Unconditional logistic regression was used to compute age-, tPSA- and free/total PSA-adjusted odds ratios (OR) and respective 95% confidence intervals (95% CI) for the association between serum VEGF and different prostatic pathologies.
Results: Prostate biopsy main diagnoses were normal or benign prostatic hyperplasia (27.3%), prostatitis (16.6%), and prostatic cancer (55.0%). The median VEGF levels (ng/mL) in these groups were 178.2, 261.3 and 266.4 (p = 0.029), respectively, but no significant differences were observed for benign vs. malignant pathologies (215.2 vs. 266.4, p = 0.551). No independent association was observed between VEGF (3rd vs. 1st third) and prostate cancer, when compared to benign conditions (adjusted OR = 1.44; CI 95%: 0.64-3.26).
Conclusions: In patients at high risk of prostate cancer, circulating VEGF levels have no clinical role in deciding which patients should be submitted to prostate biopsy. Prostatitis patients, often with higher PSA levels, also present high serum levels of VEGF, and their inclusion in control groups might explain the heterogeneous results in previous studies.

Key words: *prostate; prostatic neoplasms; vascular endothelial growth factor A*
Int Braz J Urol. 2010; 36: 430-8

INTRODUCTION

Prostate cancer is the most commonly diagnosed non dermatologic malignancy and the third leading cause of cancer mortality among men in Europe (1). Prostatic specific antigen (PSA) is widely used for prostate cancer screening, despite its low accuracy across different cut-offs (2). However, the need to avoid unnecessary biopsies and missed diagnosis has led to the study of several other biomarkers that could further contribute to decide which patients should be referred for prostatic biopsy.

Vascular Endothelial Growth Factor (VEGF) is a growth factor involved in the promotion of endothelial cell proliferation, vascular permeability and angiogenesis, which are critical stages for tumor growth and development, namely prostate cancer (3). It is synthesized by adenocarcinoma cells (4,5), and in prostatic cancer patients the prostatic gland contributes considerably to circulating VEGF levels (6). Elevated plasma VEGF levels could reflect prostatic VEGF production, making VEGF a potentially interesting tumor marker to support the decision of submitting a patient to prostatic biopsy.

Previous studies on this topic are conflicting. Some authors have found higher levels of VEGF in prostatic cancer patients (7-10), while others found no differences between subjects with benign prostatic hyperplasia (BPH) and those with malignant disease (11,12), or increased values only in patients with metastatic prostatic cancer (13) or hormone-refractory disease (14). However, most previous studies evaluated relatively small samples (8,9,11-14) and all suffered from limited-challenge bias, as prostatitis, which may interfere with the diagnostic value of VEGF, was not evaluated separately in any of the studies and in many studies the control group only included subjects with no suspicion of prostatic cancer (7,10,13).

We attempted to evaluate VEGF as a diagnostic tool for prostatic cancer, comparing its serum levels across groups of patients with suspected prostate cancer, presenting different prostatic pathologies (including BPH, prostatitis, high grade prostate intraepithelial neoplasia (HGPIN) and prostate cancer).

MATERIALS AND METHODS

Patient Selection

During 2006 we consecutively enrolled 186 candidates referred to ultrasound guided trans-rectal prostate biopsy, on the basis of abnormal rectal examination and/or elevated total PSA (tPSA) levels (≥ 2.5 ng/mL), in the Department of Urology of S. Joao Hospital. None of the patients received hormonal therapy, radiotherapy or chemotherapy before undergoing prostate biopsy.

Measurement of Biomarkers

Blood was collected from all participants prior to biopsy, and samples were allowed to clot for 30 minutes before centrifugation. Part of the serum was used for a new assessment of tPSA, free PSA (fPSA) and complexed PSA (cPSA). The remaining serum was frozen (-20ºC), and subsequently was used for VEGF quantification by ELISA (quantitative sandwich enzyme immunoassay technique) double determinations with Quantikine®, a Human VEGF Immunoassay (R&D Systems, Minneapolis, MN).

Outcome Evaluation

The final prostate pathology and the prostate cancer cases Gleason score were defined by biopsy results. The number of biopsy cores ranged from 8 to 13. All prostatic biopsies were reviewed by two different pathologists that were blinded to the patients' different PSA isoforms and VEGF values. Patients were grouped into four mutually-exclusive groups, according to the most severe diagnosis observed in the biopsy specimens, as follows (ordered by increasing severity): normal prostate or BPH (N/BPH), prostatitis, HGPIN, and prostatic cancer.

Statistical Analysis

The Kruskal-Wallis test was used to compare quantitative variables across prostate pathology groups. Spearman correlation coefficients were computed to quantify the association between VEGF and age, tPSA, cPSA and f/t PSA ratio.

A receiver operating characteristic (ROC) analysis was used to compute the area under the ROC curve (AUC) and to identify the VEGF level cut-off for which a higher proportion of patients was correctly classified when distinguishing prostatic cancer from benign diagnosis.

Unconditional logistic regression was used to compute odds ratios (OR) and respective 95% confidence intervals (95% CI) for the association between serum VEGF levels (groups defined using tertiles as cut-offs and the cut-off defined by the ROC

curve analysis) and different prostatic pathologies, crude and adjusted for age, tPSA and f/tPSA. The tPSA levels were modeled after log-transformation. Further analyses were conducted combining N/BPH, prostatitis and HGPIN in a group of benign pathology. Due to the low number of patients with HGPIN, these patients were excluded from the analyses by prostatic pathology subgroups, and considered only when comparing malign with all types of benign pathology.

Statistically significance in this study was set as $p < 0.05$. All reported p values are two-sided. Statistical analysis was performed using STATA®, version 9.2.

RESULTS

The median age of the participants was 68 years (percentile 25-percentile 75 [P25-P75]: range 62-73), the median tPSA level was 7.4 ng/mL (P25-P75: 5.4-12.1) and the median f/tPSA ratio was 0.16 (P25-P75: 0.08-0.23).

Table 1 – *Characteristics of the participants stratified by prostatic histology.*

		N / BPH	Prostatitis	HGPIN	Prostate Cancer	p Value
N		51	30	6	99	
Median Age (years)		67.0	67.0	67.0	69.0	0.678
Age (years)	≤ 60	13 (25.5%)	5 (16.7%)	1 (16.7%)	16 (16.2%)	0.766
	60-70	20 (39.2%)	15 (50.0%)	3 (50.0%)	40 (40.4%)	
	> 70	18 (35.3%)	10 (33.3%)	2 (33.3%)	43 (43.4%)	
Median tPSA (ng/mL)		5.7	9.9	6.1	8.3	< 0.001
tPSA (ng/mL)	≤ 4	11 (22.0%)	1 (3.4%)	1 (16.7%)	10 (10.1%)	0.006
	4-10	33 (66.0%)	14 (48.3%)	4 (66.7%)	51 (51.5%)	
	> 10	6 (12.0%)	14 (48.3%)	1 (16.7%)	38 (38.4%)	
Median cPSA (ng/mL)		4.0	6.7	4.6	6.4	< 0.001
cPSA (ng/mL)	≤ 4	26 (51.0%)	5 (1.2%)	3 (50.0%)	18 (18.6%)	< 0.001
	4-10	24 (47.1%)	21 (72.4%)	3 (50.0%)	53 (54.6%)	
	> 10	1 (2.0%)	3 (10.3%)	0 (0.0%)	26 (26.8%)	
Median f/tPSA ratio		0.22	0.20	0.22	0.11	< 0.001
f/tPSA ratio	≤ 0.15	14 (28.0%)	10 (34.5%)	0 (0.0%)	68 (68.7%)	< 0.001
	> 0.15	36 (72.0%)	19 (65.5%)	6 (100.0%)	31 (31.3%)	
Median VEGF (pg/mL)		178.2	261.3	251.9	266.4	0.067
VEGF * (pg/mL)	≤ 170	24 (47.1%)	6 (20.0%)	2 (33.3%)	31 (31.3%)	0.087
	171-335	18 (35.3%)	11 (36.7%)	3 (50.0%)	31 (31.3%)	
	> 335	9 (17.6%)	14 (43.3%)	1 (16.7%)	37 (37.4%)	

** Tertiles were used to define cut-offs. N / BPH = normal prostate or benign prostate hyperplasia; HGPIN = high grade prostate intraepithelial neoplasia; PSA: prostate-specific antigen; VEGF = vascular endothelial growth factor*

Prostatic biopsies revealed prostatic cancer in 99 cases (53.2%), prostatitis in 30 cases (16.1%), HGPIN in 6 cases (3.2%), BPH in 32 cases (17.2%) and normal prostate in the remaining 19 participants (10.2%). Among prostatic cancer cases the Gleason score was 6 in 21.4% patients, 7 in 51.1% and 8 or higher in 23.4%.

Table-1 summarizes participants' characteristics stratified by prostatic histology. Age was similar between groups, but tPSA and cPSA were significantly higher in patients with prostatitis (9.9 and 6.7 ng/mL, respectively) and prostatic cancer (8.3 and 6.4 ng/mL respectively) when compared to N/BPH (5.7 and 4.0 ng/mL, respectively). The median f/t PSA ratio was lower in prostatic cancer patients compared to patients with benign histology (0.11 vs. 0.21).

The median serum VEGF level in our sample was 232.3 pg/mL (range: 16.4-1648.3 g/mL; P25-P75: 144.0-339.6 pg/mL). There was a weak positive correlation between VEGF and tPSA ($r = 0.18$; $p = 0.013$) and a weak negative correlation between VEGF and f/t PSA ratio ($r = -0.17$; $p = 0.017$). No significant association was observed with age ($r = -0.04$; $p = 0.56$) or cPSA ($r = 0.15$; $p = 0.054$). These results were similar when stratified by prostatic pathology (data not shown).

As presented in Figure-1, VEGF levels were significantly higher in prostatic cancer and prostatitis than in N/BPH (median: 266.4, 261.3 and 178.2 pg/mL, respectively; $p = 0.029$), but no statistically significant difference was observed when comparing prostatic cancer with benign pathology (median: 215.2 vs. 266.4 pg/mL, respectively; $p = 0.551$). The median VEGF levels were similar when the analysis was restricted to patients with tPSA between 2.5 and 10 ng/mL (211.5 pg/mL for benign histology and 246.7 pg/mL for prostatic cancer; $p = 0.67$). These results were similar if patients with HGPIN were excluded (data not shown).

The ROC curve of VEGF serum levels for the detection of prostatic malignancy is presented in Figure-2. The AUC was 0.53 (95% CI: 0.44-0.61) and the cut-off value for which a higher proportion of patients was correctly classified (57.0%) was 266.4 pg/mL.

Higher VEGF levels (3rd third vs. 1st third) were approximately twice more likely in patients with prostate cancer compared to N/BPH, but the adjusted estimates were not significantly different from unity (OR = 2.19, 95% CI: 0.76-6.31) (Table-2).

Results comparing benign and malignant prostatic pathology are presented in Table-3. In gen-

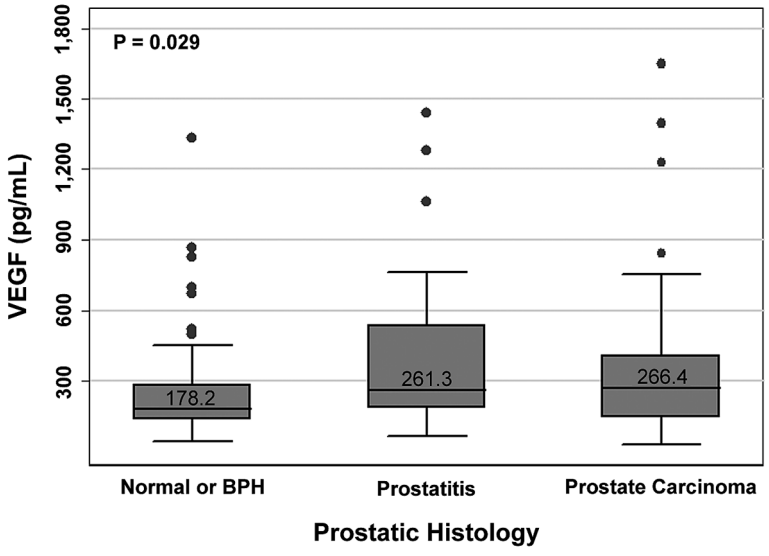

Figure 1 *– Serum levels of vascular endothelial growth factor (VEGF), according to prostate biopsy histology. BPH: benign prostatic hyperplasia.*

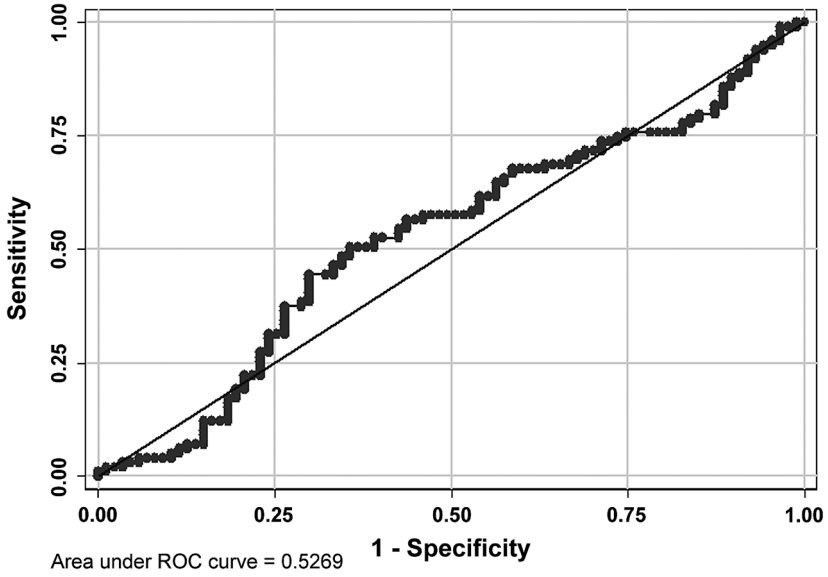

Figure 2 – *Receiver operating characteristic (ROC) curves for vascular endothelial growth factor (VEGF) serum levels as a test for diagnosis prostate carcinoma using the biopsy results as the gold standard.*

eral, OR estimates were lower than when N/BPH was used as reference. The age- and PSA-adjusted OR for the association between prostatic cancer and higher VEGF levels (3rd third vs. 1st third) was 1.44 (95% CI: 0.64-3.26).

In prostatic cancer patients, the VEGF levels were not significantly different across Gleason score groups. The median values were 258.8 pg/mL for patients with histological Gleason score 6, were 272.5 pg/mL for those with Gleason score 7 and 234.8 pg/

Table 2 – *Multivariate logistic analysis of the association between vascular endothelial growth factor (VEGF) and prostate histology.*

	N / BPH	Prostatitis			Prostate Cancer		
Serum VEGF (pg/mL)	**N** (%)	**N** (%)	**OR** (95% CI)	**OR*** (95% CI)	**N** (%)	**OR** (95% CI)	**OR*** (95% CI)
< 266.4§	37 (72.6)	16 (53.3)	1 (reference)	1 (reference)	50 (50.5)	1 (reference)	1 (reference)
> 266.4§	14 (27.4)	14 (46.7)	2.31 (0.90-5.95)	1.63 (0.53-5.03)	49 (49.5)	2.59 (1.25-5.38)	1.89 (0.81-4.38)
≤ 170†	24 (47.1)	6 (19.4)	1 (reference)	1 (reference)	31 (31.3)	1 (reference)	1 (reference)
171-335†	18 (35.3)	11 (35.5)	5.78 (1.68-19.85)	4.57 (1.00-20.94)	31 (31.3)	3.18 (1.29-7.85)	2.18 (0.79-6.05)
> 335†	9 (17.6)	14 (45.2)	2.36 (0.76-7.34)	1.05 (0.28-3.99)	37 (37.4)	2.39 (0.94-6.06)	2.19 (0.76-6.31)

** = adjusted for age; tPSA and f/tPSA; § = cut-off that optimizes proportion of patients correctly classified; † = tertiles were used to define cut-offs; N / BPH = normal prostate or benign prostate hyperplasia.*

Table 3 – *Multivariate logistic analysis of the association of vascular endothelial growth factor (VEGF) with prostate cancer.*

	Benign Histology	Prostate Cancer		
Serum VEGF (pg/mL)	**N** (%)	**N** (%)	**OR** (95% CI)	**OR*** (95% CI)
< 266.4§	56 (64.4)	50 (50.5)	1 (reference)	1 (reference)
> 266.4§	31 (35.6)	49 (49.5)	1.77 (0.98-3.19)	1.22 (0.62-2.40)
≤ 170†	32 (36.8)	31 (31.3)	1 (reference)	1 (reference)
171-335†	32 (36.8)	31 (31.3)	1.66 (0.81-3.40)	1.01 (0.44-2.31)
>335†	23 (26.4)	37 (37.4)	1.66 (0.81-3.40)	1.44 (0.64-3.26)

** = adjusted for age, tPSA and f/tPSA; § = cut-off that optimizes proportion of patients correctly classified; † = tertiles were used to define cut-offs; benign histology = including normal prostate or benign prostate hyperplasia, prostatitis and high grade prostate intraepithelial neoplasia; CI = confidence intervals; OR: odds ratio.*

mL for those with the more aggressive score Gleason score 8-10 (p = 0.716).

COMMENTS

VEGF levels are higher in subjects with prostatitis and prostatic cancer compared to patients at high prostate cancer risk but whose prostatic biopsy only revealed normal or hyperplasic tissue. However, in this consecutive series of patients eligible for prostatic biopsy there were no overall differences in VEGF serum levels between subjects with benign prostatic disease and prostate cancer cases.

Our results contribute to explain the heterogeneity observed in the literature on this topic. Prostatitis is an inflammatory condition associated with angiogenesis that raises VEGF levels, similar to the observed in prostate cancer, and may be highly prevalent in patients with increased tPSA levels. Reports of prostatitis prevalence range from 10% to 63% (15), and was 16.1% in our series. We observed no relevant difference in VEGF circulating levels between patients with benign prostatic histology and cancer, when patients with prostatitis were also considered in the latter group. The two previous studies (11,12) that evaluated participants with high risk of prostate cancer also observed no significant associations between cancer and VEGF levels.

Other studies (7-10,13,14) showed higher VEGF levels in patients with prostate cancer when compared with healthy controls or subjects with benign prostatic hypertrophy. Such comparisons however, are not clinically relevant since elevated tPSA is the most frequent indication for prostatic biopsy, and reflect limited-challenge-bias (16,17). A diagnostic test must be evaluated in a clinically relevant population, preferably in a consecutive series of individuals in whom the target condition is suspected (17). Studies using healthy controls, not representing the whole spectrum of potential diagnosis alternative to prostate cancer which are able to generate false-positive results, namely when prostatitis is present, produce inflated estimates of diagnostic accuracy (18).

Also, in some of these studies (7,10,13,14) whose controls were not suspected of having prostate cancer, the investigators did not perform any biopsy in the individuals that were categorized as healthy or only presenting BPH based on low PSA levels and a negative digital rectal examination. However, Thompson et al. (19) detected prostatic cancer in 10.1 percent among those with values of 0.6 to 1.0 ng/mL, 17.0 percent among those with values of 1.1 to 2.0 ng/mL, 23.9 percent among those with values of 2.1 to 3.0 n/mL, and 26.9 percent among those with values of 3.1 to 4.0 ng/mL. These values can lead to a differential information bias that would cause an underestimation of the true association measure.

VEGF could also be important in clinical practice if its levels were higher in patients with worst prognosis prostatic cancer (those with higher Gleason score or in higher clinical stage). In our study,

we did not find any significant associations between VEGF levels and Gleason score in the 99 prostate cancer patients, in accordance with previous reports (8,11,20,21). Only Shariat et al. (7) describe higher VEGF levels in those with higher Gleason score, and Duque et al. (13) report higher levels in patients with Gleason score ≥ 8 although no positive relation between Gleason score and plasma VEGF was observed. Differences in VEGF levels between metastatic and localized prostatic cancer have been reported (13), but we decided not to make this type of evaluation in our study due to the restricted number of cases.

To measure serum VEGF we used the kit from R&D Systems that has an intra-assay coefficient of variation of 4.5% (22). The Elisa kit has been used previously (7,12,13) and is considered adequate to measure VEGF in serum or plasma.

Circulating VEGF in serum from cancer patients may reflect an aggregate of tumor-cell and platelet-stored VEGF (23). To better reflect the disease-related circulating VEGF levels, the use of rapidly processing citrated plasma samples and additional centrifugation has been recommended (23). This has been disputed, by other authors, suggesting that both plasma and serum levels of VEGF may be equally useful (24). Nonetheless, there is a potential for an information bias in our VEGF levels that we cannot exclude, although its effects are difficult to predict.

The use of circulating VEGF to predict disease staging, patient outcome, early identifying patients at higher risk of lymph node metastases or selecting patients for early systemic intervention or adjuvant radiation therapy, sparing others from the associated morbidity with these treatment options, are still under study and can provide important advances in prostate oncology. Ultimately, a better understanding of the VEGF system should provide additional knowledge about prostatic cancer growth that would allow us to develop better molecular markers for use in clinical practice.

Our results show that VEGF levels have no clinical importance in deciding which patients suspected of having prostatic cancer should be submitted to prostatic biopsy. The exclusion of patients with prostatitis from the control group is the probable cause of the heterogeneous results in previous studies.

CONFLICT OF INTEREST

None declared.

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EDITORIAL COMMENT

Neovascularization is required to sustain growth of solid tumors and the main proteins involved in the angiogenic cascade are fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF). The possibility to detect VEGF in urine post prostate massage or in serum of men in risk to develop the neoplasia is tempting and could help the lack of specificity of prostate specific antigen (PSA), avoiding unnecessary biopsies. VEGF has already been described as an important player in prostate carcinogenesis, allowing tumor growth and dissemination. The authors studied VEGF in serum of patients with abnormalities on digital rectal examination and/or PSA $\geq$ 2.5 ng/mL showing higher levels of VEGF in cancer but also in prostatitis. Prostatitis has been related to cancer development by some authors, and Narayan et al. (1) have recently described prostate cancer induction in animal models using testosterone and N-methyl-N-nitrosourea showing abnormal expression of inflammatory mediators and VEGF. VEGF serum levels may also be elevated in patients with prostatitis in the present study should be interpreted as a link between chronic inflammation and the development of the neoplasia. VEGF expression should be an important player for tumor initiation and/or progression related to chronic inflammation. Even though the authors concluded that VEGF serum detection is not helpful to indicate prostate biopsy, their results should be used as a new trail to be explored in prostate carcinogenesis, opening a new possibility of using angiogenesis inhibitors as prostate cancer prevention.

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Does Tumor Extent on Needle Prostatic Biopsies Influence the Value of Perineural Invasion to Predict Pathologic Stage > T2 in Radical Prostatectomies?

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ABSTRACT

Purpose: Perineural invasion (PNI) on needle prostatic biopsies (NPB) has been controversial as a marker of extraprostatic extension and consequently for planning of nerve-sparing radical prostatectomy (RP). The aim of this study was to find whether tumor extent on NPB influences the value of PNI to predict stage > pT2 on RP.
Materials and Methods: This retrospective study was based on 264 consecutive patients submitted to radical retropubic prostatectomy. Their NPB were matched with whole-mount processed and totally embedded surgical specimens. Tumor extent on NPB was evaluated as the percentage of linear tissue in mm containing carcinoma in all cores. Considering the median value, patients were stratified into 2 groups: harboring less or more extensive tumors on NPB. Univariate and multivariate logistic regression analyses were used to relate stage > pT2 to PNI and other clinical and pathological variables.
Results: In patients with more extensive tumors, PNI was predictive of stage > pT2 in univariate analysis but not in multivariate analysis. In less extensive tumors, PNI showed no association between any clinical or pathological variables studied; no difference in the time to biochemical progression-free status compared to patients without PNI; and, no predictive value for pathological stage > pT2 on both univariate and multivariate analyses.
Conclusion: Tumor extent on NPB influences the predictive value of PNI for pathologic stage > pT2 on RP. With a higher number of small tumors currently detected, there is no evidence that perineural invasion should influence the decision on preservation of the nerve during radical prostatectomy.

Key words: *prostate; prostatic neoplasms; biopsy; needle; prostatectomy; prognosis*
Int Braz J Urol. 2010; 36: 439-49

INTRODUCTION

Perineural invasion (PNI) on needle prostatic biopsies as a marker of extraprostatic extension has been controversial (1-15). In almost all studies, perineural invasion has been related to extraprostatic extension in univariate analysis but in only a few studies in multivariate analysis. The practical importance relates to the decision of whether to sacrifice part or all of the neurovascular bundle on the side of the biopsy with PNI when planning nerve-sparing radical prostatectomy. The aim of this study was to determine whether tumor extent on needle biopsies significantly influences the value of PNI to predict

stage > pT2 (pT3a and/or pT3b) on radical prostatectomies.

MATERIALS AND METHODS

This retrospective study was based on 264 consecutive patients submitted to radical retropubic prostatectomy by one surgeon (UF) in the period 1997 to 2008 due to clinically localized (T1c or T2) prostate adenocarcinoma. Their needle prostatic biopsies (mean 9 cores per biopsy) were matched with whole-mount processed and totally embedded surgical specimens. A mean of 32 paraffin blocks were processed, and 6μm sections from each block were stained with hematoxylin and eosin. PNI was considered as prostate cancer extension along the perineural sheath (Figure-1). The presence of any PNI, regardless of amount, was recorded as positive for PNI. Positive surgical margins were defined as cancer cells touching the inked surface of the prostate. Extraprostatic extension (pT3a) was diagnosed whenever cancer was seen in adipose tissue and, in case of desmoplastic response, whenever a protuberance corresponding to extension of tumor into the periprostatic tissue was seen (16). Seminal vesicle invasion (pT3b) was defined as invasion of the muscular wall (17).

Extent on needle biopsy was evaluated as the percentage of linear tissue in mm containing carcinoma. Considering the median value of extent, biopsies were stratified into 2 equal groups: 132 biopsies with less extensive and 132 biopsies with more extensive tumors. Tumor extent on radical prostatectomy was estimated by use of a point-count method previously described (18,19). Grading was according to the standard Gleason system (20,21). All pathological findings were evaluated by one senior uropathologist (AB).

Clinical variables analyzed included preoperative serum prostate-specific antigen (PSA), age and clinical stage (T1c or T2). Total serum PSA was measured utilizing previous validated Immulite® PSA kit. Biochemical progression was defined as PSA ≥ 0.2 ng/mL according to recommendation of the American Urological Association (22). After radical prostatectomy, serum PSA was drawn every 3 months during the first year, every 6 months during the second year, and annually thereafter. The mean and median follow-up of the patients was 28 and 20 months, respectively. No patient of this series was treated before or after surgery.

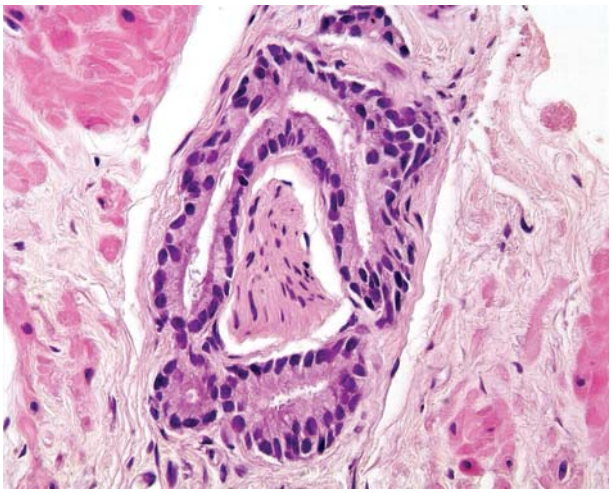

Figure 1 *– Perineural invasion on needle prostatic biopsy (HE, X400).*

The data were analyzed using the Mann-Whitney test for comparison of means and the Fishers exact test for comparison of proportions. Time to biochemical (PSA) progression-free outcome was studied using the Kaplan-Meier product-limit analysis; the comparison between the groups was done using the Log-rank test. Univariate and multivariate analyses using a logistic regression model was used to relate the outcomes of prostatectomy stage > pT2 to several clinical and pathological variables. Two-sided P value < 0.05 was considered statistically significant. All statistical analyses were performed using the commercially available SPSS 15.0.

RESULTS

Table-1 shows the clinical and pathological findings of the 264 patients studied. Perineural invasion was present in 48/264 (18.2%) biopsies. The median value of tumor extent on biopsy was 13.6%. The frequency of PNI in the group with less extent tumors (≤ 13.6% of tissue in mm containing carcinoma) was 10.6%; in the group with more extensive tumors (> 13.6% of tissue in mm containing carcinoma) the frequency of PNI was 25.8%.

Table 1 *– Clinical and pathological characteristics of the study population.*

Variable	N (%)
Age (years)	
Mean ± SD	63.2 ± 6.6
Median	64
Range	43-76
Clinical stage	
T1c	111 (44)
T2	141 (56)
Preoperative PSA (ng/mL)	
Mean ± SD	9.2 ± 5.4
Median	7.8
Range	0.6-35
Gleason score (needle biopsy)	
Mean ± SD	6.4 ± 0.6
Median	6
Range	4-9
Tumor extent in biopsy (%)	
Mean ± SD	19.7 ± 19.1
Median	13.6
Range	0.4-100
Tumor extent in radical prostatectomy (positive points)	
Mean ± SD	36.7 ± 36.4
Median	27
Range	1-225
Positive margins	
Absent	146 (55.3)
Present	118 (44.7)
Extraprostatic extension (pT3a)	
Absent	194 (73.8)
Present	69 (26.2)
Seminal vesicle invasion (pT3b)	
Absent	230 (87.1)
Present	34 (12.9)
Pathologic stage > pT2 (pT3a/pT3b)	
Absent	190 (72)
Present	74 (28)

SD = standard deviation.

Table-2 shows the association of PNI to several clinical and pathological variables comparing patients with less extensive and more extensive tumors. On biopsies showing more extensive tumors and PNI, patients showed statistically significant higher Gleason score on biopsy (p = 0.02), more extraprostatic extension (p = 0.02), and more seminal vesicle invasion (p = 0.04). On biopsies showing less extensive tumors and PNI, there was no statistical significant association to all variables studied.

Figures-2 and 3 show the Kaplan-Meier biochemical progression-free survival curve following radical prostatectomy. In patients with less extensive tumors on biopsy (Figure-2), at 5 years, 71% of the patients without PNI were free of biochemical progression compared to 83% of the patients with PNI. There was no statistically significant difference between the groups (log-rank, p = 0.24). In patients with more extensive tumors on biopsy (Figure-3), at 5 years, 43% of the patients without PNI were free of biochemical progression compared to 33% of the patients with PNI. There was no statistically significant difference between the groups (Log-rank, p = 0.26).

Table-3 shows the univariate and multivariate logistic regression analyses of several clinical and pathological variables predictive of pathological stage > T2 on radical prostatectomy in patients with less extensive tumors on biopsy. PNI was not predictive of stage > pT2 on both analyses. On univariate analysis, only extent of cancer and Gleason score on biopsy were predictive. On multivariate analysis, extent of tumor on biopsy was statistically significant and Gleason score on the level of significance.

Table-4 shows the univariate and multivariate logistic regression analyses of several clinical and pathological variables predictive of pathological stage > T2 on radical prostatectomy in patients with more extensive tumors on biopsy. PNI was predictive of stage > pT2 only on univariate analysis. Other variables statistically significant on univariate analysis were preoperative PSA, tumor extent on needle biopsy, and Gleason score on biopsy. On multivariate analysis, only extent of tumor on needle biopsy was an independent predictor of stage > pT2 (pT3a and/or pT3b) on radical prostatectomy.

COMMENTS

Our study showed that extent of tumor influences the value of PNI to predict pathological stage

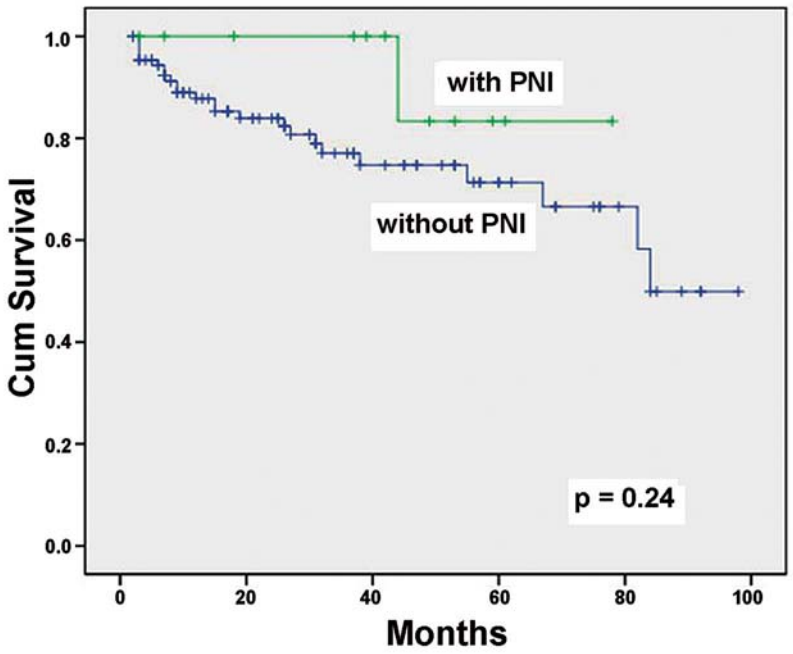

Figure 2 *– Kaplan-Meier biochemical (PSA) progression-free survival curve of patients with and without PNI on 132 biopsies with ≤ 13.6% of tissue in mm containing carcinoma (log-rank, p = 0.24).*

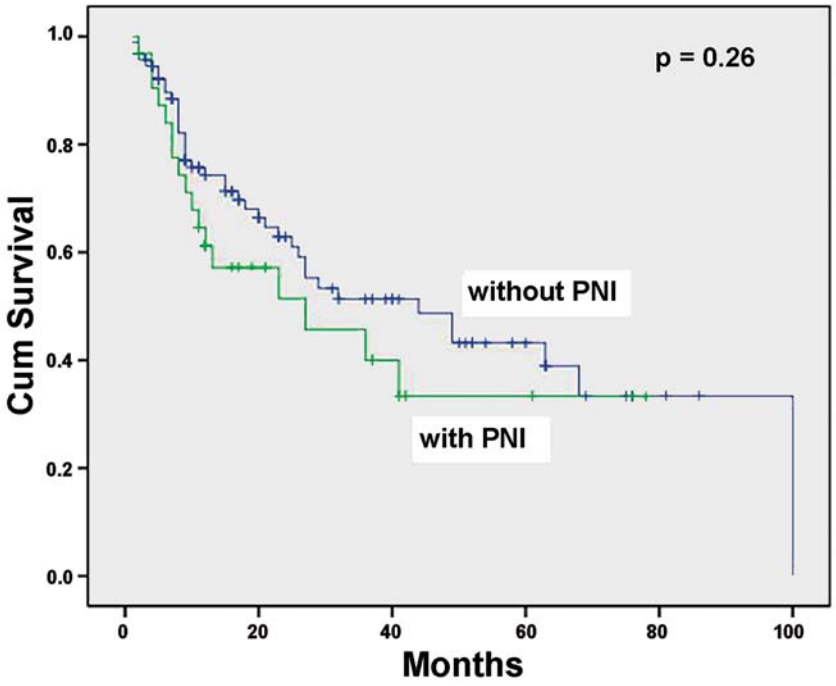

Figure 3 *– Kaplan-Meier biochemical (PSA) progression-free survival curve of patients with and without PNI on 132 biopsies with > 13.6% of tissue in mm containing carcinoma (Log-rank, p = 0.26).*

Table 2 – *Association of perineural invasion (PNI) to several clinical and pathological variables on 132 biopsies with less extensive tumors (≤ 13.6% of tissue in mm containing carcinoma) and 132 biopsies with more extensive tumors (> 13.6% of tissue in mm containing carcinoma).*

Variable	PNI in Biopsies with Less Extensive Tumors	PNI in Biopsies with More Extensive Tumors
	p Value	p Value
Age	0.94(1)	0.20(1)
Clinical stage	0.47(2)	0.66(2)
Preoperative PSA	0.36(1)	0.14(1)
Gleason score on needle biopsy	0.79(1)	0.02(1)
Tumor extent on radical prostatectomy	0.13(1)	0.09(1)
Positive margins	0.23(2)	0.16(2)
Extraprostatic extension	0.43(2)	0.02(2)
Seminal vesicle invasion	0.24(2)	0.04(2)

(1) Mann-Whitney test; (2) Fisher's exact test

Table 3 – *Univariate and multivariate logistic regression analyses of several clinical and pathological variables predictive of pathological stage > pT2 (pT3a/pT3b) on 132 biopsies with less extensive tumors (≤ 13.6% of tissue in mm containing carcinoma).*

Clinical and Pathological Variables	Univariate Analysis		Multivariate Analysis	
	Hazard ratio (95% CI)	p Value	Hazard ratio (95% CI)	p Value
Age	1.02 (0.95-1.10)	0.63	1.01 (0.93-1.09)	0.86
Clinical stage	0.59 (0.22-1.60)	0.30	0.51 (0.16-1.58)	0.24
Preoperative PSA	1.04 (0.95-1.13)	0.46	1.04 (0.93-1.17)	0.46
Extent of cancer on biopsy	1.24 (1.09-1.41)	< 0.01	1.22 (1.05-1.42)	0.01
Gleason score on biopsy	2.82 (1.27-6.26)	0.01	2.43 (1.01-5.85)	0.05
PNI on biopsy	1.52 (0.38-5.97)	0.55	1.23 (0.28-5.29)	0.78

CI = confidence interval; PNI = perineural invasion.

> T2 on radical prostatectomies. In patients with less extensive tumors on biopsy (≤ 13.6% of tissue in mm containing carcinoma) and PNI, there was no association to any one clinical or pathological variable studied; no difference in the time to biochemical (PSA) progression-free outcome compared to patients without PNI; and, no predictive value for pathological stage > pT2 on both univariate and multivariate analysis. The only study to mention the influence of extent of tumor to the predictive value of PNI is Rubins's et al. (6). In their study, PNI revealed a significant association with stage pT3 on univariate analysis. However, on multivariate analysis the association was significant only when the greatest percentage of any single biopsy involved by prostate carcinoma and the total percentage of cancer in all cores were not considered, due to a significant interaction between these measures and PNI.

Table 4 – *Univariate and multivariate logistic regression analyses of several clinical and pathological variables predictive of pathological stage > pT2 (pT3a/pT3b) on 132 biopsies with more extensive tumors (> 13.6% of tissue in mm containing carcinoma).*

Clinical and Pathological Variables	Univariate Analysis		Multivariate Analysis	
	Hazard ratio (95% CI)	p Value	Hazard ratio (95% CI)	p Value
Age	1.02 (0.97-1.08)	0.41	1.01 (0.94-1.07)	1.00
Clinical stage	1.48 (0.68-3.20)	0.32	0.88 (0.36-2.20)	0.79
Preoperative PSA	1.09 (1.03-1.15)	0.01	1.07 (1.00-1.08)	0.06
Extent of cancer on biopsy	1.06 (1.03-1.08)	< 0.01	1.05 (1.03-1.08)	< 0.01
Gleason score on biopsy	1.92 (1.13-3.30)	0.02	1.47 (0.79-2.73)	0.22
PNI on biopsy	3.32 (1.48-3.33)	< 0.01	1.67 (0.64-4.37)	0.30

CI = confidence interval; PNI = perineural invasion.

The findings with more extensive tumors on biopsy are in accordance with most of the studies in the literature. Egan and Bostwick (2) found on univariate analysis that PNI on needle biopsy was significantly associated to extraprostatic extension and seminal vesicle invasion. On multivariate analysis, however, only preoperative PSA, proportion of the biopsy involved by cancer, and Gleason score were significant. Ukimura et al. (4) found that PNI on biopsy was a good predictor among others studied for extraprostatic extension on univariate analysis but not on multivariate analysis. In the study by Vargas et al. (5) PNI was not an independent predictor of extraprostatic extension when PSA was included.

D'Amico et al. (7) evaluated the clinical use of PNI on biopsy for predicting time to PSA failure following radical prostatectomy of 750 men with clinically localized or PSA detected prostate cancer. The presence of PNI on biopsy was not a significant predictor of PSA outcome following RP for patients in the intermediate or high-risk group. O'Malley et al. (9) compared 78 biopsies with PNI with 78 matched controls without PNI and were unable to show that PNI on needle biopsy influences long-term tumor-free survival. Freedland et al. (10) studied 190 men who underwent radical prostatectomy. Percent of tissue with cancer on biopsy was the strongest predictor of biochemical recurrence on multivariate analysis. PNI was not an independent predictor of either adverse pathology or biochemical failure.

In Bismar's et al. study (11) neither presence nor absence of perineural nor number nor percentage of positive nerves were related to pathologic stage on univariate or multivariate analyses. In Tsuzuki's et al. study (12) PSA, Gleason score, digital rectal examination, percent of side specific cores with tumor and average percent involvement of each positive core but not PNI were found to be statistically significant independent predictors of extraprostatic extension in the region of the neurovascular bundle. Studying 452 consecutive patients undergoing radical retropubic prostatectomy by a single surgeon, Cannon et al. (13) concluded that although biopsy PNI alone was associated with a higher probability of extraprostatic extension, it was not predictive of bilateral nerve-sparing technique or a positive surgical margin in an individual patient.

In other studies, however, PNI was an independent predictor of final pathologic stage. de la Taille et al. (3) found that PNI, PSA and Gleason score on the biopsy independently predicted stage pT3 disease. The authors concluded that PNI is an important preoperative predictor of pathologic stage and should be reported when adenocarcinoma is diagnosed on prostate needle biopsies. In the Sebo et al. study (8) joint predictors of extraprostatic exten-

sion were the percent cores positive for carcinoma, Gleason score of 7, Gleason score of 8 or 9, serum PSA and PNI. In the Loeb et al. study (15), PNI was significantly associated with aggressive pathology and biochemical progression. On multivariate analysis, PNI was significantly associated with extraprostatic extension and seminal vesicle invasion. Bastacky et al. (1) studied 302 needle prostatic biopsies and found a sensitivity of 27% and a specificity of 96% for PNI to predict extraprostatic extension. They concluded that measuring PNI on needle biopsy helps identify extraprostatic extension and may help in planning nerve-sparing radical prostatectomy in the decision of whether to sacrifice part or all of the neurovascular bundle on the side of the biopsy. These authors did not study the predictive value on multivariate analysis.

Some factors may be responsible for the discrepancies in the literature: the number of cores examined per case may influence the rate of detection of PNI, the different methods of processing and submitting tissue from radical prostatectomy specimens (completely vs. partially embedded prostate glands) may contribute to different rates of detection of extraprostatic carcinoma, different definitions of PNI on needle biopsy tissue and extraprostatic extension, and different values of PSA for evaluation of biochemical progression-free outcome following surgery. In a search from January 1990 to December 2005 using MEDLINE, Embase, and the Web of Knowledge, Harnden et al. (14) performed a systematic review of studies that examined the association between perineural invasion and prostate cancer recurrence. These authors concluded that a considerable variation in study design, execution, and reporting precluded meta-analysis and quantitative risk estimation.

The frequency of perineural invasion on needle prostatic biopsies varies from 11% to 38% (1-6,8,10,11). Considering all biopsies in our study the frequency was 18%. In the group with less extensive tumors the frequency was 10.6% and in the group with more extensive tumors 25.8%. It is worth noting that in less extensive tumors the frequency of PNI is in the lower range of the frequency found in the literature. Studying very small tumors on biopsies (less than 1 mm in length in only one core), Thorson et al. (23) still found a frequency of 2% of PNI. We have also seen PNI invasion in autopsied patients with very small incidentally found histologic carcinomas. These findings probably suggest that PNI may be an early event. This suggestion is shared by Byar and Mostofi (24). The authors studied 208 total prostates removed using the step-section technique for early carcinoma of the prostate. The high frequency of PNI found (84.1%) suggested by the authors that the phenomenon occurs early in the course of the disease. The similarity in the survival rates for cases with and without PNI indicated little if any prognostic significance.

Our study may have some limitations. All patients were submitted to radical prostatectomy, and therefore we were unable to determine any different effect of PNI on biochemical progression-free outcome with other forms of treatment, such as watchful waiting or radiotherapy. Another limitation could be related to the fact that we only recorded the presence or absence of PNI on needle biopsy but did not quantify the extent. In addition, the mean follow-up is relatively short, and it is possible that greater differences could occur with additional follow-up. A strength of the study could be the homogeneity of the study population which was comprised of consecutive patients treated by one expert surgeon limiting any influence of variability in surgical technique and the pathological evaluation also by one senior uropathologist.

In summary, tumor extent on needle biopsies influences the predictive value of PNI for pathologic stage > pT2 (pT3a and/or pT3b) on radical prostatectomies. In patients with more extensive tumors on needle biopsy, PNI predicted pathologic stage > pT2 on radical prostatectomy on univariate analysis but on multivariate analysis did not show independent predictive value. This finding is in accordance to most of the studies reported in the literature. In patients with less extensive tumors on biopsy (≤ 13.6% of tissue in mm containing carcinoma) and PNI, there was no association between any clinical or pathological variables studied; no difference in the time to biochemical (PSA) progression-free outcome comparing to patients without PNI; and, no predictive value for pathological stage > pT2 on both univariate and multivariate analysis. With a higher number of small tumors currently detected, there is no evidence that perineural invasion should influence the decision on preservation of the nerve during radical prostatectomy.

CONFLICT OF INTEREST

None declared.

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EDITORIAL COMMENT

The importance of perineural invasion (PNI) in prostate biopsies remains controversial in the literature. The studies published since 1993 have shown an incidence of PNI variable from 11% to 38% significantly related to unfavorable histological features, specially extra-prostatic extension (EPE) and positive surgical margins (1,2). Some authors have shown a relationship between PNI and biochemical recurrence, and because of that, the description of the presence or absence of PNI in prostate biopsies plays a role in surgical pathology reports. The authors studied 264 patients, finding 18.2% of PNI related to EPE, higher Gleason score and seminal vesicles (SV) infiltration only for extensive tumors. However, they did not find any correlation between PNI and tumor progression. The most robust study was published recently by Loeb et al. (3) describing the Johns Hopkins experience with 1256 men submitted to radical prostatectomy, and they found a correlation between PNI with EPE and SV infiltration in multivariate analysis. In addition to the fact that tumor progression occurred in 10.5% of patients with PNI and in only 3.5% of patients without PNI, this aspect was not an independent risk factor for biochemical recurrence. Most importantly, it was not considered to decide nerve-sparing surgery. Since there is some agreement that PNI is related to aggressive pathologic features, we believe it is important that pathologists continue to describe the presence or absence of PNI to give the opportunity to surgeons, oncologists and radiotherapists to take this aspect in account to better plan patients' treatment.

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EDITORIAL COMMENT

The authors are to be commended on this thorough analysis of how transrectal prostate needle biopsy specimens correlate with whole mount prostatectomy specimens. This study is solid based on the large number of patients (264). There is further strength in that a single surgeon performed each radical retropubic prostatectomy, while all of the pathology was interpreted by a single pathologist. Finally, the statistical methods employed were appropriate for this type of analysis.

In the literature, there is great controversy regarding the significance of perineural invasion

(PNI). There are as many reports to suggest significance of PNI as there are reports that demonstrate no difference in patient outcomes. Specifically, can PNI predict preoperatively the presence of extracapsular extension (ECE) at the time of radical prostatectomy? Naturally, if this can be shown that a patient is at risk preoperatively for ECE, the surgeon may electively alter the technique to sacrifice part or all of the neurovascular bundle on the ipsilateral side of the disease. Additionally, this information may be useful in counseling patients pre-treatment regarding the pros and cons of various treatment options.

This report by Billis et al., does in fact demonstrate a subset of patients that have a significant risk of having a higher grade Gleason score, ECE and seminal vesicle involvement at the time of radical prostatectomy. Specifically, it was the group of patients in that more than 13.6% of linear (mm) malignant involvement was identified in the needle biopsy specimens. 25.8% of this cohort possessed PNI. Fortunately, regarding overall freedom from biochemical progression, there was no significant difference between those with and without PNI. Without question, better pretreatment patient selection will ultimately improve treatment outcomes. It is not uncommon to assume a patient has low risk criteria when in fact, they may harbor more advanced disease. In the future, clinicians will utilize more sophisticated biopsy techniques and apply known pathologic risk factors to decrease the risk of understaging prostate cancer.

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Transperitoneal versus Extraperitoneal Laparoscopic Radical Prostatectomy During the Learning Curve: Does the Surgical Approach Affect the Complication Rate?

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ABSTRACT

Purpose: To compare the perioperative complication rate obtained with the transperitoneal laparoscopic radical prostatectomy (TLRP) and with the extraperitoneal LRP (ELRP) during the learning curve (LC).
Materials and Methods: Data of the initial 40 TLRP (Group 1) were retrospectively compared with the initial 40 ELRP (Group 2). Each Group of patients was operated by two different surgeons.
Results: The overall surgical time (175 min x 267.6 min; $p < 0.001$) and estimated blood loss (177.5 mL x 292.4 mL; $p < 0.001$) were statistically better in the Group 1. Two intraoperative complications were observed in Group 1 (5%) represented by one case of bleeding and one case of rectal injury, whereas four complications (10%) were observed in Group 2, represented by two cases of bleeding, one bladder and one rectal injuries ($p = 0.675$). Open conversion occurred once in each Group (2.5%). Overall postoperative complications were similar (52.5% x 35%; $p = 0.365$). Major early postoperative complications occurred in three and in one case in Group 1 and 2, respectively. Group 1 had two peritonitis (fecal and urinary), leading to one death in this group.
Conclusions: No statistical differences in overall complication rates were observed. The transperitoneal approach presented more serious complications during the early postoperative time and this fact is attributed to the potential chance of intraperitoneal peritonitis not observed with the extraperitoneal route.

Key words: *prostate; prostatic neoplasms; prostatectomy; laparoscopy*
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INTRODUCTION

Since 1998, laparoscopic radical prostatectomy (LRP) has gained worldwide popularity, based on the Montsouris initial publication with the transperitoneal LRP (TLRP) (1) followed by the Brussels initial publication with the extraperitoneal approach (ELRP) (2).

It has been ten years and so far a debate related to the approach for performing LRP, mainly during the learning curve (LC), still remains. Some authors prefer the transperitoneal approach based on the larger working space and better luminosity and others prefer the extraperitoneal counterpart, based on the lack of contact with the intraperitoneal organs (3-6).

Analyzing the comparative studies for LRP during the LC, it can be noted that the vast majority of groups started their programs using the transperitoneal approach, switching later on to the extraperitoneal route (7-9). This observation generates a bias on results, once the findings of worse results with the transperitoneal approach when compared with the extraperitoneal approach is overlooked. The results observed with the TLRP in these studies reflect the suboptimal results found during the initial phase of a LRP program and the results achieved with the extraperitoneal approach are the reflex of an "already" well-trained surgical team.

The aim of this study was to compare the initial perioperative complications obtained with both approaches used to perform LRP and identify, if possible, a more suitable approach to use during the initial stages of a LRP program.

MATERIALS AND METHODS

This retrospective study was approved by each institutional research and ethical committee. The data of the first 40 TLRP performed between March, 2004 and November, 2007 (Group 1) performed at Getúlio Vargas Hospital of Recife were recorded and compared with the first 40 ELRP performed between August, 2003 and June, 2006 (Group 2) at Clinics Hospital of State University of São Paulo.

Each group was operated by only one experienced laparoscopic urologic surgeon in two uro-laparoscopic referral centers in this country. By the time of this study, each surgeon had already performed more than 250 laparoscopic surgeries, including partial nephrectomies, radical nephrectomies, donor nephrectomies, adrenalectomies, pyeloplasties and others. Inclusion criteria were age $\leq$ 75 years old, clinically localized prostate tumors (cT1 and cT2N0M0), total PSA $\leq$ 10 ng/dL and Gleason score $\leq$ 7.

Surgical Technique

All TLRP were performed by the Montsouris technique (10) while the ELRP were done by the Brussels technique (2) with some modifications.

Briefly, in the Montsouris technique, the vas deferens and seminal vesicles were firstly dissected through the retrovesical space. After entering the Retzius space and opening the endopelvic fascia, the dorsal vein complex (DVC) was tied. The bladder neck was incised, reaching the pre-dissected vas deferens. Bilaterally, the prostatic pedicles were controlled and an interfascial neurovascular bundle dissection was performed whenever possible (11). Afterwards, the DVC and urethra were cut, leaving the prostate apart for later removal. A running urethrovesical (UV) anastomosis was made in all cases of Group 1 as described by van Velthoven et al. (12).

In the Brussels technique, the Retzius space was digitally created, avoiding transperitoneal entering. The other stages were about the same as transperitoneal approach, differing only in the straight access to vas deferens and seminal vesicles after bladder neck incision. An interrupted figure of "X" UV anastomosis was performed in all patients in Group 2.

Pathological Evaluation

All fine-needle biopsies and specimens were evaluated by the uro-pathology service of each institution. Positive surgical margin (PSM) was defined as the presence of tumor at the inked margin. Tumors were graded according to the Gleason score and pathological staging was based on TNM 1997 classification.

Statistical Analysis

Analysis of variance was used to compare continuous outcome variables between both groups. The Student's-t-test was used for homogeneous variances in each group and the Chi-square and Fisher's exact test were used to compare categorical outcome variables. Statistical significance was defined as P value < 0.05.

RESULTS

Preoperative data are shown in Table-1. There was a statistical difference between groups 1 and 2,

Table 1 – *Preoperative results.*

	Group 1 (n = 40)	**Group 2** (n = 40)	**p Value**
Age (years)	59.8 ± 6.8 (46-73)	63.6 ± 7.9 (51-75)	p = 0.011
PSA (ng/dL)	5.4 ± 2.02 (2.0-10.0)	5.9 ± 1.96 (2.0-9.9)	p = 0.255
Prostatic weight (g)	36.2 ± 14.3 (20-80)	35.4 ± 20.5 (15-140)	p = 0.846
Clinical stage - n (%)			p = 0.013
T1c	32 (80%)	20 (50%)	
T2a	7 (17.5%)	15 (37.5%)	
T2b	1 (2.5%)	5 (12.5%)	
Gleason score - n (%)			p = 0.001
4 (2+2)	-	1 (2.5%)	
5 (3+2)	-	2 (5%)	
6 (3+3)	20 (50%)	32 (80%)	
7 (3+4)	14 (35%)	2 (5%)	
7 (4+3)	6 (15%)	3 (7.5%)	

PSA = prostate-specific antigen.

related to the patient's age, clinical stage and Gleason score. Clinical stage T1c was more common in Group 1 (80%) while cT2 was prevalent in Group 2 (50%). On the other hand, the Gleason score 7 was more prevalent in Group 1 (50% x 12.5%).

The intraoperative data are described in Table-2. Overall surgical time (175 min x 267.6 min; $p < 0.001$) and estimated blood loss (177.5 mL x 292.4 mL; $p < 0.001$) were statistically significant better in the Group 1. Two complications (5%) were observed in Group 1, represented by a bleeding from the DVC and rectal injury. The first one was controlled after conversion to the open approach and the last one was treated with intracorporeal suture. Four complications (10%) occurred in Group 2, represented by two cases of bleeding (5%), one bladder (2.5%) and one

Table 2 – *Comparison of intraoperative data.*

	Group 1 (n = 40)	**Group 2** (n = 40)	**p Value**
Overall operative time (min)	175.0 ± 48.4 (110-360)	267.6 ± 70.57 (160-540)	p < 0.001
Blood loss (mL)	177.5 ± 148.5 (50-1000)	292.4 ± 173.7 (10-900)	p < 0.001
Open conversion - n (%)	1 (2.5%)	2 (5%)	p = 1.000
Complications - n (%)			
Overall	2 (5%)	4 (10%)	p = 0.675
Complication type - n (%)			
Bleeding	1 (2.5%)	2 (5%)	
Rectal injury	1 (2.5%)	1 (2.5%)	
Bladder injury	-	1 (2.5%)	

Table 3 – *Comparison of postoperative data.*

	Group 1 (n = 40)	**Group 2** (n = 40)	**p Value**
Time to discharge - (days)*	3.0 (3-35)	3.0 (2-17)	p = 0.042
Early urinary continence - n (%)	27 (69.2%)	34 (85%)	p = 0.033
Sexual intercourse - n (%)	18 (45%)	17 (42.5%)	p = 0.368
Follow-up (months)	9.3 ± 7.7 (1-36)	32.9 ± 12.6 (8-50)	p < 0.001

** Median*

rectal injury (2.5%). Both bleedings came from the DVC. The first one was controlled after conversion to the open approach and the last one was treated with intracorporeal suture. The bladder injury was recognized and treated by intracorporeal suture. On the other hand, the rectal injury was unrecognized in the intraoperative time and evolved with bloody anal discharge on postoperative day one, which led to an open colostomy.

Postoperative data are described in Table-3. Median time to discharge, early urinary continence and follow-up time were statistically better for Group 2. No statistical difference was observed on early postoperative sexual function, evaluated by the vaginal penetration rate whether or not using sildenafil 100 mg.

The Table-4 shows no statistical difference in overall postoperative complications (52.5% x 35%; p = 0.365). Nonetheless, sub-stratifying the complications, a statistical difference was observed by comparing the minor complications during the early and late postoperative time for each group.

The main complications observed in Group 1 were one case each of urinary sepsis, fecal and urinary peritonitis. The sepsis occurred on postoperative day (POD) 8 by Klebsiella pneumoniae despite preoperative negative urine culture and trans-operative use of parental ceftriaxone. The patient was readmitted and had an uneventful recovery after appropriate parenteral antibiotic therapy. The fecal peritonitis occurred on POD 4 due to fecal leakage by the rectal suture line performed intraoperatively. The patient evolved with peritonitis, sepsis and died on POD 35 even after colostomy and parenteral antibiotic therapy. The urinary peritonitis occurred due to urinary leakage from the posterior aspect of the UV anastomosis, leading to a 1500 cc urine peritoneal collection. After open laparotomy and peritoneal drainage, the patient had an uneventful recovery.

In the Group 2, seven urinary leakages originating from the UV anastomosis occurred and were treated by prolonged bladder catheterization. Of those, six evolved with urinary strictures (bladder neck- 03; bulbar urethra- 02; and meatal urethra- 01), and had an uneventful recovery after appropriate treatment. A further urinary leakage due to the UV anastomosis evolved with a large retroperitoneal infiltration and was treated with open drainage, positioning of a tubular drain and prolonged bladder catheterization.

Lastly, the Table-5 shows the final oncological data. Comparing the results between groups 1 and 2, statistical difference was observed in the biochemical recurrence rate (5% x 20%; p = 0.043), overall incidence of PSM (10.3% x 32.5%; p = 0.016) and pathological stages (pT2: 94.8% x 70% and pT3: 5.2% x 30%; p = 0.005). Nonetheless, no difference was observed when the incidence of PSM was correlated with the pathological stages. The majority of PSM in Group 1 occurred in pT2c (75%), while this observation was more prevalent in pT3a in Group 2 (61.5%). For pT3b, 100% of PSM occurred in both groups.

COMMENTS

According van Velthoven et al. (4) and Gill et al. (13), about 92% of uro-laparoscopic centers that currently use the extraperitoneal approach, started their laparoscopic programs using the transperitoneal

Table 4 – *Comparison of postoperative complications and reoperations.*

	Group 1 (n = 40)	**Group 2** (n = 40)	**p Value**
Overall complications (before 30th POD):	21 (52.5%)	14 (35%)	p = 0.365
Early complications (before 30th POD):			
Minor - n (%)			p < 0.001
Perineal pain	4 (10%)	-	
Abdominal wall hematoma	2 (5%)	-	
Urinary leakage	-	7 (17.5%)	
Major - n (%)			p = 0.241
Fecal peritonitis (death)	1 (2.5%)	-	
Urinary peritonitis	1 (2.5%)	-	
Urinary sepsis	1 (2.5%)	-	
Retroperitoneal urinary infiltration	-	1 (2.5%)	
Late complications (after 30th POD)			
Minor - n (%)			p = 0.004
UTI	9 (22.5%)	-	
Bladder neck stricture	2 (5%)	3 (7.5%)	
Urethral stricture	1 (2.5%)	2 (5%)	
Urethral meatus stricture	-	1 (2.5%)	
Major - n (%)	0	0	p = 1.000
Reoperations: n (%)	2 (5%)	2 (5%)	p = 1.000
Death	1 (2.5%)	-	

POD = postoperative day; UTI = urinary tract infection.

route. In general, this observation can cause a bias on results when these accesses are compared in the same series. Such discrepancy in results can even be greater during the initial phase of a LRP program.

Perhaps the best way to overcome the LC in LRP is the incorporation of robotics in clinical practice (14). However, even in robotic LRP, a LC does exist and so far, controversies remain about the choice of the approach to use is these cases. Moreover, the high costs associated with this technique, make it a distant reality for developing countries. Therefore, continuous improvements in LRP technique are mandatory and identification of factors that can improve and shorten the LC is imperative to achieve better results.

The main goal of this retrospective study was to compare the perioperative complication rates of two distinct groups of patients operated each by the transperitoneal and extraperitoneal approaches during the initial phases of a LRP program. For this, each group was operated by only one urologist, having each a wide experience in retropubic radical prostatectomies and in more than 250 uro-laparoscopic surgeries. This study model, despite some points of criticism, was adopted to analyze the influence of the LC over the incidence of complications and to identify factors that could improve the results in this phase. Only Machado et al. (15) performed a similar study and observed better results with the extraperitoneal approach when compared with the transperitoneal route.

Observing the intraoperative data, the patients in Group 1 reached better surgical time and bled less than the ones in the Group 2 and these findings can be associated with the better working space and luminosity achieved with the transperitoneal access.

Table 5 – *Postoperative pathological data.*

	Group 1 (n = 39)	**Group 2** (n = 40)	**p Value**
Biochemical recurrence	2 (5%)	8 (20%)	p = 0.043
Gleason score - n (%)			p = 0.365
6 (3+3)	14 (35%)	21 (52.5%)	
7	24 (60%)	16 (40%)	
7 (3+4)	19 (47.5%)	12 (30%)	
7 (4+3)	5 (12.5%)	4 (10%)	
8 (3+5)	1 (2.5%)	2 (5%)	
8 (4+4)	-	1 (2.5%)	
BPH	1 (2.5%)	-	
Positive surgical margins - n (%)	4 (10.3%)	13 (32.5%)	p = 0.016
Pathological stage - n (%)			p = 0.005
T2	37 (94.8%)	28 (70%)	
T3	2 (5.2%)	12 (30%)	

BPH = benign prostatic hyperplasia.

Nonetheless, two major complications were observed in Group 1, causing a urinary and fecal peritonitis, leading to the death of one patient. These results are the real reflex of the LC effect over each LRP program without any previous experience with LRP.

Urinary leakage can occur up to 28% in LRP during the LC (16-19). Of note, all seven early minor complications observed in the Group 2 were represented by urinary leakages, while only one was observed in the Group 1, which was considered a major complication. The majority of these cases evolved to urinary strictures and needed surgical treatment. In general, urinary leakages occur due to non-well aligned suture lines, surgery in prostates > 60 grams, use of interrupted sutures and when the extraperitoneal access is chosen (18,19). In general, tension over the UV anastomosis is considered higher when the extraperitoneal access is used instead of the transperitoneal route, because the bladder remains stacked on the abdominal wall by the urachus (20). In fact, based in these observations, the authors recommend the use of the UV running suture since the initial phases of the LC. Likewise, to rule out urinary leakage, filling the bladder in with 200 cc of saline after finishing the UV anastomosis is recommended.

Each group had one major complication on early postoperative time related to urinary leakage, as well as one rectal injury. In Group 1, both complications evolved to peritonitis needing reoperation, culminating in one death. On the other hand, these findings in the Group 2 evolved with less severity and went well after reoperation.

The incidence of rectal injury occurs in 1.8%-6% (8,21) and is more common during the LC (8,21). According Touijer et al. (22) and Martinez-Piñeiro et al. (19), the majority of injuries occur during the apical dissection. Although the rectal injury had been recognized and sutured during the intraoperative time in one patient in Group 1, the injury presented a fecal leakage on POD 4, leading to peritonitis and death. This fact was attributed to the use of the harmonic shears to dissect the posterior aspect of the prostate, near the apex. Probably, an invisible thermal injury occurred in the rectal wall during the surgery and a later wall necrosis developed, leading to the fecal leakage (19,22).

Important to notice that rectal injury can occur whatever the approach, but this complication tends to have a worse outcome when the transperitoneal route is adopted. The authors strongly recommend the

use of cold shears instead of the use of any kind of thermal shears to dissect this area to avoid this major complication, no matter which laparoscopic approach chosen.

Bladder injury is considered a rare event and is more common during the LC, reaching 8% (17,23). It can occur with both approaches and usually the injury is recognized and sutured during the surgery. In general, all injuries have an uneventful recovery after appropriate treatment.

Perineal pain is a rare event and was observed in four patients in Group 1 (10%). This was attributed to hyper abduction of legs in order to place the laparoscopic rack in between. No further cases of this type of complication were observed after discontinuation of this practice.

Epigastric artery injury occurs in about 2% - 6.2% of cases and generally is associated with trocar insertion during transperitoneal surgeries (17,23). This injury rarely occurs during ELRP, because the vessels are easily seen after the extraperitoneal space has been created. The authors suggest puncturing before the site of trocar placement with a fine needle in order to verify the route, avoiding this injury. Also is recommended to have a Carter-Thomason device readily to use if necessary.

Finally, urinary tract infection occurs in 1.4% - 2.8% in all cases of LRP, despite of antibiotic prophylaxis (2,24). Generally, these infections are caused by prolonged indwelling catheter use and/or inappropriate antibiotic prescription. Currently, the authors suggest the use of quinolones for 14 days after the hospital discharge and the urethral catheter removal as soon as possible, around the postoperative day 7.

The LRP is considered the most challenging laparoscopic surgery in urology. The greatest drawback of this surgery is its steep LC and consequently the possibility of major complications to occur and weak functional results during this time. Moreover, the initiation of a LRP program demands great caution in order to not overcome the main objective of this surgery: the cure. Therefore, continuous improvements and training are mandatory to achieve better outcomes. Based in our results, there was no difference in the incidence of perioperative complications whatever the approach used to operate both groups during the LC. The incidence and severity of major complications were higher when the transperitoneal approach was adopted.

CONCLUSIONS

The overall complication rate was similar in both approaches. Minor complications occurred in both groups and tended to complete resolution after appropriate treatment. The higher incidence of urinary leakage in Group 2 was directed associated with the interrupted UV anastomosis and indirectly linked with the extraperitoneal route. The transperitoneal approach presented more serious complications during the early postoperative time and this fact is attributed to the potential chance of intraperitoneal peritonitis not observed with the extraperitoneal approach.

CONFLICT OF INTEREST

None declared.

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Cross-cultural Adaptation of the Dysfunctional Voiding Score Symptom (DVSS) Questionnaire for Brazilian Children

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ABSTRACT

Purpose: To translate and culturally adapt the Dysfunctional Voiding Symptom Score (DVSS), questionnaire into Brazilian Portuguese.

Materials and Methods: The 10-item Dysfunctional Voiding Symptom Score (DVSS) was translated into Brazilian Portuguese according to a standard methodology: translation, synthesis, back-translation, Expert Committee, and pre-testing. After the translation process the final version was pre-tested and patient responses were analyzed to identify necessary modifications. Reliability was evaluated using the test-retest method, and internal consistency was assessed using Cronbach's alpha.

Results: The Cronbach's alpha coefficient was calculated in the test and retest phases. Internal consistency was found to be satisfactory, as confirmed by a Cronbach's alpha coefficient of 0.76 for the test and 0.77 for the retest. A high degree of stability was found in the test/retest, with an intraclass correlation coefficient (ICC) of 0.960 ($p < 0.001$; 95% CI: 0.943-0.972).

Conclusions: The cross-cultural adaptation process of the Dysfunctional Voiding Symptom Score questionnaire to be used on Brazilian children was successfully completed following internationally accepted methodologies.

Key words: *urinary bladder; urinary incontinence; questionnaires; urinary tract infection*
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INTRODUCTION

Dysfunctional voiding (DV) is a common clinical problem seen in approximately 40% of patients presenting to the pediatric urologist (1). Females are predominantly affected with a female-to-male ratio of 5:1 (1,2). It is characterized by numerous symptoms, including recurrent urinary tract infections, urinary incontinence, constipation and encopresis. It is learned behavior that suppresses bladder contractions by inappropriately contracting the pelvic floor muscles (external urinary sphincter) during urination. This eventually becomes an involuntary process, resulting in functional obstruction of the urinary stream during voiding (detrusor/external urinary sphincter incoordination) (3).

The Standardization Committee of the International Children's Continence Society (4) defined

dysfunctional voiding as "over activity of the urethral sphincter during the voiding contraction of the detrusor in neurologically normal children". There exists great variability in the clinical presentation of voiding dysfunction in children. This variability translates into different approaches for defining the problem and even treatment modalities. Although behavioral modification remains the cornerstone of treatment, pharmacological and biofeedback techniques have been used (5).

The various treatment outcome data published for pediatric dysfunctional voiding are difficult to compare secondary to the lack of a universally accepted reproducible means of reporting symptoms and improvement. Urologists are familiar with symptom scorings. The International Prostate Symptom Score has been widely accepted and is currently the most popular way of grading benign prostatic hyperplasia symptoms in men.

Farhat et al. (6) described validated symptom scoring for wetting and functional disorders in children called the dysfunctional voiding symptom score (DVSS). The DVSS includes 10 quantitative and qualitative urological variables assessed by age-appropriate questions for children, and has been used as an objective instrument to grade voiding dysfunction in children (Appendix-1).

Since this instrument was originally written in English and no similar validated questionnaire about this theme existed in Brazil, a cultural adaptation was necessary.

The cross-cultural adaptation process had to follow international guides to maintain the equivalence between the original and target versions (7,8).

The aim of the present study was to translate and culturally adapt the questionnaire entitled Dysfunctional Voiding Symptom Score into Brazilian-Portuguese language.

MATERIALS AND METHODS

Permission to translate the DVSS into Brazilian Portuguese was obtained from one of the DVSS's authors, Dr. Walid Farhat. The translation of this phase was carried out according to methods recommended in the literature (Figure-1). The translation of the instrument into Brazilian Portuguese was done independently by two Brazilians translators, who knew the study's objectives. The two Portuguese versions generated one version after authors' consensus (DVSS 1) and then, it was submitted to back-translation, done by two other translators, natives of the USA and England, who lived in Brazil, had full mastery of Portuguese and knowledge about Brazilian culture. The translators were neither informed about the study objectives, nor about the concepts involved and the goal of the instrument. The result of this phase was the DVSS version 2.

Subsequently, it that was submitted to a committee of experts, composed of 5 pediatric urology specialists, with English as their first language. They compared each item of the original instrument and the translated version in relation to semantic/idiomatic equivalences in order to assure the correct translation. Also, they had to assure the cultural equivalence, which is related to the context and the life experiences in Brazilian population, and conceptual equivalence, which is the verification of the original instrument concept maintenance in the translated version. The questionnaire items were considered as a good agreement by the Committee only when the agreement percentage was approximately 90%.

For the qualitative analysis, a discussion about the judges' suggestions was performed. The goal was to consolidate all the versions of the instrument and indicate which characteristics should be considered in the pre-final version. A consensus was reached achieving semantic, idiomatic, experiential, and conceptual equivalence. After all suggestions made by the judges, the pre-final version was developed for field testing.

A pre-test was carried out to verify the cultural adequacy of the instrument, and the answer "I didn't understand the question" was added to all of the items. Data collection was conducted with 40 children who answered the instrument. Pre-test data analysis showed that none of the items was above the 15th percentile of incomprehension, which attested to the instrument's adequacy with no need for changes to its content or a new pre-test.

After the conclusion of the pre-test, the final Brazilian-Portuguese version was obtained. The primary developer of the DVSS (Dr. Walid Farhat) was informed at all stages of the translation process and

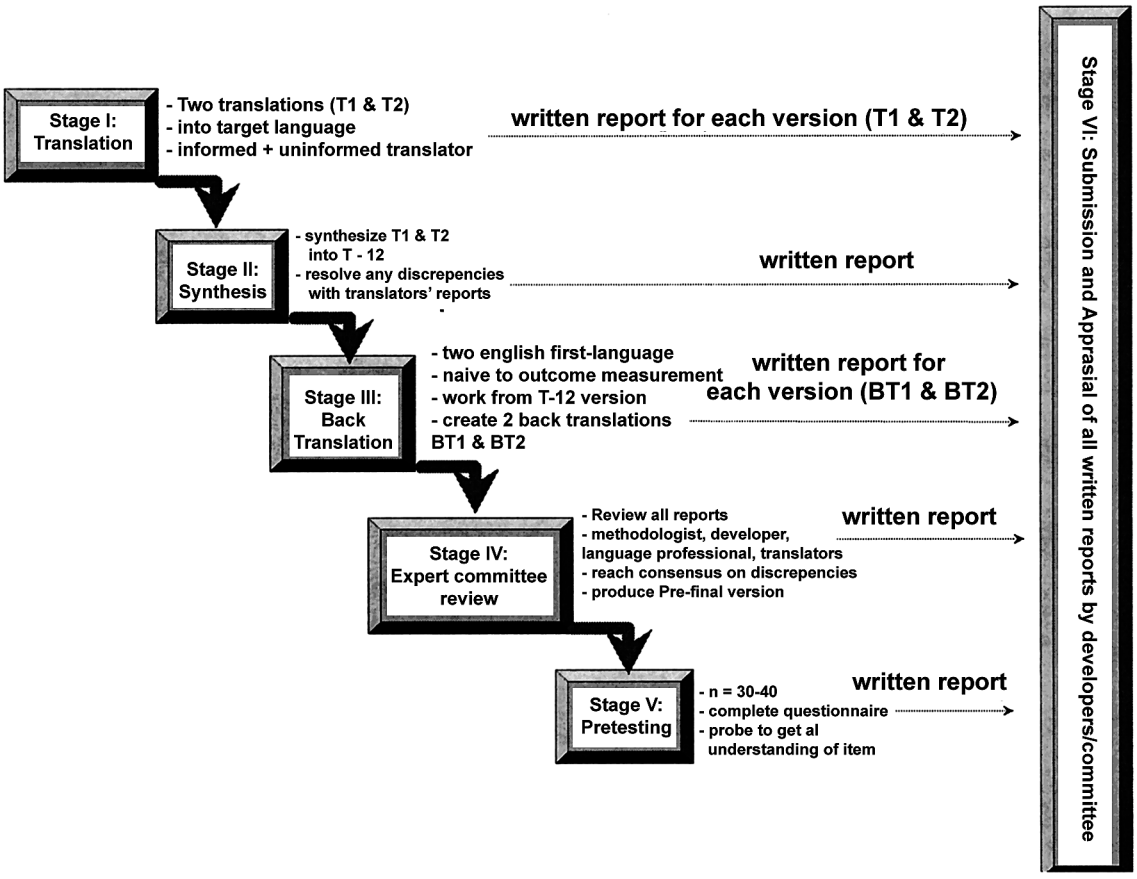

Figure 1 *– Graphic representation of the stages of cross-cultural adaptation recommended.*

approved the final version of the questionnaire and from now on will be referred to as the DVSS Brazilian version.

All patients who participated in this study were asked to provide written informed consent prior to enrollment. The full protocol received the approval of the Local Ethics and Research Committee.

The questionnaire data were recorded in commercial statistical software (Excel/2003 for Microsoft Windows). Descriptive statistics were used to describe the sample, to verify the content validity of the adapted instrument, and to determine the pretest results the following analyses were performed:

- Cronbach's coefficient alpha: to verify reliability. Cronbach alpha values > 0.70 were established as constituting evidence of satisfactory internal consistency.
- Intraclass correlation coefficient (ICC): used to verify reliability with reference to the stability of the instrument (test-retest). ICC values ≥ 0.90 were considered evidence of stability.

RESULTS

The team of specialists which analyzed the translations pointed out that there was a correspondence between the items translated, semantic equivalence between the two translations and no translation difficulty. Adjustments were made for the few verbal differences. Therefore, the counter-translation compared to the original version did not did not require any changes in grammatical structure, when the Portuguese version was translated back into English.

In the pretest phase, the questionnaire was answered by a sample of 40 parents of children with dysfunctional voiding symptoms. This group was characterized by the fact that the majority of subjects

were female (80%, 32/40) with a mean age of 6.2 years. Following application of the instrument, the patients were interviewed to evaluate the difficulties found in filling out the questionnaire and to identify any questions or words that were difficult to understand.

The Cronbach's alpha coefficient was calculated in the test and retest phases. Internal consistency was found to be satisfactory, as confirmed by a Cronbach's alpha coefficient of 0.76 for the test and 0.77 for the retest. A high degree of stability was found in the test/retest, with an intraclass correlation coefficient (ICC) of 0.960 ($p < 0.001$; 95% CI: 0.943-0.972). Table-1 presents the final version of the Brazilian DVSS.

Table 1 – *Portuguese Brazilian version of the Dysfunctional Voiding Symptom Score.*

Durante os Últimos 30 Dias	**Nunca ou Quase Nunca**	**Menos Que Metade do Tempo**	**A Metade do Tempo**	**Quase Todo o Tempo**
1. Seu(a) filho(a) tem molhado de xixi a roupa durante o dia?	0	1	2	3
2. Quando seu(a) filho(a) se molha de xixi, a cueca ou calcinha fica ensopada?	0	1	2	3
3. Com que frequência seu(a) filho(a) não faz cocô todos os dias?	0	1	2	3
4. Seu(a) filho(a) tem que fazer força para fazer cocô?	0	1	2	3
5. Com que frequência seu(a) filho(a) só vai ao banheiro fazer xixi uma ou duas vezes por dia?	0	1	2	3
6. Seu(a) filho(a) segura o xixi cruzando as pernas, agachando ou dançando?	0	1	2	3
7. Quando seu(a) filho(a) precisa fazer xixi tem que ir rápido ao banheiro? (não consegue esperar)	0	1	2	3
8. Seu(a) filho(a) tem que fazer força para fazer xixi?	0	1	2	3
9. Seu(a) filho(a) disse que sente dor quando faz xixi?	0	1	2	3
10.Seu(a) filho(a) passou por alguma situação estressante como as dos exemplos abaixo nos últimos 30 dias? Marque ao lado sim ou não. • Bebê novo em casa • Mudança de casa • Mudança de escola • Problemas escolares • Abuso (sexual/físico) • Problemas em casa (divórcio/morte) • Eventos especiais (aniversário) • Acidente / ferimento • Outros		Não (0)	Sim (3)	

Appendix 1 *– Original dysfunctional voiding symptom score.*

Over the Last Month	Almost Never	Less than Half the Time	About Half the Time	Almost Every Time	Not Available
1 - I have had wet clothes or wet underwear during the day.	0	1	2	3	NA
2 - When I wet myself, underwear is soaked.	0	1	2	3	NA
3 - I miss having a bowel movement every day.	0	1	2	3	NA
4 - I have to push for my bowel movements to come out.	0	1	2	3	NA
5 - I only go to the bathroom one or two times each day.	0	1	2	3	NA
6 - I can hold onto my pee by crossing my legs, squatting or doing the "pee dance".	0	1	2	3	NA
7 - When I have to pee. I cannot wait.	0	1	2	3	NA
8 - I have to push to pee.	0	1	2	3	NA
9 - When I pee it hurts.	0	1	2	3	NA
10 - Parents to answer. Has your child experienced something stressful like to example below? • New baby. • New home. • New school. • School problems. • Abuse (sexual/physical). • Home problems (divorce/death). • Special events (birthday). • Accident/injury. • Others.		NO (0)		YES (3)	
Total					

COMMENTS

This study provides the first adaptation into the Brazilian context of a specific instrument for the voiding dysfunction in children. To date, there is no consensus in the literature regarding the best strategy to perform transcultural adaptations. The process chosen was based on the script proposed by Guilemin et al. (8). In addition, this method has been already successfully applied in Brazil by other researchers.

At the present time, there are a great number of questionnaires developed on a certain culture. The process of translation and cultural adaptation has been considered essential for comparisons between studies from different countries, languages, and cultures. A good linguistically accurate translation is not sufficient because items must also be adapted culturally to preserve the conceptual meaning of the questionnaire.

The translations should be mainly evaluated in terms of conceptual equivalence so that necessary grammatical changes can be conceptually similar to another culture. With regard to the cross-cultural adaptation, cultural factors such as habits and activities of a population should be considered because an

activity which is not common in a certain population can make the instrument's adaptation invalid. In the present study, no obstacles were found either in the Brazilian Portuguese language or regarding the cultural aspects which could render unviable the applicability of the translation and the cross-cultural adaptation of the analyzed instrument.

The cross-cultural adaptation process of the Dysfunctional Voiding Symptom Score to Brazilian-Portuguese language followed standardized guides: translation, synthesis, back-translation, Expert Committee, and pre-testing (7,8).

The evaluation of the reliability showed satisfactory internal consistency as indicated by a Cronbach's alpha coefficient of 0.76 for the test and 0.77 for the retest.

The changes made on the adapted version were authorized by the author of the original instrument after previous consultation.

After all the stages had been achieved, the entire process of the cross-cultural adaptation was considered completed.

However, this study did not include the assessment of measurement equivalence. Therefore, further studies should perform this task comparing the psychometric properties of the Portuguese version of the DVSS to those of the original instrument.

CONCLUSION

The cross-cultural adaptation process of the Dysfunctional Voiding Symptom Score questionnaire to be used on Brazilian children was successfully completed following internationally accepted methodologies.

CONFLICT OF INTEREST

None declared.

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Urinary Proteomics Evaluation in Interstitial Cystitis/Painful Bladder Syndrome: A Pilot Study

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ABSTRACT

Purpose: Interstitial cystitis/painful bladder syndrome (IC/PBS) is characterized by chronic pain, pressure and discomfort felt in the pelvis or bladder. An in-depth shotgun proteomics study was carried out to profile the urinary proteome of women with IC/PBS to identify possible specific proteins and networks associated with IC/PBS.
Materials and Methods: Urine samples from ten female IC/PBS patients and ten female asymptomatic, healthy control subjects were analyzed in quadruplicate by liquid chromatography-tandem mass spectrometry (LC-MS/MS) on a hybrid linear ion trap-orbitrap mass spectrometer. Gas-phase fractionation (GPF) was used to enhance protein identification. Differences in protein quantity were determined by peptide spectral counting.
Results: α-1B-glycoprotein (A1BG) and orosomucoid-1 (ORM1) were detected in all IC/PBS patients, and ≥ 60% of these patients had elevated expression of these two proteins compared to control subjects. Transthyretin (TTR) and hemopexin (HPX) were detected in all control individuals, but ≥ 60% of the IC/PBS patients had decreased expression levels of these two proteins. Enrichment functional analysis showed cell adhesion and response to stimuli were down-regulated whereas response to inflammation, wounding, and tissue degradation were up-regulated in IC/PBS. Activation of neurophysiological processes in synaptic inhibition, and lack of DNA damage repair may also be key components of IC/PBS.
Conclusion: There are qualitative and quantitative differences between the urinary proteomes of women with and without IC/PBS. We identified a number of proteins as well as pathways/networks that might contribute to the pathology of IC/PBS or result from perturbations induced by this condition.

Key words: *interstitial cystitis; painful bladder syndrome; urine proteomics*
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INTRODUCTION

Interstitial cystitis/painful bladder syndrome (IC/PBS) is defined by chronic pain, pressure and discomfort felt in the lower pelvis or bladder, which are unrelated to any identifiable cause. Urinary urgency and frequency are also common symptoms of IC/PBS. Despite years of intense research, the underlying etiology, pathophysiology, and risk factors for developing and perpetuating this syndrome remain unclear. Diagnosis is based on symptoms and exclusion of other conditions, due to the lack of characteristic pathological findings, well-defined disease phenotypes, or objective biomarkers. Because of

these barriers, the diagnosis of IC/PBS is frequently delayed, and treatment frequently requires a multimodal approach (1).

One of the hypotheses proposed for the pathophysiology of IC/PBS is disruption of the urothelial barrier leading to symptoms. Bladder surface mucus, composed of glycosaminoglycans (GAGs) and proteoglycans, creates a highly impermeable barrier that is a key to maintain bladder function. Destruction of this barrier leads to tissue infiltration of urinary solutes, in particular potassium, which depolarizes nerves and muscles and causes tissue injury (2). Neurogenic inflammation has also been proposed as a pathophysiologic mechanism in IC/PBS (3). In response to stimuli, urothelial cells could activate neural circuits, releasing factors that cause chronic pain. Both hypotheses could conceivably result in urinary protein byproducts, which could then potentially serve as biomarkers for IC/PBS.

The lack of biomarkers that can be used for diagnosis of IC/PBS or to track treatment efficacy contributes to the clinical burden. Thus, identification of biomarker(s) for IC/PBS would represent a major advance in the field. Cataloging biomolecules present in complex biological samples has become increasingly important in clinical research for the purpose of identifying disease specific biomarkers. In the case of proteins, proteomics uses mass spectrometry to qualitatively and quantitatively catalog proteins. Application of proteomics to human diseases is challenging because about 35,000 human genes could translate into over 1,000,000 functional protein entities due to post-translational modifications as well as sequence variations (4). In spite of these complexities urinary biomarker discovery holds considerable promise because it has been recently shown that the urinary proteome contains approximately 1,500 proteins (5). This makes the urinary proteome far less complex than the blood proteome where biomarkers are also being sought for various human diseases (4).

In this pilot study, we applied proteomic strategies and related methodologies to profile the urinary proteome of patients with IC/PBS. The potential benefits of this study include a greater understanding of possible causes and underlying mechanisms of IC/PBS.

MATERIALS AND METHODS

Urine Sample Collection and Processing

Human urine acquisition was carried out with our institution's Ethics Committee approval. Ten women with a clinical diagnosis of IC/PBS, being treated and followed in the Female Urology Clinic, were enrolled in the study. All women had symptoms of IC/PBS for at least one year, and all had undergone extensive evaluation to exclude reversible, identifiable causes for their pelvic pain and urinary symptoms. Control urine was obtained from ten asymptomatic, pain-free, healthy female subjects, age-matched to the IC/PBS group. One protease inhibitor cocktail tablet (Roche, Indianapolis, IN) was added per 50 mL urine to avoid proteolysis after urine collection. The urine was centrifuged at 2,000 x g for 10 min at 4°C to remove cells and debris. The supernatant was collected and processed for protein purification by TCA (trichloroacetic acid) precipitation (10% w/v). Protein concentration was measured by BCA™ protein assay (Thermo Fisher, Waltham, MA). Proteins, 200µg each per subject, were reduced, alkylated, digested with trypsin (Promega, Madison, WI), and then desalted.

Mass Spectrometry Analysis

Peptide digests were analyzed by electrospray ionization on a hybrid linear ion trap-orbitrap mass spectrometer (Thermo Fisher). For each liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis, approximately 0.5µg of peptides were loaded on the column and eluted in acetonitrile gradient (6). To maximize protein identification without protein fractionation, ions were selected via a data-dependent process from 400-2,000 Th or by gas-phase fractionation (GPF) from 400-521, 516-690, 685-968, and 963-2,000 Th (6). Each experiment was acquired in quadruplicate.

Database Search and Protein Identification

Acquired tandem mass spectra (MS/MS) were searched for sequence matches against the Interna-

tional Protein Index (IPI) human protein database using SEQUEST. PeptideProphet and ProteinProphet, which compute a probability likelihood of each identification being correct, were used for statistical analysis (7). Only proteins identified by more than one unique peptide sequence were included in the analysis. Differences in protein expression were calculated using peptide spectral counting algorithms that use MS/MS data to estimate changes in relative abundance of proteins (8).

Western Blot Analysis

Ten µg of pooled IC/PBS or control urine protein was resolved on 4-12% NuPAGE® gel (Invitrogen, Carlsbad, CA) and transferred to PVDF membrane for incubation with primary antibodies, followed by HRP-conjugated secondary antibodies (Amersham, Piscataway, NJ). Reactivity was visualized by enhanced chemiluminescence (Amersham).

RESULTS

IC/PBS and Control Urinary Proteomes

GPF increased protein identification by more than 60% over the use of one large m/z range in both sets of urine samples (Figure-1A). A total of 889 IC/PBS and 1003 control proteins with Protein Probability ≥ 0.8, with error rates ≤ 0.023 and ≤ 0.022 respectively, were identified. Recently, the normal urine proteome was extensively analyzed revealing more than 1,500 proteins (5). A comparative analysis of our IC/PBS and control urines, and normal urine data by Adachi et al. (5) is shown in a Venn diagram (Figure-1B) created by ProteinCenter (www.proxeon.com). According to this analysis, 165 proteins appeared to be unique to IC/PBS. However, proteins identified in only one mutually exclusive subset may be due to under-sampling in other samples or result from data filtering (7).

Identified urine proteins were annotated with Gene Ontology (GO) (9), which assign probable subcellular compartmentalization and molecular functions. Approximately 50% of the proteins identified were annotated as secreted or membrane-associated proteins, which may be a characteristic of the urine proteome (5).

Proteins Associated with IC/PBS

A total of 78 proteins with P-value ≤ 0.1 were considered to be statistically significant for differential expression between IC/PBS and control for this study (Table-1). This P-value was chosen to cast a wider net that includes the most of the differentially expressed proteins. By quantitative analysis, 19 were found up-regulated in IC/PBS compared to control, and 59 were down-regulated. Among these, we focused on proteins identified in all ten IC/PBS subjects and were up-regulated in at least 60% of this cohort by spectral counts. Similarly, proteins that were found in all ten control subjects, and were down-regulated in more than 60% of IC/PBS cohort were also investigated. Using these criteria, two up-regulated and two down-regulated proteins were found. The two up-regulated were α-1B-glycoprotein (A1BG) and orosomucoid-1 (ORM1) both of which are glycoproteins. This is promising in that many current biomarkers like prostate specific antigen (PSA) are glycoproteins (7). A1BG is a plasma glycoprotein of unknown function but over-expression of this protein in pancreatic adenocarcinoma patients has been reported (10). ORM1 is an acute phase plasma protein that increases as a result of acute inflammation (11). The two down-regulated proteins were transthyretin (TTR) and hemopexin (HPX). TTR is a thyroid hormone-binding protein. Defects in TTR are the cause of amyloidosis (12). HPX protects low-density lipoprotein against hemoglobin-induced oxidation (13). Differential expression of these proteins was further validated by Western blot analysis of pooled IC/PBS and control samples (Figure-2). Among the other up-regulated proteins, afamin (APF), osteopontin (SPP1), pancreatic secretory trypsin inhibitor (SPINK1), proactivator polypeptide (PSAP), and apolipoprotein (LPA) were found from all ten IC/PBS patients.

Enrichment functional analysis, which ranks the most relevant cellular processes among the differentially expressed proteins, was performed by MetaCore™ pathway analysis tool (www.genego.com). Cellular process networks such as inflammatory response, tissue degradation, and wounding response

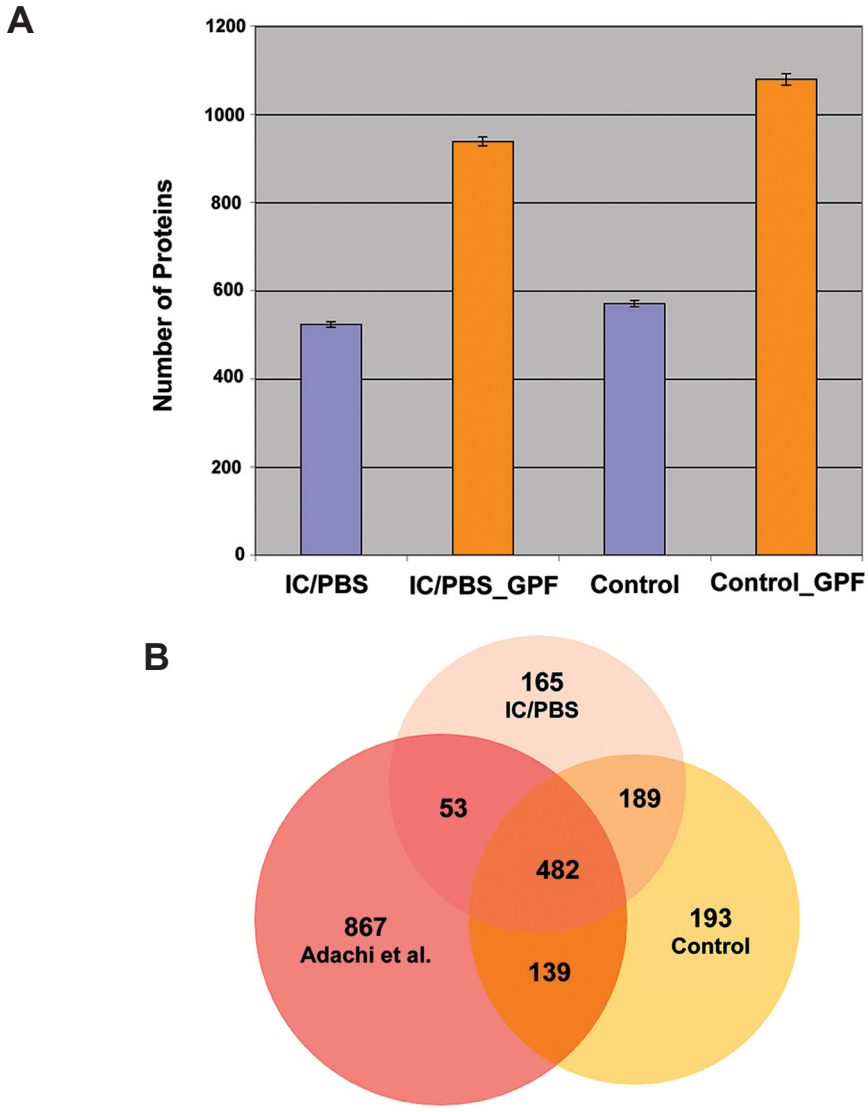

Figure 1 - IC/PBS and control urine protein identifications. (A) Average number ± standard deviation (on 4 technical replicates) of identified proteins with 2 or more peptides (multiple hits) using single m/z or 4 gas-phase m/z fractions for IC/PBS and control urine. (B) Our urinary proteome of IC/PBS and controls were compared to the previously reported normal urine proteome (Adachi et al. 2006). The Venn diagram shows overlapped proteins (621) between our asymptomatic control proteins and previously published healthy urine proteins. This comparative analysis also identified 165 proteins that may be unique to the IC/PBS proteome. However, proteins identified in one sample only may be due to under-sampling in other sample or result from data filtering.

were found up-regulated in IC/PBS, whereas cell adhesion, extra cellular matrix remodeling, and stimulus response were found to be down-regulated.

When the 165 IC/PBS proteins (Figure-1B) were queried for pathways, neurophysiological GABA-A receptor life cycle pathway was mapped with the most number of proteins. Gamma-amino butyric acid receptors (GABA-A) mediate fast synaptic inhibition in the brain and spinal cord (14). Alterations in neuronal surface receptors modulate the synaptic strength, leading to changes in sensitivity to neurotransmitters (15). When a similar network analysis was performed for the 193 control proteins,

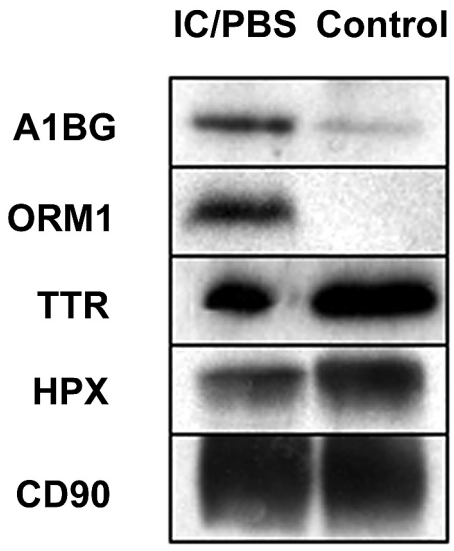

Figure 2 *– Western blot analysis of differentially expressed proteins. Ten μg of pooled proteins were resolved by gel electrophoresis and probed by antibodies. The expression level as indicated by the band intensity correlated well with the spectral count quantification method. Shown are the results for α-1B-glycoprotein (A1BG), orosomucoid-1 (ORM1), transthyretin (TTR), and hemopexin (HPX). CD90 (Thy-1) served as the control for sample loading. CD90 is a GPI-anchored protein also found secreted.*

DNA damage regulation pathway was one of the most activated pathways, suggesting a lack of DNA damage regulation and repair functions in IC/PBS.

In Silico Analysis of Tissue Specific Expression

Although the proteins identified in this study are found in urine, some of the proteins identified may be more highly expressed in a specific tissue type, and their tissue specificity could enhance understanding of the mechanisms underlying IC/PBS. Among the differentially expressed proteins, cell adhesion molecule with homology to L1CAM (CHL1) is highly expressed in the cortex, brain, and spinal cord based on UniProt tissue classifications (www.uniprot.org). CHL1 is a neural recognition molecule involved in signal transduction pathways, and loss of this gene is responsible for mental defects (16). In our datasets, CHL1 was down-regulated in IC/PBS.

Protein-protein Interaction Network

Protein-protein interactions are important in signal transduction, which plays a fundamental role in many biological processes and diseases. The differentially expressed proteins were investigated for novel protein-protein interactions using MetaCore™. Direct and indirect protein interactions were ranked and interpreted in terms of GO processes. Two novel protein network modules with potential importance in IC/PBS were identified: 1) glucose metabolic process and positive regulation of natural killer cell-mediated immune response to tumor cells, and 2) response to external stimulus, cell adhesion, wounding, and stress.

COMMENTS

Much effort has been devoted to the search for useful biomarkers for IC/PBS diagnosis, phenotyping, and for predicting response to treatment (17). Initial attempts to develop a urinary biomarker concentrated on mediators of pain such as substance P (18). Other proposed pain biomarkers have included uroplakin III-δ4 mRNA (19), and heparin-binding epidermal growth factor-like growth factor (HB-EGF) (20). Antiproliferative factor (APF) is another candidate (21). To date, none of these has been shown to definitively correlate with IC/PBS symptoms, clinical course, or response to treatment. In a recent urine biomarker evaluation study, no robust association among urinary IL-6, cyclic guanosine monophosphate, HB-EGF, epidermal growth factor, APF, and bladder biopsy was found in IC/PBS (22). Previously, a urinary proteomic method was applied to identify biomarkers from age-, race-, and gender-matched IC/PBS and control subjects (23). Three up-regulated proteins (uromodulin and two kininogens) in the control and one up-regulated protein (inter-α-trypsin inhibitor heavy chain H4) in the IC/PBS were found. These proteins showed a correlation to IC severity on IC-specific quality-of-life scales. All four proteins were also found in our study but their differential expressions were not statistically significant in our datasets.

The goal of this study was to use an in-depth proteomic approach to identify specific proteins or

Table 1 *– Differentially expressed proteins identified from IC/PBS and control urine samples. The protein IPI ID, annotation, ProteinProphet probability score, gene, expression ratio (IC/control, log2), non-adjusted P-value, and GO biological process, GO molecular function, and GO cellular compartment are tabulated. The list is sorted by P-values. ProteinProphet probability 1 represents the highest score of correct identification. NA = not available.*

Up-regulated proteins in IC/PBS

Protein	Annotation	Probability	Gene	IC/ Control	p Value	Biological Process	Molecular Function	Cellular compartment
IPI00019943	Afamin	1	AFM	1.69	0.00	transport	NA	extracellular
IPI00889156	Immunoglobulin κ variable 3-20	1	IGKV3-20	1.91	0.01	immune response	antigen binding	extracellular
IPI00022895	α-1B-glycoprotein	1	A1BG	1.10	0.01	NA	NA	extracellular
IPI00010402	Uncharacterized protein	1	SH3BGRL3	0.79	0.03	NA	NA	nucleus
IPI00018136	Vascular cell adhesion protein 1	1	VCAM1	3.09	0.03	cell-cell adhesion	protein binding	membrane
IPI00784430	Ig κ chain V-III region VG	1	IGKV3D-11	2.17	0.04	immune response	antigen binding	extracellular
IPI00292150	Latent-transforming growth factor β-binding protein 2	0.99	LTBP2	3.00	0.04	signaling pathway	calcium ion binding	extracellular
IPI00021000	Osteopontin	1	SPP1	1.00	0.05	ossification	cytokine activity	extracellular
IPI00022417	Leucine-rich α-2-glycoprotein	1	LRG1	1.97	0.05	NA	protein binding	membrane
IPI00884926	Orosomucoid 1	1	ORM1	1.66	0.05	acute-phase response	protein binding	extracellular
IPI00020687	Pancreatic secretory trypsin inhibitor	1	SPINK1	1.11	0.05	NA	serine-type endopeptidase inhibitor activity	extracellular

Table 1 – continued

IPI00018236	Ganglioside GM2 activator	1	GM2A	1.41	0.06	glycolipid catabolic process	sphingolipid activator protein activity	lysosome
IPI00007726	Kallikrein-13	0.93	KLK13	1.32	0.07	proteolysis	serine-type endopeptidase activity	cytoplasm
IPI00012503	Proactivator polypeptide	1	PSAP	0.54	0.07	glycosphingolipid metabolic process	α-galactosidase activity	lysosome
IPI00020091	α-1-acid glycoprotein 2	1	ORM2	1.24	0.08	acute-phase response	binding	extracellular
IPI00160384	Protein δ homolog 1	1	DLK1	0.95	0.08	multicellular organismal development	calcium ion binding	membrane
IPI00217236	Tubulin-specific chaperone A	0	TBCA	3.17	0.09	post-chaperonin tubulin folding pathway	unfolded protein binding	cytoskeleton
IPI00029168	Apolipoprotein (a)	1	LPA	1.15	0.09	proteolysis	serine-type endopeptidase activity	extracellular
IPI00290856	Lymphatic vessel endothelial hyaluronic acid receptor 1	1	LYVE1	1.09	0.09	cell-matrix adhesion	hyaluronic acid binding	membrane

Table 1 – continued

Down-regulated proteins in IC/PBS								
Protein	**Annotation**	**Probability**	**Gene**	**IC/ Control**	**p Value**	**Biological Process**	**Molecular Function**	**Cellular Component**
IPI00022432	Transthyretin	1	TTR	-1.05	0.00	thyroid hormone generation	thyroid hormone transporter activity	extracellular
IPI00016334	Cell surface glycoprotein MUC18	1	MCAM	-1.49	0.01	cell adhesion	protein binding	membrane
IPI00015199	T-cell antigen CD7	1	CD7	-2.32	0.01	calcium ion transport	receptor activity	membrane
IPI00153049	Matrix-remodeling-associated protein 8	1	MXRA8	-1.55	0.01	NA	NA	membrane
IPI00240345	C-type lectin domain family 14 member A	1	CLEC14A	-3.91	0.01	NA	sugar binding	membrane
IPI00019157	Chondroitin sulfate proteoglycan 4	1	CSPG4	-2.09	0.01	angiogenesis	tyrosine phosphatase signaling	membrane
IPI00183445	Latrophilin-1	1	LPHN1	-2.50	0.01	neuropeptide signaling pathway	G-protein coupled receptor activity	membrane
IPI00293057	Carboxypeptidase B2	1	CPB2	-1.14	0.02	proteolysis	zinc ion binding	extracellular
IPI00291867	Complement factor I	1	CFI	-0.84	0.02	proteolysis	serine-type endopeptidase activity	membrane
IPI00218834	Low affinity Ig γ Fc region receptor III-A	1	FCGR3A	-2.32	0.02	immune response	IgG binding	membrane
IPI00298971	Vitronectin	1	VTN	-1.08	0.02	immune response	heparin binding	extracellular

Table 1 *– continued*

IPI00300786	α-amylase 1	1	AMY1A, AMY1B	-1.30	0.02	carbohydrate metabolic process	α-amylase activity	extracellular
IPI00297124	IL-6 receptor subunit β	1	IL6ST	-1.91	0.02	signal transduction	IL-6 receptor activity	membrane
IPI00387119	Ig κ chain V-III region POM	1	IGKV3	-1.93	0.03	NA	NA	NA
IPI00026270	Carboxypeptidase M	1	CPM	-1.25	0.03	proteolysis	zinc ion binding	membrane
IPI00218914	Retinal dehydrogenase 1	1	ALDH1A1	-3.17	0.03	aldehyde metabolic process	retinal dehydrogenase activity	cytosol
IPI00022488	Hemopexin	1	HPX	-0.83	0.03	cellular iron ion homeostasis	iron ion binding	extracellular
IPI00026944	Nidogen-1	1	NID1	-1.04	0.04	protein-chromophore linkage	calcium ion binding	membrane
IPI00299059	Neural cell adhesion molecule L1-like protein	1	CHL1	-1.91	0.04	nervous system development	protein binding	membrane
IPI00020996	Insulin-like growth factor-binding protein complex acid labile chain	1	IGFALS	-2.14	0.04	signal transduction	insulin-like growth factor binding	soluble fraction
IPI00646689	Thioredoxin domain-containing protein 17	1	TXNDC17	-3.58	0.04	cell redox homeostasis	NA	cytoplasm

Table 1 – continued

IPI00025476	Pancreatic α-amylase	1	AMY1C, AMY2A	-1.41	0.05	carbohydrate metabolic process	α-amylase activity	extracellular
IPI00169383	Phosphoglycerate kinase 1	1	PGK1	-1.22	0.05	glycolysis	ATP binding	cytoplasm
IPI00032532	Growth arrest-specific protein 6	1	GAS6	-1.91	0.05	regulation of cell growth	calcium ion binding	extracellular
IPI00031065	Deoxyribonuclease-1	1	DNASE1	-0.80	0.05	DNA catabolic process	Deoxyribonuclease I activity	nucleus
IPI00027493	4F2 cell-surface antigen heavy chain	1	SLC3A2	-1.62	0.05	calcium ion transport	calcium:sodium antiporter activity	melanosome
IPI00301579	Epididymal secretory protein E1	1	NPC2	-1.26	0.05	cholesterol homeostasis	cholesterol binding	lysosome
IPI00001759	Oxidized low-density lipoprotein receptor 1	1	OLR1	-0.68	0.05	proteolysis	receptor activity	membrane
IPI00219622	Proteasome subunit α type-2	1	PSMA2	-2.00	0.05	protein catabolic process	threonine endopeptidase activity	nucleus
IPI00015525	Multimerin-2	1	MMRN2	-1.00	0.05	NA	NA	extracellular
IPI00073772	Fructose-1,6-bisphosphatase 1	1	FBP1	-1.03	0.06	gluconeogenesis	phosphatase activity	cytosol
IPI00220271	Alcohol dehydrogenase	1	AKR1A1	-1.74	0.06	glucose metabolic process	aldehyde reductase activity	NA
IPI00032179	Antithrombin III variant	1	SERPINC1	-1.29	0.06	blood coagulation	serine-type endopeptidase inhibitor activity	membrane
IPI00816555	IGLV2-14 protein	1	IGLV2-14	-1.58	0.06	NA	NA	NA
IPI00291136	Collagen α-1(VI) chain	1	COL6A1	-0.70	0.07	phosphate transport	protein binding	soluble fraction

Table 1 – continued

IPI00442294	Neurotrimin variant 3	1	HNT	-3.32	0.07	neuron recognition	protein binding	membrane
IPI00293088	Lysosomal α-glucosidase	1	GAA	-1.25	0.07	diaphragm contraction	α-glucosidase activity	lysosome
IPI00029275	Melanotransferrin	1	MFI2	-1.08	0.07	cellular iron ion homeostasis	ferric iron binding	membrane
IPI00465248	α-enolase	1	ENO1	-1.12	0.07	glycolysis	transcription corepressor activity	nucleus
IPI00034319	Protein CutA	1	CUTA	-0.91	0.08	response to metal ion	enzyme binding	membrane
IPI00329801	Annexin A5	1	ANXA5	-1.28	0.08	anti-apoptosis	phospholipase inhibitor activity	cytoplasm
IPI00219525	6-phosphogluconate dehydrogenase	1	PGD 6	-1.05	0.08	pentose-phosphate shunt, oxidative branch	phosphogluconate dehydrogenase	NA
IPI00232571	Glypican-4	1	GPC4	-1.74	0.08	cell proliferation	NA	membrane
IPI00032294	Cystatin-S	1	CST4	-3.58	0.08	NA	cysteine protease inhibitor activity	extracellular
IPI00295777	Glycerol-3-phosphate dehydrogenase	1	GPD1	-2.32	0.08	glycerol-3-phosphate catabolic process	dehydrogenase	cytosol
IPI00219425	Poliovirus receptor	1	PVR	-0.77	0.08	cell adhesion	receptor activity	membrane
IPI00000792	Quinone oxidoreductase	1	CRYZ	-3.17	0.09	visual perception	quinone reductase activity	cytoplasm
IPI00182728	Vacuolar protein sorting-associating protein 4B	1	VPS4B	-2.17	0.09	intracellular cholesterol transport	ATPase activity	membrane

Table 1 – continued

IPI00024284	Basement membrane-specific heparan sulfate proteoglycan core protein	1	HSPG2	-0.53	0.09	cell adhesion	protein binding	membrane
IPI00742696	Vitamin D-binding protein	1	GC	-0.66	0.09	vitamin transport	actin binding	extracellular
IPI00007221	Plasma serine protease inhibitor	1	SERPINA5	-0.65	0.09	transport	serine-type endopeptidase inhibitor activity	membrane
IPI00646304	Peptidyl-prolyl cis-trans isomerase B	1	PPIB	-1.49	0.09	protein folding	peptidyl-prolyl cis-trans activity	melanosome
IPI00099670	Carboxyl ester lipase	1	CEL	-1.24	0.09	triacylglycerol metabolic process	sterol esterase activity	cytoplasm
IPI00215980	Poliovirus receptor-related protein 2	1	PVRL2	-0.82	0.09	homophilic cell adhesion	coreceptor activity	membrane
IPI00439446	Mannosidase, α, class 1A, member 1	1	MAN1A1	-1.18	0.10	glycosylation	mannosidase activity	Golgi membrane
IPI00550640	IGHG4 protein	1	IGHG4	-1.11	0.10	immune response	copper ion binding	membrane
IPI00016786	Cell division control protein 42 homolog	0.94	CDC42	-1.58	0.10	positive regulation of pseudopodium formation	GTPase activity	cytosol
IPI00437186	Probable G-protein coupled receptor 116	1	GPR116	-3.17	0.10	neuropeptide signaling pathway	G-protein coupled receptor activity	membrane
IPI00000073	Pro-epidermal growth factor	1	EGF	-0.58	0.11	positive regulation of phosphorylation	EGF receptor activating ligand activity	nucleus

protein networks that may be involved in IC/PBS pathogenesis. A number of urine proteins were found to be differentially expressed between IC/PBS and control. Among the up-regulated proteins in IC/PBS, A1BG and ORM1 were present in all ten IC/PBS patients, with up-regulated expression in ≥ 60% of the cohort. TTR and HPX were found down-regulated in ≥ 60% of the IC/PBS cohort, and were present in all control urines. None of these four proteins have been previously implicated in IC/PBS pathogenesis. Two of these, A1BG and ORM1, are glycoproteins. Currently, many clinical biomarkers and therapeutic targets are glycoproteins, e.g. Her2/neu, PSA, and CA125.

165 and 193 proteins were found either in the IC/PBS or control urines, respectively. One interesting finding from these datasets was that pathway and network analyses identified possible activation of neurophysiological processes involved in synaptic inhibition, and lack of DNA damage repair in IC/PBS. One of the most important findings in pain research has been the identification of changes in the central nervous system (CNS), which may explain the perpetuation of pain in chronic pain syndromes (24). For example, in male chronic pelvic pain syndrome, responses to painful stimuli are changed, and evidence of nervous system alterations is present (25). CHL1 is known to be abundantly expressed in the CNS (e.g., brain, and spinal cord). Overall expression level of CHL1 was down-regulated in IC/PBS. Although no conclusions are being made as a result of these data, altered response to stimuli taken together with down-regulation of CNS proteins such as CHL1 may represent neurophysiological changes that contribute to IC/PBS pathogenesis.

Our strategy identified many differentially expressed proteins not previously associated with IC/PBS and this led to hypotheses around several novel network modules. These included glucose metabolism, alteration of which has been linked to human diseases (26). Natural killer cell mediated immune response has been well documented in various human diseases including prostate cancer (27). Little is known about involvement of glucose metabolism and natural killer cell immune response in IC/PBS. However, an association of glucose metabolism in IC/PBS has been recently detected by gene array analysis of experimentally induced IC in mice (28). Although the implication of these networks needs to be further investigated, any alteration to protein-protein interactions could impact the natural cascade signaling process.

Although there is clear correspondence between pathological events and changes in protein expression in relevant networks and modules, whether any of the differentially expressed proteins are true markers for IC/PBS will require further investigation. In urine analysis, an individual's lifestyle, diet, medication history, and time of urine collection can influence the proteome profile; none of these factors were considered in urine collection in this or other studies. Another limitation of this study is the lack of detailed phenotyping of the subjects, which might aid data interpretation. However, this is a pilot study, and we were attempting to determine if our methods held merit for identifying selected proteins; furthermore, this small cohort would likely preclude any conclusions based on demographic or clinical variables.

CONCLUSION

Our preliminary data indicate that there are qualitative and quantitative differences between the urinary proteomes of women with and without IC/PBS. We identified a number of proteins as well as pathways/networks that might contribute to the pathology of IC/PBS or result from perturbations induced by this condition.

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CONFLICT OF INTEREST

None declared.

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EDITORIAL COMMENT

I will say in order that biological meaning may be derived and testable hypotheses may be built from proteomic experiments in relation to IC/PBS, assignments of proteins detected by mass spectrometry must be supplemented with additional notation, such as information on known protein functions, protein-protein interactions, or biological pathway associations.

Visualizing this bulk of proteomic information and summarization the resulting significant differential expressed proteins underlying IC/PBC in an easy to navigate tabular formats, including meta-information on those proteins in addition to complementary gene ontology (GO) terminology, is also important so that in-house expertise on particular proteins may be integrated into the larger datasets.

Furthermore, proteins of interest underlying IC/PBC can be exported and matched to allow for re-searching of mass spectrometry data, and gene names corresponding to the proteins underlying IC/PBS for further characterization, including pathway analysis. Therefore, I am hoping future published

articles can make use of certain proteomic mapping and comparison tools (1-4) further investigating mass spectrometry and proteomic outputs in order to derive insight into the signaling pathway underlying IC/PBS.

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EDITORIAL COMMENT

Interstitial cystitis (IC) is a debilitating chronic disease caused by undetermined and unknown factors, which impedes the development of accurate diagnostic methods, therefore delaying the treatment of patients that otherwise would promptly be cared for. Currently, the diagnosis of IC in most cases is given by exclusion mainly because this pathology lacks adequate biologic markers in blood and urine.

Exact etiology of interstitial cystitis (IC) is unknown, however the impermeability of the urothelial barrier of the bladder just as an alteration in the production of urine proteins could play important physiological roles in lower urinary tract dysfunction (1).

Recently, the study of proteome has been introduced as a diagnostic tool for inflammatory diseases of difficult diagnoses. In diabetic nephropathy the proteomic marker can be a prognostic and/or a therapeutic factor (2).

In a preliminary study, the authors showed that there were qualitative and quantitative differences between the urinary proteomes of women with and without IC/PBS. Furthermore, future studies researching the proteome characteristics associated with IC could provide not only a better comprehension of physiopathology but also lead to further development of new drugs or therapies for treatment and/or prevention of IC and related disorders.

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Diminution of Oxalate Induced Renal Tubular Epithelial Cell Injury and Inhibition of Calcium Oxalate Crystallization in vitro by Aqueous Extract of Tribulus terrestris

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ABSTRACT

Purpose: Recurrence and persistent side effects of present day treatment for urolithiasis restrict their use, so an alternate solution, using phytotherapy is being sought. The present study attempted to evaluate the antilithiatic properties of Tribulus terrestris commonly called as "gokhru" which is often used in ayurveda to treat various urinary diseases including urolithiasis.

Materials and Methods: The activity of Tribulus terrestris was investigated on nucleation and the growth of the calcium oxalate (CaOx) crystals as well as on oxalate induced cell injury of NRK 52E renal epithelial cells.

Results: Tribulus terrestris extract exhibited a concentration dependent inhibition of nucleation and the growth of CaOx crystals. When NRK-52E cells were injured by exposure to oxalate for 72 h, Tribulus terrestris extract prevented the injury in a dose-dependent manner. On treatment with the different concentrations of the plant, the cell viability increased and lactate dehydrogenase release decreased in a concentration dependent manner.

Conclusion: The current data suggests that Tribulus terrestris extract not only has a potential to inhibit nucleation and the growth of the CaOx crystals but also has a cytoprotective role. Our results indicate that it could be a potential candidate for phytotherapy against urolithiasis.

Key words: *phytotherapy; urolithiasis; calcium oxalate; NRK 52E, Tribulus terrestris*
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INTRODUCTION

Nephrolithiasis is common, affecting up to 10% of the population at some point during their lifetime (1). Calcium-containing stones are the most commonly occurring to an extent of 75-90% followed by magnesium ammonium phosphate (Struvite) to an extent of 10-15%, uric acid 3-10% and cystine 0.5-1% (2). Calcium oxalate stones are found in two different varieties, calcium oxalate monohydrate (COM) or Whewellite, and calcium oxalate dihydrate (COD) or Weddellite. COM, the thermodynamically most stable form, is observed more frequently in clinical stones than COD and it has a greater affinity for renal tubular cells, thus responsible for the formation of stones in the kidney (3).

Various authors have suggested the role of crystal induced cell injury in the development of

kidney stones by providing the sites for crystal attachment and retention within the kidneys (4,5).

Oxalate, a metabolic end product and a major constituent of the majority of renal stones, has been shown to be toxic to renal epithelial cells of cortical origin (6). It has been observed that exposure of renal epithelial cells to oxalate which is a constituent of most kidney stones leads to a disruption of the normal activities of the renal epithelial cells such as altered membrane surface properties and cellular lipids, changes in gene expression, disruption of mitochondrial function, formation of reactive oxygen species and decreased cell viability (7).

Various mechanisms have been proposed to explain crystal retention (8). As a result of crystal growth and agglomeration, particles may be formed that are too large to freely pass the renal tubules. Alternatively, relatively small crystals could be retained by adhering to the surface of the urothelial lining and then increase in size (8).

The surgical methods available to treat kidney stones like extracorporeal shock wave lithotripsy have serious side effects. Therefore, it is worthwhile to look for an alternative for the management of urolithiasis. Many medicinal plants have been employed during ages to treat urinary stones though the rationale behind their use is not well established through systematic and pharmacological studies, except for some composite herbal drugs and plants (9-12). Plant medicines are in great demand both in the developed as well as developing countries for primary health care because of their wide range of biological and medicinal activities, higher safety margin and low cost.

Fruits of Tribulus terrestris (Zygophyllaceae) locally named as "gokhru" in India are commonly used in folklore to treat urolithiasis. So far, its diuretic properties have been documented in literature and it is actively used in various drug formulations of kidney stone treatments.

The present study aimed at investigating the efficacy of Tribulus terrestris on calcium oxalate crystal nucleation and growth in vitro as well as further examining the potency of Tribulus terrestris on oxalate induced injury in NRK 52E (rat renal tubular epithelial) cells.

MATERIALS AND METHODS

Preparation of the Tribulus terrestris Extract

The dried and matured fruits of Tribulus terrestris were obtained from "Natural Remedies Pvt. Ltd." at Bangalore in India. A collection of voucher specimens is available at the company.

The air-dried fine powdered plant fruits were boiled in distilled water. The extract was then filtered using Whatman No. 1 filter paper and the filtrate was evaporated in vacuum and dried using a rotary evaporator at 60° C (13). The final dried samples were stored in labeled sterile bottles and kept at -20° C. The various concentrations of the plant sample tested for their inhibitory potency were 25 µg/mL, 50 µg/mL, 100 µg/mL, 200 µg/mL, 400 µg/mL and 1000 µg/mL, which were prepared at the time of experiment and were referred to as aqueous extract of Tribulus terrestris.

For cell culture studies a stock solution of the dried aqueous Tribulus terrestris extract was dissolved in dimethyl sulfoxide (DMSO) [final concentration of the DMSO in the highest concentration of plant extract tested did not exceed 0.4% (v/v) and did not affect the cell proliferation]. Further dilutions of the stock were done using serum free DMEM (Dulbecco's Modified Eagle's Media) and filtered by 0.3 mm syringe filter (14).

Nucleation Assay

The method used was similar to that described by Hennequin et al. with some minor modifications (15). Solutions of calcium chloride and sodium oxalate were prepared at the final concentration of 3 mmoL/L and 0.5 mmoL/L, respectively, in a buffer containing Tris 0.05 moL/L and NaCl 0.15 moL/L at pH 6.5. Both solutions were filtered through a 0.22 µm filter; 33 mL of calcium chloride solution was mixed with 3.3 mL of the aqueous extract at different concentrations. Crystallization was started by adding 33 mL of sodium oxalate solution. The final solution was magnetically stirred at 800 rpm using a PTFE-coated

stirring bar. The temperature was maintained at 37o C. The absorbance of the solution was monitored at 620 nm after every 1 min. The percentage inhibition produced by the herb extract was calculated as [1-(Tsi/Tsc)] X 100, where Tsc was the turbidity slope of the control and Tsi the turbidity slope in the presence of the inhibitor.

Growth Assay

Inhibitory activity against CaOx crystal growth was measured using the seeded, solution-depletion assay described previously by Nakagawa and colleagues (16). Briefly, an aqueous solution of 10 mM Tris-HCl containing 90 mM NaCI was adjusted to pH 7.2 with 4 N HC1. Stone slurry (1.5 mg/mL) was prepared in 50 mM sodium acetate buffer (pH 5.7). CaOx monohydrate crystal seed was added to a solution containing 1 mM CaCl2 and 1 mM sodium oxalate (Na2C2O4). The reaction of CaCl2 and Na2C2O4 with crystal seed led to deposition of CaOx (CaC2O4) on the crystal surfaces, thereby decreasing free oxalate that is detectable by spectrophotometry at λ214 nm. When aqueous extract is added into this solution, depletion of free oxalate ions will decrease if the test sample inhibits CaOx crystal growth. Rate of reduction of free oxalate was calculated using the baseline value and the value after 30-second incubation with or without test sample. The relative inhibitory activity was calculated as follows: % Relative inhibitory activity = [(C-S)/C] × 100, where C is the rate of reduction of free oxalate without any test sample and S is the rate of reduction of free oxalate with a test sample.

Cell Culture

Normal rat epithelial derived renal tubular epithelial (NRK 52E) cells were obtained from National Centre of Cell Sciences (NCCS, Pune). The cells were maintained as monolayers in Dulbecco's Modified Eagle's Medium (DMEM) with 2.0 mM L-glutamine adjusted to contain 3.7 g/L sodium bicarbonate, 4.5 g/L glucose. Media was supplemented with 1% Penicillin (100 units/mL)-Streptomycin (10,000 µg/mL) and 10% fetal bovine serum. Cells were cultured in 25 cm2 tissue-culture treated flasks at 37o C and 5% CO2 in humidified chambers.

Oxalate-induced Cell Injury

NRK 52E cells were incubated in DMEM containing 1 mM sodium oxalate in the presence of different concentrations of the aqueous extract of the test sample (10 µg/mL, 25 µg/mL and 50 µg/mL) for 72 hours (14,17). Cell injury was assessed by measuring the cell viability through trypan blue and monitoring the lactate dehydrogenase (LDH) leakage into the medium.

Cytotoxicity - Trypan Blue Assay

The cytotoxicity of the aqueous extract of T. terrestris was assessed by cell viability using trypan blue exclusion method. For the determination of cell viability, cells were plated at the density of 4 × 104 cells/well and cultured for 72 h. The medium was replaced with serum-free medium and the cells were treated with various concentrations of the plant extracts (10 µg/mL, 25 µg/mL and 50 µg/mL) for a further 72 h. The percentage viability for the cells was calculated as (live cells/total cells) x 100.

LDH Leakage Assay

LDH leakage assay was performed by the method described by Wagner et al. (18). Briefly, 6.6 mM NADH and 30 mM sodium pyruvate were prepared in Tris (0.2M, pH 7.3). Reaction was initiated with the addition of 50 µL of the test sample and the disappearance of NADH was monitored at 340 nm, for 5 min at an interval of 1 min. The percentage of LDH release was calculated by dividing the activity of LDH in the supernatant by the LDH activity measured after complete cell lysis achieved by sonication.

Statistical Analysis

Data were expressed as mean values of three independent experiments (each in triplicate) and ana-

lyzed by the analysis of variance ($p < 0.05$) to estimate the differences between values of extracts tested.

RESULTS

Inhibition of Nucleation of CaOx Crystals by Tribulus terrestris Extract

Figure-1 displays the effect of the different concentration of the aqueous extract of Tribulus terrestris on the nucleation of calcium oxalate crystals. As regards control (with no plant sample), the percentage inhibition was constant at 71.4 ± 0.001 with increase in the concentration of Tribulus terrestris extract of 25 µg/mL, 50 µg/mL and 100 µg/mL. As the concentration of Tribulus terrestris extract was increased to 200 µg/mL, the percentage inhibition increased to 100 ± 0.001 but was reduced to 85.7 ± 0.002 for 400 µg/mL. The percentage inhibition was restored to 100 ± 0.001 with 1000 µg/mL of the extract.

Inhibition of CaOx Crystal Growth by Tribulus terrestris Extract

Figure-2 demonstrates the percentage inhibition shown by Tribulus terrestris on the calcium oxalate crystal growth. Tribulus terrestris extract showed inhibition in a concentration dependent manner. The percentage inhibition with 25 µg/mL of plant sample was 17.6 ± 0.004. With 50 µg/mL, 100 µg/mL and 200 µg/mL, the inhibition was almost constant in the range of 65-70% but inhibition increased significantly with 400 µg/mL and 1000 µg/mL of Tribulus terrestris extract to 126.4 ± 0.001 and 169.2 ± 0.001 respectively.

Diminution of Oxalate-induced Renal Tubular Epithelial Cell Injury by Tribulus terrestris Extract

Figure-3 depicts the protective effect of the aqueous extract of Tribulus terrestris towards the renal tubular epithelial cells. The oxalate induced a significant injury to the cells which could be ascertained by a decrease in viability from 100% in the controls (untreated cells) to 73.9%. However, the injury due to oxalate was significantly reduced in those cells treated with the Tribulus terrestris extracts. As the concentration of the extract increased from 10 µg/mL to 50 µg/mL, the percentage viability improved showing that the plant has an inhibitory activity towards the oxalate which caused injury to the renal cells in a concentration dependent manner. The plant extract alone (50 µg/mL, containing 0.4% DMSO) had no

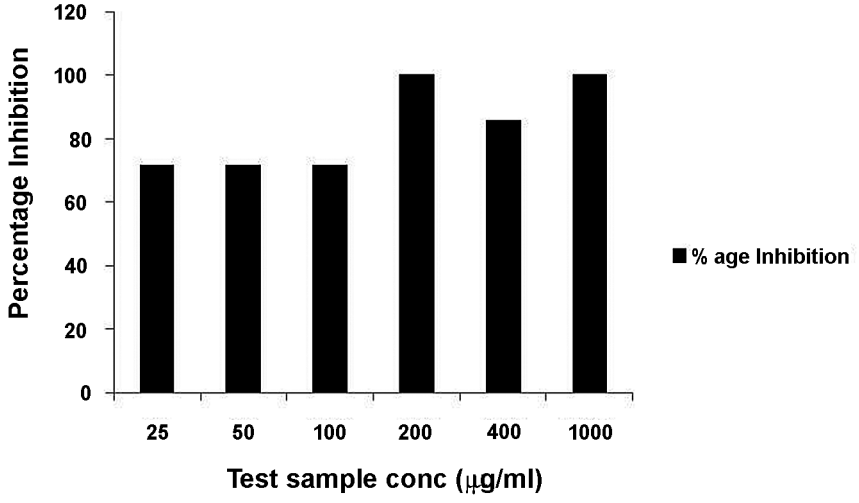

Figure 1 *– Effect of Tribulus terrestris on nucleation of CaOx.*

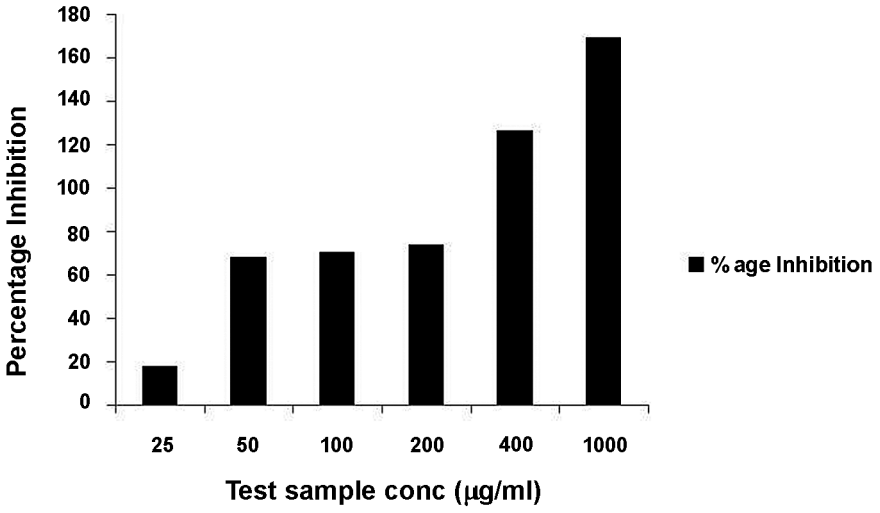

Figure 2 *– Effect of Tribulus terrestris on the growth of CaOx.*

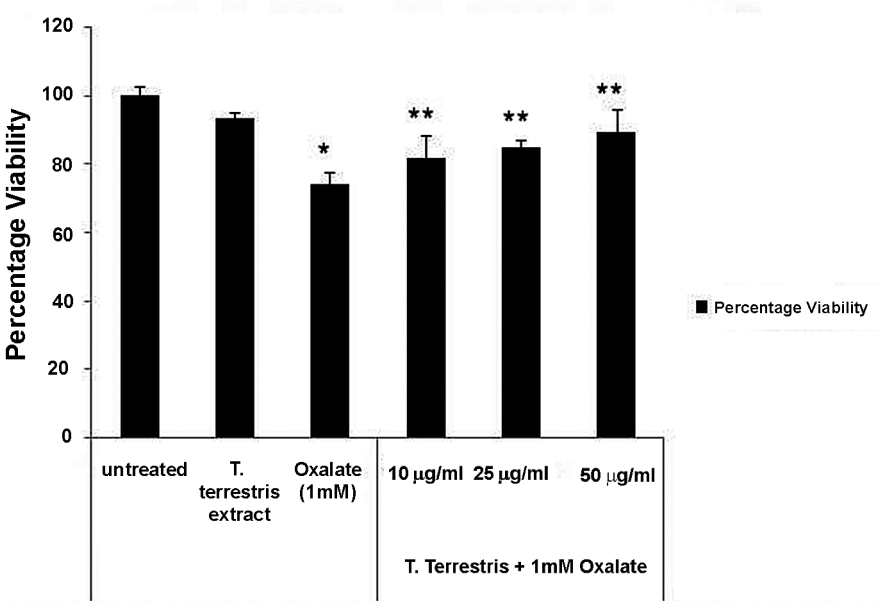

Figure 3 *– Effect of Tribulus terrestris on the viability of NRK 52E. Data are mean ± SEM of three independent observations. * $p < 0.05$ versus untreated control, ** $p < 0.05$ versus oxalate control.*

effect on the cell injury in the absence of oxalate indicating that even at the highest concentration of DMSO used there was no cytotoxicity to the cells. The percentage viability with 10 µg/mL, 25 µg/mL and 50 µg/mL was 81.6 ± 6.9, 84.9 ± 1.9 and 89.1 ± 6.9 respectively.

Lactate dehydrogenase is a stable cytosolic enzyme that is released when the cell is lysed or there is any injury on the cell membrane. A significant increase in LDH release was seen when the NRK 52E cells were exposed to oxalate alone. When NRK 52E cells were treated with the plant extract at varying

concentrations(10, 25 and 50 µg/mL) along with oxalate (1 mM) for 72 h, a reduction in oxalate-induced cell injury was observed as assessed by a decreased LDH release (Figure-4), Again it was seen that the plant extract alone had no significant effect on the measures of cell injury in the absence of oxalate. The percentage LDH release for 10 µg/mL, 25 µg/mL and 50 µg/mL was observed to be 126.5 ± 4.2, 112.6 ± 5.2 and 109.8 ± 1.0 respectively after treatment with oxalate and the plant extract with respect to control.

COMMENTS

There is growing evidence that CaOx nephrolithiasis is associated with renal injury. Hyperoxaluria is a major risk factor for calcium oxalate nephrolithiasis, and calcium oxalate urinary stones are the most common type of urinary stone. High level of oxalate produced a variety of changes in the renal epithelial cells, such as an increase in free radical production and a decrease in antioxidant status, followed by cell injury and cell death. These changes are significant predisposing factors for the facilitation of crystal adherence and retention (5,14).

Due to significant side effects and failure to prevent recurrence by the present day treatment procedures for urolithiasis, alternative treatment modalities using herbal products have assumed importance. A dramatic advancement in using phytotherapy for urolithiasis treatments has been observed in recent years and many investigators have proposed to further scientific study on its efficacy. Many medicinal plants have been employed for centuries to treat urinary stones though the rationale behind their use is not well established.

In the present study, the anticalcifying properties of Tribulus terrestris commonly called "gokhru" were explored in vitro. The inhibitory potency of the plant was tested on the nucleation and growth of the most commonly occurring kidney stones, calcium oxalate monohydrate. A concentration dependent trend of inhibition was observed using Tribulus terrestris extract with maximum inhibition of 100% and 170% for CaOx nucleation and the growth assay respectively with 1000 µg/mL of the extract.

In our study with NRK 52E, Tribulus terrestris proved to have a protective effect towards the renal epithelial cells again in a concentration dependent manner. When NRK-52E cells were injured

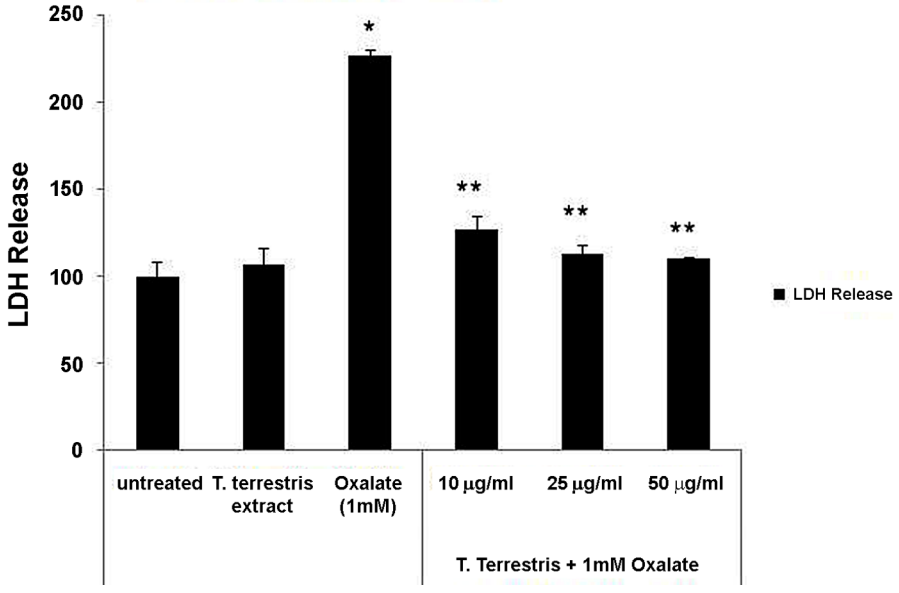

Figure 4 *– Effect of Tribulus terrestris on the % LDH release. Data are mean ± SEM of three independent observations.*
** $p < 0.05$ versus untreated control, ** $p < 0.05$ versus oxalate control.*

by exposure to oxalate for 72 h, the plant extract prevented the injury in a dose-dependent manner. The mechanism of inhibition /reduction in the injury needs to be studied further. Studies have shown that inhibition of the inflammatory response induced by injury due to crystal formation helps in restoring normalcy.

Beghalia et al. (19) have suggested in studies using certain Algerian medicinal plants that the herb extract may contain substances that inhibit the growth of COM crystals. This property of plant extracts could be important in preventing kidney stone formation; the agglomeration of particles is a critical step in urinary stone formation, as larger crystals are less likely to pass spontaneously in the urinary tract (8,20). They (19) further postulated that the plant extracts may contain substances that inhibit CaOx crystal aggregation and also the binding of the crystals to the renal epithelial surface. This could explain a decrease in LDH release as seen in the cells treated with the plant extract compared to those treated with oxalate alone.

Our studies are in agreement with the studies previously reported as regards the anti-urolithiatic potency of Tribulus terrestris on the growth COM crystals using double diffusion gel growth technique (21). The anti-urolithiatic ability of the plant is also currently being evaluated in animal models and has exhibited dose-dependent anti-urolithiatic activity and almost completely inhibited stone formation further supporting our results (22,23).

Recently several plants including Herniaria hirsuta (24), Phyllanthus niruri (25) and Bergenia linguata (26) are being explored for their anti-urolithiatic properties on the basis of their usage in the traditional medicine. Herniaria hirsuta, a plant from Morocco is also known to exhibit the antilithiatic activity. The adhesion of the radioactive COM crystals to the Madin Darby canine kidney cells was studied in the presence and the absence of the aqueous extract. COM crystal binding to the cells was inhibited by the extract in a concentration dependent manner (24). In vitro effect of an aqueous extract of Phyllanthus niruri L., a plant used in Brazilian folk medicine for the treatment of urolithiasis, on a model of CaOx crystal endocytosis by Madin-Darby canine kidney cells was investigated by Campos and Schor. The extract exhibited a potent and effective non-concentration-dependent inhibitory effect on the CaOx crystal internalization. This response was present even at very high (pathologic) CaOx concentrations and no Phyllanthus niruri L.-induced toxic effect could be detected (25). Bergenia ligulata is a widely used plant in South Asia, mainly India and Pakistan, as a traditional medicine for treatment of urolithiasis. The crude aqueous-methanolic extract of Bergenia ligulata rhizome was studied using in vitro and in vivo methods and the extract showed the anti-urolithic activity through CaOx crystal inhibition, diuretic, hypermagneseuric and antioxidant effects (26). Also in our laboratory, antilithiatic potency of Dolichos biflorus (27) and Trachyspermum ammi (28) has been evaluated in vitro and in vivo. The most active protein fraction was isolated from these plants and thus adds a new perspective to study plant fractions for their therapeutic use as antilithiatic proteins.

CONCLUSION

In conclusion, the aqueous extract of Tribulus terrestris has been shown to possess an ability to inhibit CaOx crystallization in vitro. In addition this extract has also shown cytoprotective properties towards the NRK 52E cells by lowering LDH leakage and increasing the cell viability. Our study suggests the possibility of using Tribulus terrestris as a therapeutic agent to treat urolithiasis and further characterization of its active compound(s) could lead to a new candidate drug for patients with urolithiasis.

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CONFLICT OF INTEREST

None declared.

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EDITORIAL COMMENT

Kidney stone disease is a major health problem in modern societies. As technology evolved, surgical options have gained more acceptance as they provide less invasive approaches, more efficacious results and lesser collateral effects. However, the costs involved are significant and an increasing effort should be continuously made in order to optimize prevention. The article presented by Aggarwal et al. clarifies the efficacy of the herbal Tribulus terrestris on the inhibition of calcium oxalate calculi formation. Herbal medicine has been long used to treat different health conditions including stone disease. However, only more recently efforts began to be made to determine the mechanisms involved and their objective efficacy. In the present evidence-based medicine era this is of utter importance. Herbal medicines may be an alternative to the currently existing medicines providing the additional advantage of minimal or inexistent collateral effects. Other herbal medicines should undergo evaluations in vitro to amplify the urologist's clinical armamentarium to combat kidney stones.

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EDITORIAL COMMENT

In this paper, the authors addressed the potential use of Tribulus terrestris as the therapeutic agent to treat urolithiasis. Urolithiasis is characterized by high recurrence rate and among the treatments used are extracorporeal shock wave lithotripsy and drug treatment, although there is no satisfactory drug to use in clinical therapy. Thus the prevention of this disease or its recurrence would be of great interest. Phytotherapy is a common method used in folk medicine as an alternative for primary health care in many countries and particularly the potential effect of many plants to treat urolithiasis has been reported over the past years. The precipitation of calcium oxalate (CaOx) inside the renal tubules and the interaction between CaOx crystals and tubular epithelium plays an important role in the genesis and evolution of urolithiasis, since renal tubular cells selectively bind and uptake CaOx crystals, a phenomenon followed by a series of intracellular events that culminate in a cell damage and death. It was shown that aqueous extract of Tribulus terrestris was able to inhibit CaOx crystallization in vitro and showed cytoprotective properties increasing the cell viability.

The extract of plants with antilithiatic properties (Tribulus terrestris, Phyllanthus niruri, Herniaria hirsute, etc.) has been shown effective to prevent calculi development in the experimental models in vivo and in vitro, showing significant effects on many stages of stone formation including crystallization, aggregation, cellular adherence and adsorption of macromolecules into the calculi, however, its effects in lithiatic patients are much less clear. Many reasons can be raised for this difference such as the treatment onset, number of patients, time of treatment, adhesion to the treatment, etc. Moreover, it was previously shown (1) that rats with already formed vesical calculi, the administration of Phyllanthus niruri had no effect on the calculi size or elimination rate but it induced a shift in the calculi shape toward a smoother surface and probably more fragile form, which could contribute to elimination and/or dissolution of calculi. Overall the available data point to a useful therapeutic application of these plants, including Tribulus terrestris in lithiatic patients, mainly as prophylactic agent in those persons who are at high risk to develop stones since they can potentially interfere with the pathogenesis of urolithiasis and may represent an attractive alternative for the prevention of lithiasis of the urinary tract.

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Experimental Model of Human Corpus Cavernosum Smooth Muscle Relaxation

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ABSTRACT

Purpose: To describe a technique for en bloc harvesting of the corpus cavernosum, cavernous artery and urethra from transplant organ donors and contraction-relaxation experiments with corpus cavernosum smooth muscle.
Materials and Methods: The corpus cavernosum was dissected to the point of attachment with the crus penis. A 3 cm segment (corpus cavernosum and urethra) was isolated and placed in ice-cold sterile transportation buffer. Under magnification, the cavernous artery was dissected. Thus, 2 cm fragments of cavernous artery and corpus cavernosum were obtained. Strips measuring 3 x 3 x 8 mm^3 were then mounted vertically in an isolated organ bath device. Contractions were measured isometrically with a Narco-Biosystems force displacement transducer (model F-60, Narco-Biosystems, Houston, TX, USA) and recorded on a 4-channel Narco-Biosystems desk model polygraph.
Results: Phenylephrine (1μM) was used to induce tonic contractions in the corpus cavernosum (3 - 5 g tension) and cavernous artery (0.5 - 1g tension) until reaching a plateau. After precontraction, smooth muscle relaxants were used to produce relaxation-response curves (10^{-12}M to 10^{-4} M). Sodium nitroprusside was used as a relaxation control.
Conclusion: The harvesting technique and the smooth muscle contraction-relaxation model described in this study were shown to be useful instruments in the search for new drugs for the treatment of human erectile dysfunction.

Key words: *penis; cavernous artery; penile erection; experimental; erectile dysfunction*
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INTRODUCTION

Erectile dysfunction (ED) affects approximately 150 million people worldwide. The prevalence of ED in Brazil is high: more than 40% of Brazilian men between 40 and 70 years of age suffer from ED and more than a million new cases are registered annually (1,2).

Although phosphodiesterase type-5 (PDE-5) inhibitors have revolutionized the treatment of erectile dysfunction, many patients, mostly those with endothelial dysfunction (56% of cases), do not benefit from this form of therapy (3).

At present many studies are being carried out using nitric oxide (NO) donors, guanylyl cyclase activators (both soluble intracellular and membrane-bound isoforms), ion channel agonists and RhoA-kinase inhibitors in order to formulate new drugs with different mechanisms of action to treat this patient population (4,5).

The vast majority of these studies employ rat and rabbit corpus cavernosum due to the difficulty in

obtaining samples of human tissue (5-7). However, at our Urology Service, experimental studies on ED have been in progress since 2004 using human corpus cavernosum tissue from organ donors.

The purpose of the present study was to provide a detailed description of the technique used for en bloc harvesting of the corpus cavernosum, cavernous artery and urethra of transplant organ donors and the methods used in contraction-relaxation experiments with corpus cavernosum smooth muscle.

MATERIALS AND METHODS

All study protocols were previously approved by the Human Subjects Research Ethics Committee of the Federal University of Ceará and by the National Research Ethics Committee of the Brazilian Ministry of Health.

Following authorization from the family, human corpus cavernosum was obtained from cadaver donors (< 40 years) during surgery for organ transplantation.

After removal of the heart, liver and kidneys and through the same incision (xiphoid pubic), the corpus cavernosum was located above the pubic symphysis by digital hypodermic approach. The corpus cavernosum was dissected to the point of attachment with the ischiopubic ramus (crus penis) (Figure-1).

A 3 cm segment including the corpus cavernosum and urethra was isolated en bloc (Figure-2). No additional external incision was made at the end of procedure. Subsequently, the tissues were placed in ice-cold sterile transportation buffer (Collins solution) and processed within 1 hour after collection.

The samples were processed under stereoscopic magnification. The entire cavernous artery in the center of the corpus cavernosum was dissected and isolated from the surrounding cavernous tissues (Figure-3). Then cavernous tissues were separated from connective tissues and the tunica albuginea. Thus, 2 cm fragments of each cavernous artery and corpus cavernosum were obtained.

The corpus cavernosum fragments were cut into strips measuring approximately 3 x 3 x 8 mm^3 and mounted vertically under 1g resting tension. The cavernous artery was cut into 5 mm rings and mounted

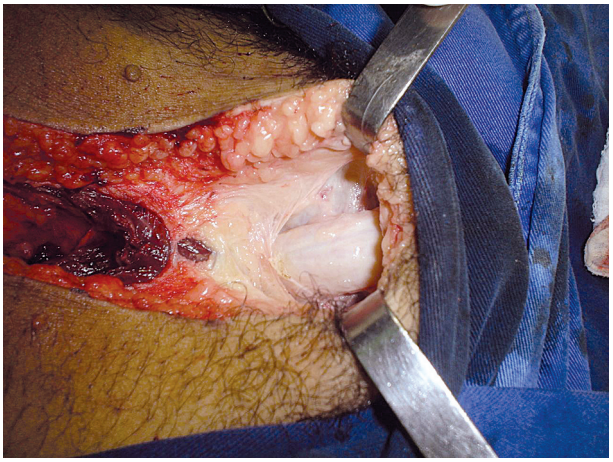

Figure 1 – *Dissection of the corpora cavernosa to the point of attachment with the crus penis.*

horizontally under 0.2g resting tension. The tissues were maintained in 5 mL organ chambers containing Krebs-Henseleit medium composed of 114.6 mM NaCl, 4.96 mM KCl, 1.3 mM $MgSO_4$, 2.0 mM $CaCl_2$, 1.23 mM NaH_2PO_4, 25 mM $NaHCO_3$ and 3.6 mM glucose, enriched with 10 μM guanethidine and 10 μM indomethacin (pH 7.4, 37ºC, gassed with 5% CO_2 and 95% O_2).

The tissues were allowed to equilibrate for 90 min with washing at 15 min intervals. The ten-

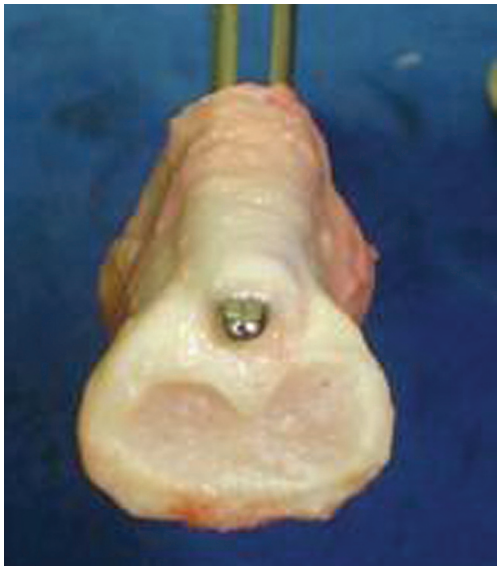

Figure 2 – *Corpora cavernosa and urethra.*

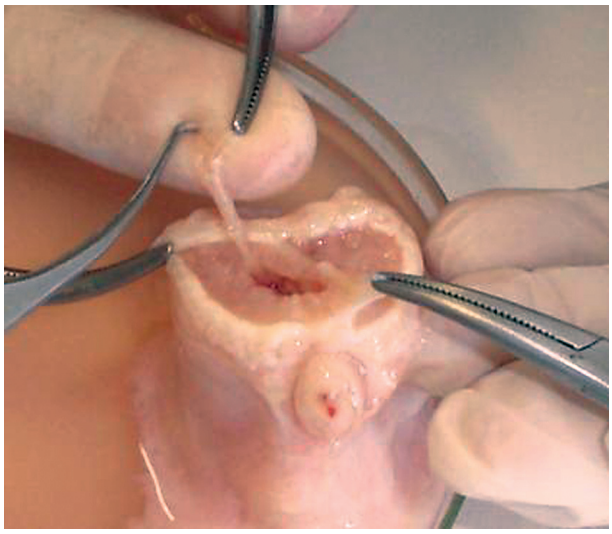

Figure 3 – *Cavernous artery.*

sion was measured by an isometric transducer (F-60 Narco-Biosystems connected to a 4-channel desk model polygraph) (Figure-4).

One micromole phenylephrine was added to the baths to obtain 60-70% submaximal smooth muscle contractions. Subsequently, concentration-response curves (10^{-8}M to 10^{-2} M) to smooth-muscle relaxants or sodium nitroprusside (SNP), a nitric oxide donor, were plotted to check for endothelial functional integrity.

Figure 4 – *Isolated bathing system.*

RESULTS

Phenylephrine (1μM) was used to induce tonic contractions in the corpus cavernosum (3 - 5g tension) and cavernous artery (0.5 - 1g tension) until reaching a plateau. After precontraction, smooth muscle relaxants were used to produce relaxation-response curves (10^{-12}M to 10^{-4} M). SNP was used as a relaxation control.

A number of chemical substances have been used in our laboratory to induce smooth muscle relaxation, including Ru[(NH3)4(caffeine)(NO)]C13, a nitric oxide donor. It completely relaxes the human corpus cavernosum and cavernous artery achieving an E_{max} of 100% and an EC_{50} of 6.4 ± 0.14 (Figure-5).

Using preparations of corpus cavernosum with intact endothelium from human donors under 40 with no history of erectile dysfunction or cardiovascular risk factors (e.g. diabetes, hypertension and dyslipidemias), this physio-pharmacological model proved to be an attractive instrument in the search for new drugs for the treatment of human erectile dysfunction.

COMMENTS

PDE-5 inhibitors have revolutionized the treatment of erectile dysfunction. However, many patients with ED also suffer from endothelial dysfunction (56%) and are therefore unresponsive to this class of drugs (3).

Endothelial dysfunction is often observed in patients with comorbidities such as arterial hypertension and diabetes mellitus. It is characterized by a deficiency in the endogenous production of NO (8).

In true ED, diabetes, hypertension, and dyslipidemia (components of the metabolic syndrome) tend to be associated with endothelial dysfunction. ED has also been reported to be a marker for cardiovascular arterial disease (9).

The search for new drugs capable of increasing the availability of endogenous NO has been a considerable challenge. Several experimental models have been used over the past decades based on rat, rabbit and human corpus cavernosum (6,7,10,11).

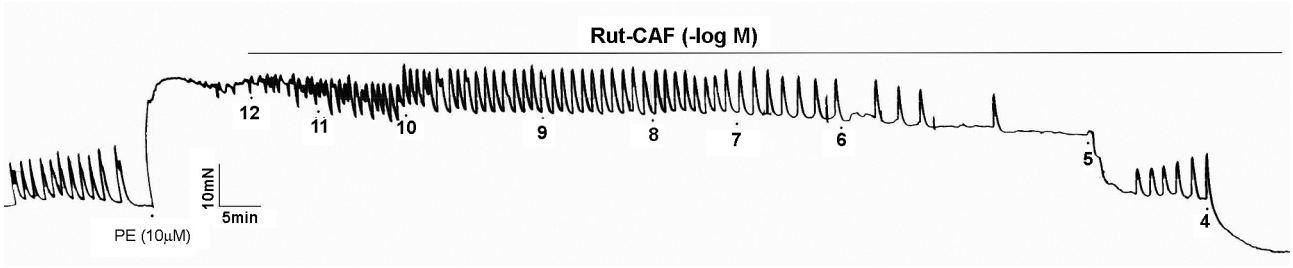

Figure 5 – *Physiographic tracing of the Rut-CAF (10^{-12} - 10^{-4} M) effect in human corpus cavernosum strips placed in Kreb-Henseleit (37ºC; pH 7.4, 95% O_2 and 5% CO_2) solution, under a 1g tension and pre-contracted with phenylephrine. Vertical scale = 10 mN and horizontal scale = 5 min.*

Using corpus cavernosum in vivo and other tissues (e.g. platelets) from species such as rats, rabbits and humans, Peng Wang et al. (12) concluded that, in spite of similar kinetics and enzymatic features, different PDEs have different sensitivities to inhibitors. This should be taken into account when working with experimental models of this type.

Our experimental model employed healthy corpus cavernosum tissues from young cadaver donors killed by trauma or stroke in order to minimize the concern about distortion of results caused by sample tissues of poor condition.

In contrast, in a study using a similar human corpus cavernosum model for the evaluation of the effect of sildenafil on enzymatic PDE inhibition and consequent smooth muscle relaxation, samples were obtained from patients with ED during surgery for penile prosthesis implantation, so it seems likely that in this case most of the subjects presented endothelial injury to some degree (6).

Another concern in this field of research is the availability of tissues to perform the experiments. In Ceará, eight organ transplantations are carried out every month, making it possible to complete studies without major interruptions.

Seidler et al. (4) worked on a similar model using corpus cavernosum donated by patients undergoing sex reassignment surgery as treatment for transsexualism and gender identity disorder. In spite of the good condition of the tissues, the small number of men submitting to this type of procedure limits the possibility of collecting sufficient tissue for experimental work.

The present paper presents a comprehensive model for harvesting human corpus cavernosum tissues and for carrying out smooth muscle relaxation experiments in vivo. Healthy human corpus cavernosum is removed from cadaver donors and subjected to experiments in isolated baths. The technique allows to dissect and isolate the corpus cavernosum, cavernous artery and urethra.

The importance of the technique lies in that it makes it possible to test a range of new drugs, including stable NO donors, guanylyl cyclase activators and RhoA-kinase inhibitors, on smooth muscle corpus cavernosum, penile arteries and urethra (7,12-15).

CONCLUSION

This experimental model involves the dissection, harvesting, isolation and conservation of the human corpus cavernosum, cavernous artery and urethra under ideal conditions along with the accompanying physio-pharmacological studies. The feasibility and reproducibility of the model makes it an attractive instrument in the search for new drugs for the treatment of human erectile dysfunction.

CONFLICT OF INTEREST

None declared.

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EDITORIAL COMMENT

Penile erection is a complex neurovascular event that relies on vasodilatation of erectile tissues due to neuronal and endothelial derived nitric oxide (NO) released by activation of parasympathetic nerves on sexual stimulation of the cavernous endothelial lining (1).

This sexual stimulus brings about blood flow into the corpus cavernosum and the consequent penile rigidity is maintained by means of a veno-occlusive mechanism.

This is enabled by the particular micro-architecture of the corpus cavernosum, which consents a sophisticated hemodynamic system.

Otherwise, the tunica albuginea plays a key role in the erectile function.

Being rich in elastic fibers it is able to resist overstretching of the corpus at raised levels of intracavernous pressure, compressing the trans-albugineal effluent veins, as well providing on inextensible protective structure to the arteriole and to the intracavernous nerves.

This function is possible due to its structure made of collagenic fibers linked by elastic fiber bridges (2,3).

Therefore, it is very important to keep its integrity to maintain its fundamental role in the erectile mechanism.

The presence of structural disorders like an excessive collagen deposition gives rise to the formation of a plaque, fibrotic first and then calcified, as can be found in Peyronie's disease.

Moreover, there is a significant decrease of elastic fiber concentration as well in these patients affected by induration penis plastica (4).

Similar changing were found in patients who underwent radical prostatectomy, where the trabecular elastic fibers and smooth muscle fibers were decreased and collagen content was significantly increased (5).

As age advances the gonadal steroid hormones, and in particular, testosterone production decreases (6), nerve conduction slows down and the efficiency of the vascular microcirculation of the penis is reduced.

Androgens are essential for the development, growth and maturation of erectile tissues, acting on the hemostatis in the corpora cavernosum, regulating the growth of smooth muscle and protein synthesis of the connective tissues.

Therefore, a decrease in their production could give rise to the switch from elastic fibers to collagen fibers, which is the basis of cavernosal fibrosis (7,8).

Recent studies have shown that testosterone also regulates the expression of phosphodiesterasis type 5 (PDE5) (9).

It is known that erectile dysfunction (ED) affects 150 million people worldwide.

Until few years ago, it was thought that 90% of ED had a psychogenetic etiology.

Moreover, further neurophysiological, hemodynamical and pharmacological studies have helped us to understand better the complex biochemical and micro-anatomical mechanism of the erectile function, showing us that 50% of ED has an organic etiology (10).

On the other hand, even psychogenetic ED could be the consequence of an increase of adrenergic stimulation and having itself an organic origin (11).

The past 20 years have witnessed remarkable changes in the treatment of ED.

The emergence and the success of PDE5 inhibitors as effective therapy for erectile dysfunction is remarkable considering the intent behind the development of the original compound: initially designed as an antianginal agent, it quickly became apparent that the first PDE5 inhibitor on the market, sildenafil, displayed erectogenesis as a side effect, and the drug was soon recognized as a potential revolutionary treatment for ED..

Furthermore, sildenafil has been shown to prevent the progression of fibrosis of the corpus cavernosum in prostatectomized patients. Its efficacy seems to result from an anti-proliferative effect exerted on fibroblasts (12).

It is known that PDE-5 inhibitors have revolutionized the treatment of erectile dysfunction and changed the life of million people worldwide.

However there still a high percentage of patients with ED that are also affected by endothelial dysfunction (56%) and subsequently they are unresponsive to this class of drugs (13).

Although already extensively studied, NO donors continue to be an important topic as regards ED.

Many studies have been carried out to find new NO donors or new guanylyl cyclase activators to try to find new drugs to treat these patients who are non-responders to PDE-5 inhibitors (14).

The present work shows us a model for harvesting human corpus cavernosum tissues and for making smooth muscle relaxation experiments in vivo.

The healthy corpus cavernosum taken from young cadaver donors killed by trauma or stroke offer tissues in good condition.

In the literature, we have not found a similar approach due the difficulty to obtain samples of human tissues.

With this technique is possible to test new drugs, like NO donors, guanylyl cyclase activators and RohA-Kinase inhibitors on human smooth muscle tissues in vivo rather then using corpus cavernosum in vivo from animals like rats, or rabbits as it has been performed by Pen Wang et al. (15).

Finally, this harvesting technique and smooth muscle contraction-relaxation model could be a very useful instrument to help us to find new drugs to treat ED.

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UROLOGICAL SURVEY

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STONE DISEASE

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Ureteroscopic ultrasound technology to size kidney stone fragments: proof of principle using a miniaturized probe in a porcine model

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J Endourol. 2010; 24: 939-42

Purpose: A prototype ultrasound-based probe for use in ureteroscopy was used for in vitro measurements of stone fragments in a porcine kidney.
Methods: Fifteen human stones consisting of three different compositions were placed deep in the collecting system of a porcine kidney. A 2 MHz, 1.2 mm (3.6F) needle hydrophone was used to send and receive ultrasound pulses for stone sizing. Calculated stone thicknesses were compared with caliper measurements.
Results: Correlation between ultrasound-determined thickness and caliper measurements was excellent in all three stone types ($r(2) = 0.90$, $p < 0.0001$). All 15 ultrasound measurements were accurate to within 1 mm, and 10 measurements were accurate within 0.5 mm.
Conclusion: A 3.6F ultrasound probe can be used to accurately size stone fragments to within 1 mm in a porcine kidney.

Editorial Comment

The authors report a good correlation with stone-size to within 1mm. As a 0.5mm discrepancy represents a 1.5F difference for stone extraction through a 12F inner diameter ureteral access sheath, one could argue that the limit for error be placed at 0.5mm - in which case the probe is accurate in only 2/3 of cases. Though the authors tested stones 3-7mm in size, the critical clinical question is posed by those stones 4-5mm in size - a report to the operator that the stone is less than or greater than 4.5 mm in size is required with a high level of accuracy.

The authors utilized a rigid probe - they do not state what length probe was utilized, or if it would be easy to modify to the typical 42cm length of a rigid ureteroscope, or preferably to a flexible configuration for a flexible ureteroscope. One limitation of the device is that it provides unidimensional sizing capabilities - if the largest dimension of the stone was not captured, then the risk of engaging a stone too large to extract would not be mitigated. Often air bubbles are transmitted to the collecting system through the irrigation fluid; one might anticipate that interference with the stone-fluid interface may pose a challenge in these cases for stone sizing.

The technology provides the potential sizing stones prior to engaging them in a basket - thereby decreasing the risk of ureteral injury - this would be particularly appealing for stones that are impacted or partially embedded. In addition, this technology may aid in the identification of submucosal calculi and calculi in a calyceal divericulum, and facilitate unroofing with the holmium laser.

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Management of ureteral calculi

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This is the fourth in a series of articles on an electronic survey of practicing urologists in the United States and elsewhere that concerns endourologic management of a variety of conditions. There were 416 e-mail responses to the survey, which was conducted in late 2007 and early 2008. The electronic mail lists of the American Urological Association and the Endourological Society were used. A little more than half of the respondents were from the United States, with 15% from Europe and 12% from Asia. Further details regarding the survey can be found in a previous publication.1

The present report focuses on questions from the survey that concern the treatment of patients with ureteral calculi:

1. Do you have access to the holmium laser for ureteroscopic stone treatment? (Answer choices, yes or no)
2. Which treatment would you recommend for an uncomplicated patient with a symptomatic distal ureteral stone that was 4 (or 8 or 15) mm? (Answer choices, ureteroscopic stone removal, extracorporeal shock wave lithotripsy (SWL) or ureteral stent placement alone)
3. Which treatment would you recommend for an uncomplicated patient with a symptomatic midureteral stone that was 4 (or 8 or 15) mm? (Same answer choices as question 2)
4. Which treatment would you recommend for an uncomplicated patient with a symptomatic proximal ureteral stone that was 4 (or 8 or 15) mm? (Same answer choices as question 2)

Editorial Comment

The international sample of urologists responding to the questionnaire may not be reflective of the general practicing urologist in those countries - they are selected by their membership in international organizations (AUA) or membership in societies for those with specific interest and/or expertise in endourology (Endourology Society). As such, the survey may overestimate the availability of holmium laser in these countries and the dissemination of advanced endoscopic procedures.

The authors did not stratify responses based on gender of the patient - one might anticipate a higher percentage of urologists may tackle a midureteral or proximal ureteral stone in female than in a male patient due to the ability to access the stone with a semi-rigid ureteroscope.

Interestingly, non-North American urologists were more likely (15%) than North American urologists (4%) to stent only for a small 4mm stone. This may represent a higher inclination to give the patient an opportunity to pass the stone spontaneously once stented - a two edged sword as it may increase the likelihood for a secondary procedure down the road.

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ENDOUROLOGY & LAPAROSCOPY

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How do young residents practice laparoscopic surgical skills?

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Objectives: To investigate whether a training system using a dry box is feasible for training young urologists. Despite laparoscopic surgery being widely indicated for several urological diseases, a laparoscopic training system for young urologists has not been fully established yet. However, the learning curve for laparoscopic surgery has not yet been ascertained.
Methods: We continued to test 11 sixth-year residents (postgraduate year: PGY6) and third-year residents (PGY3) in our department in terms of surgical skills using a dry box. We gave them several tasks (cutting and suturing) and let them practice until task completion. We continued to test all participants by these tasks for 16 weeks.
Results: At the beginning of the present study, the PGY6 residents achieved significantly better scores than the PGY3 residents. However, the difference between the 2 groups became insignificant over time. Furthermore, statistical analysis revealed that a practice time of 100 minutes per week was the only significant factor affecting the last test score. For the final test, the mean practice time for all participants was 79.1 minutes per week.
Conclusions: These results suggest that laparoscopic surgical skills can definitely be polished by adequate voluntary practice.

Editorial Comment

Although genitor-urinary laparoscopy has been established as surgical technique for treatment of urological diseases; training of residents and surgeons has been challenging due to the lack of validated teaching protocols and techniques. The authors have focused on a dry lab exercises that involved mentors and residents in an intensive training schedule demonstrating improvement in laparoscopic surgical skills despite the level of academic training. According to the authors, the only variable significant to improvement of skills was the practice time of 100 min/week practicing a set of laparoscopic exercises defined by the authors. Age, clinical experience, and laparoscopic experience did not affect the outcome of the final evaluation. Certainly, clinical experience in laparoscopy must be correlated to these findings; moreover, a validated skill development program must be created to train our residents and surgeons.

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Evaluating urinary continence and preoperative predictors of urinary continence after robot assisted laparoscopic radical prostatectomy

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Purpose: We evaluated urinary continence using a validated questionnaire in a series of consecutive patients who underwent robot assisted laparoscopic radical prostatectomy, and identified the preoperative predictors of the return to urinary continence.
Materials and Methods: The clinical records of 308 consecutive patients who underwent robot assisted laparoscopic radical prostatectomy for clinically localized prostate cancer at a tertiary academic center were prospectively collected. All patients were continent before surgery. Urinary continence was evaluated using the International Consultation on Incontinence Questionnaire-Urinary Incontinence Short Form instrument. All of the patients reporting no leak in response to the question, "How often do you leak urine?" were defined as continent.
Results: A total of 273 patients (90%) were continent 12 months after robot assisted laparoscopic radical prostatectomy. Continent patients were significantly younger (61.4 +/- 6.4 vs 64.1 +/- 6.1 years, p = 0.02) than those who were incontinent. On univariable regression analysis patient age at surgery (OR 1.075, p = 0.024) and Charlson comorbidity index (OR 1.671, p = 0.007) were significantly associated with 12-month continence status. On multivariable analysis age (OR 1.076, p = 0.027) and Charlson comorbidity index (OR 1.635, p = 0.009) were independent predictors of continence rates.
Conclusions: Using the International Consultation on Incontinence Questionnaire-Urinary Incontinence Short Form 90% of patients undergoing robot assisted laparoscopic radical prostatectomy reported no urine leak 12 months after surgery. Patient age at surgery and Charlson comorbidity index were independent predictors of the return to urinary continence, whereas notably no variable related to prostate cancer was significantly correlated with urinary continence.

Editorial Comment

Reports of urinary continence rates post robotic laparoscopic radical prostatectomy ranged from 30% to 89% at 3 months, from 50% to 95% at 6 months and from 62% to 97% at 12 months. Different investigators suggested that urinary continence maybe related to oncological characteristics, i.e.; location and aggressiveness of prostate cancer causing different dissection and excision techniques influencing the rates of urinary incontinence.

This study analyzed the predictors of return to urinary continence in a RALP series. The authors evaluated urinary continence in a series of consecutive patients who underwent RALP using a validated questionnaire and identified the preoperative predictors of return to urinary continence. Based on the ICIQ-UI questionnaire 90% of patients undergoing RALP reported no urine leak 12 months after surgery, with patient age and Charlson comorbidity index being the only independent predictors of the return to urinary continence. Interestingly, no variable concerning patient comorbidity, PCa or surgical treatment was significantly correlated with the return to urinary continence.

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IMAGING

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Diffusion-weighted MRI of peripheral zone prostate cancer: comparison of tumor apparent diffusion coefficient with Gleason score and percentage of tumor on core biopsy

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AJR Am J Roentgenol. 2010; 194: W316-22.

Objective: The objective of our study was to determine the relationship between the apparent diffusion coefficient (ADC) value on diffusion-weighted imaging (DWI) and Gleason score of prostate cancer and percentage of tumor involvement on prostate core biopsy.
Materials and Methods: We performed a retrospective study of 57 patients with biopsy-proven prostate cancer who underwent endorectal MRI with DWI between July 2007 and March 2008. Regions of interest (ROIs) were drawn on ADC maps at sites of visible tumor on DW images and ADC maps. A hierarchic mixed linear model was used to compare the ADC value of prostate cancer with the Gleason score and the percentage of tumor on core biopsy.
Results: Eighty-one sites of biopsy-proven prostate cancer were visible on DW images and ADC maps. The least-squares mean ADC for disease with a Gleason score of 6 was 0.860 x 10(-3) mm(2)/s (standard error of the mean [SEM], 0.036); Gleason score of 7, 0.702 x 10(-3) mm(2)/s (SEM, 0.030); Gleason score of 8, 0.672 x 10(-3) mm(2)/s (SEM, 0.057); and Gleason score of 9, 0.686 x 10(-3) mm(2)/s (SEM, 0.067). Differences between the mean ADC values for a prostate tumor with a Gleason score of 6 and one with a Gleason score of 7 ($p = 0.0096$) and for a prostate tumor with a Gleason score of 6 and one with a Gleason score of 8 ($p = 0.0460$) were significant. Comparison between the ADC and percentage of tumor on core biopsy showed a mean ADC decrease of 0.006 (range, 0.004-0.008 x 10(-3) mm(2)/s) for every 1% increase in tumor in the core biopsy specimen.
Conclusion: DWI may help differentiate between low-risk (Gleason score, 6) and intermediate-risk (Gleason score, 7) prostate cancer and between low-risk (Gleason score, 6) and high-risk (Gleason score > 7) prostate cancer. There is an inverse relationship between the ADC and the percentage of tumor involvement on prostate core biopsies.

Editorial Comment

In prostate cancer occurs significant reduction in the diffusion properties of water protons thus resulting in a reduction in the measured apparent diffusion coefficient (ADC) value relative to normal prostatic tissue. Several reports have been showing the utility of this technique. The combination of anatomic information obtained with conventional T2-weighted image with functional information, obtained with diffusion-weighted image, offers significant advantage over the use of either one of these techniques separately. This association significantly improves cancer detection and the accuracy in predicting the volume of cancer of the peripheral zone. This combined technique however is more effective in tumors larger than 0.5 cm3.

In this publication the authors show that the ADC values of prostate cancer may help differentiate between low-risk (Gleason, 6) and intermediate-risk (Gleason score 7 disease and between low-risk and high-risk (Gleason> 7). The ADC values in this study were compared with results of prostate biopsy. In other words, higher cellular density found in poorly differentiated tumors is responsible for more restricted movement of water protons and thus will present lower mean ADC values. They also showed that lower ADC values are associated with a higher percentage of cancer on core biopsy and higher Gleason score. They predicted that this feature could be useful to further direct patient treatment.

We have to remember however that the reported sensitivity and specificity of DWI with ADC maps for detecting prostate cancer on MRI performed at 1.5 T, range from 54% to 94% and from 61% to 100%, respectively. In this study, the authors had a relatively poor detection rate, since 56% of biopsy-proven sites of prostate cancer were not visible on DWI. Sites containing tumor and visible on DWI had higher percentage of tumor on core biopsy (mean 52%) than those not visible (mean 19%) and higher Gleason score.

The results of this work further support our opinion that the best way to detect prostatic cancer by imaging is using a multiparametric MRI examination, which combines T2-weighted images, spectroscopy, diffusion-weighted image and dynamic contrast enhanced technique. Since each one of these techniques has inherent advantages and disadvantages, efforts have been made in order to determine which combination will present higher accuracy.

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Kidney and urinary tract imaging: triple-bolus multidetector CT urography as a one-stop shop--protocol design, opacification, and image quality analysis

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Purpose: To retrospectively evaluate renal, vascular, and urinary tract visualization following a single post-contrast multidetector computed tomographic (CT) urographic sequence performed with three limited-volume bolus injections.
Materials and Methods: The institutional review board approved this retrospective study. Patient informed consent was waived. Triple-bolus multidetector CT urography was performed in 110 patients. Triple-bolus protocol consisted of 30 mL of contrast material at 2 mL/sec at 0 seconds, 50 mL at 1.5 mL/sec at 435 seconds, 65 mL at 3 mL/sec at 488 seconds, with total abdominal scanning time of 510 seconds. Two independent readers rated urinary tract opacification and qualitatively and quantitatively assessed renal parenchymal and vascular contrast enhancement. Upper urinary tract (UUT) distention was measured by one reader. Interobserver agreement was assessed by using kappa statistics.
Results: Complete opacification of the intrarenal collecting system and proximal ureter was achieved in 91% (184 of 202) (kappa = 0.62) and 82% (166 of 202) (kappa = 0.94) of segments, respectively. The distal ureter was not opacified in 21% of the cases (kappa = 0.92), and the bladder was not opacified in 20% of the cases. Mean distention was higher for proximal (3.9 mm) than for distal (3.7 mm) segments. Image quality of renal parenchymal enhancement was excellent in 76% of cases. Arteries showed better contrast enhancement than veins (excellent rating in 89% vs 59% of the cases). Radiation dose calculated for triple-bolus acquisition was 9.8 mSv.
Conclusion: Triple-bolus multidetector CT urography is a dose-efficient protocol acquiring corticomedullary-nephrographic-excretory and vascular enhancement phases in a single acquisition and provides sufficient opacification and distention of the UUT. Simultaneously, adequate image quality of renal parenchyma and vascular anatomy is achieved.

Editorial Comment

Multidetector computed tomography urography (MDCTU) has become the method of choice for investigation patients with hematuria. For the adequate characterization of parenchymal, urothelial or vascular abnormalities a three-phase MDCT urographic protocols is usually necessary. With this protocol, following an unenhanced phase a single-bolus contrast material injection is made and nephrographic, and excretory phases are obtained. Using this three-phase protocol the effective radiation dose to the patient range from 15-18 mSv. If visualization of the renal arteries and branches are necessary, an additional arterial phase is obtained, thus increasing the radiation dose to 18-20 mSV. For this reason radiologist should always perform a tailored MDCT-urography protocol adequate for each patient clinical indication. The authors' presents a triple-bolus protocol designed to show all renal contrast-enhancement phases in a single acquisition. Good results were obtained with this technique, which allows the demonstration of the renal parenchyma, the renal arteries and veins and all portions of urinary tract. After an enhanced phase, a single postcontrast MDCT urographic sequence is performed with three limited-volume bolus injections. The first bolus of intravenous contrast material is for the opacification of the urinary tract, the second bolus is for the opacification of the venous system and the last bolus is performed for the opacification of the arterial system. We have found that this protocol is excellent for evaluation of potential renal donors, a characteristic group of healthy and young patients that are benefited with the use of a low-dose protocol (11-13 mSv).

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PATHOLOGY

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Low-grade papillary urothelial carcinoma of the urinary bladder: a clinicopathologic analysis of a post-world health organization/international society of urological pathology classification cohort from a single academic center

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Context: Few large cohort studies have addressed outcome in patients with noninvasive low-grade papillary urothelial carcinoma (LG-UrCa) following implementation of the 2004 World Health Organization/International Society of Urological Pathology (WHO/ISUP) consensus classification.
Objective: To evaluate our cohort of LG-UrCa cases classified according to 2004 WHO/ISUP to reassess outcome and interobserver agreement.
Design: Files were searched for all patients diagnosed with LG-UrCa between 1998 and 2008. All sections were reevaluated for accuracy of classification.
Results: A total of 112 cases initially diagnosed as LG-UrCa were identified. Of those, 8 of 55 cases (15%) initially diagnosed by nonurologic pathologists were reclassified as high-grade papillary urothelial carcinoma and were excluded. The mean length of follow-up was 40.1 months (range, 2-113 months). Tumor recurrence was encountered in 56 of 104 patients (53.8%), including 37 (35.6%) with LG-UrCa or lower-grade tumors and

19 (18.3%) with high-grade papillary urothelial carcinoma. Of the 19 patients demonstrating grade progression, 7 (37%) also developed stage progression (invasive carcinoma, n = 5; metastatic carcinoma, n = 2). Seven patients eventually underwent radical cystectomy. None of the 104 patients died of bladder cancer. The mean number of recurrence episodes was 3.11. The mean durations of time to first recurrence and time to grade progression were 13.9 months and 25.1 months, respectively. The mean size of initial tumors was 1.73 cm. There was no significant correlation between tumor size, patient age, sex, or smoking history and the likelihood for recurrence or grade progression. A significantly higher rate of recurrence was seen in patients with multiple tumors at initial diagnosis (P = .04).

Conclusions: A tendency to underdiagnose high-grade papillary urothelial carcinoma continues to exist. More than half (53.8%) of patients with LG-UrCa developed recurrence, with an 18.3% incidence of grade progression and a 6.7% incidence of stage progression. Patients with multiple initial tumors had significantly higher risk of developing recurrence.

Editorial Comment

This is a large cohort study of outcome of patients with noninvasive low-grade urothelial carcinoma using the World Health Organization/International Society of Urological Pathology (WHO/SIPU) consensus classification. From a total of 104 patients, 53.8% developed recurrence, with an 18.3% incidence of grade progression and a 6.7% incidence of stage progression. Patients with multiple initial tumors had significantly higher risk of developing recurrence.

The World Health Organization/International Society of Urological Pathology (WHO/SIPU) consensus classification was held in Boston in 1998 during the United States and Canadian Academy of Pathology (USCAP) meeting. The results were published in the American Journal of Surgical Pathology (1). The most important recommendations of the meeting were:

1. It was recommended not to use the traditional term “dysplasia”. For the term moderate dysplasia was recommended to use low-grade intra-urothelial neoplasia and for severe dysplasia or flat carcinoma in situ the term high-grade intra-urothelial neoplasia. Cases with slight dysplasia should be reported by the pathologists.
2. The 1, 2 and 3 grading by the World Health Organization was replaced by low-grade or refer to grade 1, and high-grade to refer to grades 2 or 3.
3. “Urothelial papilloma” without qualifiers refers to the exophytic variant of papilloma, defined as a discrete papillary growth with a central fibrovascular core lined by urothelium of normal thickness and cytology. It is a rare benign condition typically occurring as a small, isolated growth commonly, but not exclusively, in younger patients.
4. It was introduced the term “papillary urothelial neoplasm of low malignant potential”. This is a papillary urothelial lesion with an orderly arrangement of cells within papillae with minimal architectural abnormalities and minimal nuclear atypia irrespective of cell thickness. Patients with these tumors are at risk of developing new bladder tumors of a similar histology. However, occasionally these subsequent lesions manifest as urothelial carcinoma, such that follow-up of the patient is warranted. In the standard classification this tumor corresponds to the “papillary urothelial carcinoma, grade 1 (low-grade), pTa”. This new category avoid labeling a patient as having cancer, which has psychosocial and financial (e.g. insurance) implications, but neither is a benign lesion (e.g., papilloma) diagnosed, so the patient might be adequately followed (2).

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Intensity of stromal changes predicts biochemical recurrence-free survival in prostatic carcinoma

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Abstract: The reactive stroma of prostate cancer contains a mixture of myofibroblasts and fibroblasts, while fully differentiated smooth-muscle cells are very rare or absent. In experimental prostate cancer models, prostatic stromal cells promote angiogenesis and stimulate prostate tumorigenesis. The aim of this study is to analyse whether the intensity of stromal changes can predict survival in patients with prostatic carcinoma. Material and methods: Stromal reaction was quantified histochemically and imunohistochemically in 50 patients treated with radical prostatectomy for clinically localized prostate carcinoma and its relationship with established prognostic factors was assessed.
Results: Kaplan-Meier analysis showed a significant association between the pattern of vimentin and desmin expression and the length of disease-free period; patients with a higher vimentin or lower desmin expression had a shorter disease-free period. On multivariate analysis only vimentin expression (odds ratio 4.06, 95% confidence interval 1.01-16.26, p = 0.049) was a significant predictor of biochemical recurrence. In patients with identical Gleason pattern and Gleason score the level of vimentin expression could identify patients with a higher risk of disease recurrence.
Conclusions: Intensity of stromal changes could serve as an independent prognostic factor in the assessment of biochemical recurrence-free survival. Among prostate cancer patients with an identical Gleason score, it could identify patients with a higher risk of biochemical recurrence. Thus, stromal changes and their intensity could serve as a novel marker for the recognition of patients with an increased risk of disease recurrence.

Editorial Comment

There is evidence that prostate carcinogenesis is influenced and controlled by cellular interactions derived from a complex relationship between stromal, epithelial and extracellular matrix components. In prostate cancer as well as in many other cancers, the stromal microenvironment is different from the corresponding normal stroma. There is increased microvessel density, inflammatory cells and modified fibroblasts. The latter are called myofibloblasts or cancer-associated fibroblasts and are considered to play a central role in the complex process of tumor-stroma interaction and consequently in the tumor growth, spread and metastasis, and could also represent an important target for cancer therapies.

The study by Tomas' et al. showed that the intensity of stromal changes could serve as an independent prognostic factor in the assessment of biochemical recurrence-free survival in patients submitted to radical prostatectomy. The only other group studying the predictive value of reactive stroma (desmoplasia) for biochemical

recurrence following surgery is from Baylor College in Houston, Texas. Yanagisawa N et al. (1) have shown that reactive stromal grading in biopsies was correlated with adverse pathological parameters in the prostatectomy and independent predictor of biochemical recurrence. The authors concluded that quantitation of reactive stroma and recognition of the desmoplastic cancer in H & E-stained biopsies is useful to predict biochemical recurrence in prostate carcinoma patients independent of Gleason grade and prostate-specific antigen. Obviously more studies are needed for definitive conclusions.

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RECONSTRUCTIVE UROLOGY

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Internal urethrotomy and intraurethral submucosal injection of triamcinolone in short bulbar urethral strictures

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Objectives: In clinical practice, internal urethrotomy is an easy procedure and is offered as a first modality for treatment of short urethral strictures. Internal urethrotomy refers to any procedure that opens the stricture by incising or ablating it transurethrally. The most common complication of internal urethrotomy is stricture recurrence. The curative success rate of internal urethrotomy is approximately 20%. Triamcinolone has antifibroblast and anticollagen properties. This study evaluated the efficacy of triamcinolone in the prevention of anterior urethral stricture recurrence after internal urethrotomy.
Methods: Fifty male patients with anterior urethral stricture were randomized to undergo internal urethrotomy with or without urethral submucosal injection of triamcinolone. Using general anesthesia urethrotomy was performed. Triamcinolone (40 mg) was injected submucosally at the urethrotomy site in 25 patients. The patients were followed for at least 12 months and the stricture recurrence rate was compared between the two groups.
Results: 23 patients in the triamcinolone group and 22 in the control group completed the study. There were no significant differences in the baseline characteristics of the patients or the etiology of the stricture between the two groups. Mean follow-up time was 13.7 ± 5.5 months (range: 1-25 months). Urethral stricture recurred in five patients (21.7%) in the triamcinolone group and in 11 patients (50%) in the control group (P = 0.04).
Conclusions: Injection of triamcinolone significantly reduced stricture recurrence after internal urethrotomy. Further investigations are warranted to confirm its efficacy and safety.

Editorial Comment

In this study men were randomized to steroid injection vs. no injection after internal urethrotomy for short (< 1.5 cm), treatment-naïve bulbar urethral strictures. Follow-up was with urethrography and urethroscopy every 6 months or when symptoms recurred. Treatment failure was defined as need for repeat treatment. Patients were similar in the two groups in all respects. Recurrence rate was 22% in the treatment group and 50% in the control group at mean follow-up of just over a year. These results are encouraging and we look forward to future reports with longer follow-up. Some shortcomings of this study deserve mention and point to areas for improvement in the design of future studies which attempt to answer this question. The study was not blinded and there was no placebo. Future studies should blind the surgeon and patient to injection with steroid vs. saline. The study outcome is fairly subjective. A more objective outcome would be a standardized assessment such as the ability to pass a cystoscope.

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Antegrade endourethroplasty with free skin graft for recurrent vesicourethral anastomotic strictures after radical prostatectomy

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Purpose: To investigate the efficacy of the antegrade endourethroplasty technique for the management of frequently recurrent vesicourethral anastomotic strictures that develop after retropubic radical prostatectomy.
Patients and Methods: Between January 2006 and February 2008, endoscopic antegrade urethroplasty was performed in 11 patients with recurrent vesicourethral anastomotic strictures that developed after retropubic radical prostatectomy (RRP). The mean age of the patients was 64.6 years. In the first step of this two-step procedure, the graft bed was prepared by transurethral resection of the vesicourethral anastomotic stricture region. In the next step, after 3 days, an Amplatz sheath was placed in the urinary bladder suprapubically. Then, an endobronchial catheter was inserted from the external urethral meatus and extended out of the body from the suprapubic region through the Amplatz sheath. A graft taken from anteromedial section of the arm was tubularized on the catheter balloon. The graft was placed into the bladder neck antegradely under endoscopic vision. Subsequently, the graft carrier catheter was fixed by previously placed two polypropylene sutures inserted into the proximal and distal part of the stricture zone percutaneously from the perineum. The transurethral catheter was taken out delicately on postoperative day 21.
Results: Urethral patency succeeded in 6 of the 11 (54.5%) patients, and maximum flow rate was more than 13mL/s in follow-up. Graft necrosis occurred in two patients, and the stricture recurred in three patients in two months postoperatively.
Conclusion: Antegrade endourethroplasty may be a suitable alternative to open surgical reconstruction in selected patients with recurrent bladder neck stricture following RRP. Further studies, including more patients with modifications, are needed to improve the success rate.

Editorial Comment

A minimally-invasive approach is appropriate in surgery when it offers results that are similar to those obtained with an open approach but with less morbidity. For this reason, the recalcitrant bladder neck contracture is the ideal stricture site for the development of the endoscopic urethroplasty. The alternatives are either unsuccessful (repeat dilation or urethrotomy) or are associated with high morbidity (urethral stent or open reconstruction). Still, concerns remain with this approach. First, a successful graft requires a healthy graft bed. Certainly, the recently resected and fulgurated TUR area is not an ideal graft bed. Second, results with tubular grafts or flaps in open urethral reconstruction have been poor. It is unclear why they should be any better with an endoscopic approach. Still, these initial results are encouraging and given the alternatives, a 55% success rate and flow rates of only 13-18cc/s are pretty good in these complex patients with few alternatives.

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UROLOGICAL ONCOLOGY

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Long-term efficacy results of EORTC Genito-Urinary Group randomized phase 3 study 30911 comparing intravesical instillations of epirubicin, bacillus Calmette-Guérin, and bacillus Calmette-Guérin plus isoniazid in patients with intermediate- and high-risk stage Ta T1 urothelial carcinoma of the bladder

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Eur. Urol. 2010; 57: 766-773

Background: Intravesical chemotherapy and bacillus Calmette-Guérin (BCG) reduce the recurrence rate in patients with stage Ta T1 urothelial bladder cancer; however, the benefit of BCG relative to chemotherapy for long-term end points is controversial, especially in intermediate-risk patients.
Objective: The aim of the study was to compare the long-term efficacy of BCG and epirubicin.
Design, Setting, and Participants: From January 1992 to February 1997, 957 patients with intermediate- or high-risk stage Ta T1 urothelial bladder cancer were randomized after transurethral resection to one of three treatment groups in the European Organization for Research and Treatment of Cancer Genito-Urinary Group phase 3 trial 30911.
Intervention: Patients received six weekly instillations of epirubicin, BCG, or BCG plus isoniazid (INH) followed by three weekly maintenance instillations at months 3, 6, 12, 18, 24, 30, and 36.
Measurements: End points were time to recurrence, progression, distant metastases, overall survival, and disease-specific survival.
Results and Limitations: With 837 eligible patients and a median follow-up of 9.2 yr, time to first recurrence ($p<0.001$), distant metastases ($p=0.046$), overall survival ($p=0.023$), and disease-specific survival ($p=0.026$) were significantly longer in the two BCG arms combined as compared with epirubicin; however, there was no difference for progression. Three hundred twenty-three patients with stage T1 or grade 3 tumors were high risk,

and the remaining 497 patients were intermediate risk. The observed treatment benefit was at least as large, if not larger, in the intermediate-risk patients compared with the high-risk patients.
Conclusions: In patients with intermediate- and high-risk stage Ta and T1 urothelial bladder cancer, intravesical BCG with or without INH is superior to intravesical epirubicin not only for time to first recurrence but also for time to distant metastases, overall survival, and disease-specific survival. The benefit of BCG is not limited to just high-risk patients; intermediate-risk patients also benefit from BCG.

Editorial Comment

The efficacy of BCG against recurrences in high-risk patients has long been recognized. Previous papers and meta-analyses have also shown that BCG favorably acts against progression of disease. Even further data supported the notion that this was also true for intermediate-risk patients. However, many authors were not satisfied with the database for these conclusions and in recent times, others have contributed with opposite results.

This EORTC paper now, with its high number of patients (975 randomized and 837 eligible) and its inherent quality of statistical analysis puts an end to many of these questions (and poses several new ones). In this randomized study of BCG against epirubicin (EPI) the results clearly show an advantage of BCG in terms of time to first recurrence, and even more important, in terms of distant metastases and survival (!). Moreover, the favorable results were even more pronounced in the intermediate-risk group. The question still remaining is why time to progression was not different between the treatment groups. According to the authors, BCG appeared to reduce the risk of progression (hazard ratio 0.56) but there were too few progressions (n=25) to make meaningful comparisons.

Certainly, this paper will have a significant impact on the guidelines on non-muscle invasive bladder cancer and on the routine clinical treatment of this disease.

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Detrusor muscle in the first, apparently complete transurethral resection of bladder tumour specimen is a surrogate marker of resection quality, predicts risk of early recurrence, and is dependent on operator experience

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Eur. Urol. 2010; 57: 843-849

Background: An European Organisation for Research and Treatment of Cancer analysis of multicentre trials found significant interinstitutional variability in recurrence rates at first follow-up cystoscopy (RR-FFC) and attributed this to variable transurethral resection of bladder tumour (TURBT) quality.
Objective: To determine whether resection of detrusor muscle (DM) in the first, apparently complete TURBT is a surrogate marker of quality and whether the presence of DM is dependent on a surgeon's experience.
Design, Setting, and Participants: Over a 2-yr period, patients with new bladder tumours that were judged to have been completely resected were recruited from our prospectively maintained bladder tumour database. Strict exclusion criteria were applied.

Measurements: Prospectively recorded tumour size, tumour multiplicity, surgeon category, DM status, grade and stage of tumour, and findings at first follow-up cystoscopy (at 3 mo) and at early re-TURBT were evaluated. Surgeons were stratified into seniors (consultants and year 5 or year 6 trainees) and juniors (trainees lower than year 5). Early recurrence (for calculating RR-FFC) was defined as pathologically confirmed tumour on early re-TURBT or recurrence at the first follow-up cystoscopy. Logistic regression multivariate analyses were carried out to determine associations between variables.
Results and Limitations: In a total of 356 patients, DM was present in 241 patients (67.7%). Multivariate analyses revealed that large tumours, high-grade tumours, and surgery by senior surgeons was independently associated with the presence of DM in the resected specimens. The RR-FFCs when DM was absent and present were 44.4% and 21.7%, respectively (odds ratio: 2.9; 95% confidence interval: 1.6-5.4; p=0.0002). The absence of DM and resection by less experienced surgeons independently predicted a higher RR-FFC. This association was also seen in small and low-grade tumours. The number of patients in this study appears modest, and further validation may be required.
Conclusions: DM absence or presence in the first, apparently complete TURBT specimen appears to be a surrogate marker of resection quality by independently predicting the RR-FFC, which is also dependent on surgeon experience.

Editorial Comment

The quality of surgery is an important fact. This retrospective analysis of resection quality and analysis of early tumor recurrences now gives some hard arguments in favor of a thorough and radical, deep transurethral resection including detrusor muscle (DM). Through all groups analyzed senior surgeons had better results than junior surgeons in terms of detrusor muscle included in specimen. The important fact is that this directly translated into early tumor recurrence at three months. The absence of DM was associated with a significantly higher risk of both early recurrence at first follow-up cystoscopy and residual disease at early re-TURBT. In patients with TaG1 and TaG2 tumors, the risk of early recurrence was 34.5% in the absence of DM, compared to 14.5% when DM was present (p=.005). In patients with G3 tumors, the overall risk of recurrence was 5-fold higher when DM was absent (< 0.001). In patients with T1 disease the recurrence rates were 81.3% and 34.9% when DM was absent or present, respectively (p=.002).

Therefore, do a good job and mind the presence of detrusor muscle in your TURBT specimen!

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NEUROLOGY & FEMALE UROLOGY

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Urethral diverticula in women: discrepancies between magnetic resonance imaging and surgical findings

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J Urol. 2010; 183: 2265-9

Purpose: Some groups consider magnetic resonance imaging the gold standard to diagnose urethral diverticula with up to 100% reported sensitivity. We describe cases contradicting this paradigm and identify reasons for discrepancies.
Materials and Methods: We searched a database for women who underwent urethral diverticulum surgery from 1998 to 2008 and also underwent preoperative magnetic resonance imaging. Images were reviewed by a blinded panel of urologists and a radiologist. They came to consensus on the presence or absence, site and anatomy of urethral diverticulum or cancer, and compared operative findings. Discrepancies were classified as errors in urethral diverticulum or cancer diagnosis and errors in urethral diverticulum anatomy or site.
Results: Of 76 patients who underwent diverticulectomy 41 also underwent magnetic resonance imaging, of whom 10 (24.4%) had a discrepancy between magnetic resonance imaging and surgical findings. In 6 of these cases there were diagnosis errors and diverticula were not seen on magnetic resonance imaging in 3. One urethral diverticulum each was misdiagnosed as Bartholin's cyst and as a typical post-collagen injection appearance. A sterile abscess was incorrectly diagnosed as a urethral diverticulum. In 2 patients magnetic resonance imaging did not detect cancer within the diverticulum. A major discrepancy in anatomy made intraoperative decision making difficult in 2 patients.
Conclusions: In cases clinically suspicious for urethral diverticulum magnetic resonance imaging had a 24.4% error rate. Serious consequences are failure to detect cancer and suboptimal treatment for urethral diverticulum. The reason for the high magnetic resonance imaging accuracy rate in other series may be that in the absence of radiological confirmation some surgeons may choose not to perform surgery. Magnetic resonance imaging is useful to assess urethral diverticula but physicians should be aware of its limitations.

Editorial Comment

A thoughtful presentation questioning the acceptance of MRI of the urethra as the absolute sensitive and specific test to identify, localize, and characterize urethral diverticula. The authors found an approximate 25% rate of diagnostic discrepancy or misdiagnosis in those patients who had undergone MRI for the diverticulum. Of keen interest was that almost 10% of the patients ultimately found to have a diverticulum were noted to have a negative MRI. The authors provide an excellent discussion reviewing their thoughts on why the MRI may fail to either identify or properly characterize a urethral diverticulum.

A good take home message after reading this work is that when evaluating for a urethral diverticulum, one should not abandon clinical judgment and suspicion or forget historical studies such as the double balloon retrograde urethrogram in the face of a negative MRI.

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Requiem for the suburethral tape

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BJU Int. 2010; 105: 445-8

In this article, the author discusses the treatment options for women with bothersome stress urinary incontinence (SUI). He comments on the strong connection between the urethra and anterior vaginal wall and the complications related to the synthetic suburethral tapes. Further, the use of a vaginal wall support procedure with a pubovaginal sling and a mesh in the vaginal wall to treat UI are discussed.

Editorial Comment

An intellectual commentary discussing suburethral tape procedures, the potential reasons for their success as well as their complications. The authors note that based on the integral theory, the suburethral sling will help restore "deficient pubo-urethral ligaments to their normal anatomy (sic)". They note that in their personal experience they have not identified a distinct pubourethral ligament. This observation has been discussed in previous editorials in this journal (1) and may explain the ability to maintain urinary continence after a supra-meatal transvaginal urethrolysis (2). In addition, the article does quote a very candid if not surprising lower level of success with suburethral sling placement based on their Level 1 evidence.

This review is a worthwhile read for the surgeon interested in expanding both central and peripheral understanding of sub midurethral sling surgery.

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PEDIATRIC UROLOGY

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Infant communicating hydroceles -- do they need immediate repair or might some clinically resolve?

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J Pediatr Surg. 2010; 45: 590-3

Purpose: Infant hydroceles that are communicating by history (fluctuation in size) or examination (reducible fluid) are often repaired soon after presentation. We have followed a series of infant boys with such hydroceles and reviewed their early natural history.
Materials and Methods: Since 1998, we have followed 174 infant boys presenting with an apparent communicating hydrocele without immediate surgical repair. All boys were initially seen before 18 months of age and most (168) by 12 months. Most had been full term at delivery, although 32 had been premature (<37 weeks' gestational age) and 11 extremely so (<32 weeks). Most boys (120) had bilateral hydroceles at presentation.
Results: Of the 110 boys followed to disposition, 69 (62.7%) had complete resolution without surgery by a mean age of 11.7 months. Forty-one patients (37.3%) underwent surgery for correction at a mean age of 14 months

because of persistence in size or development of a hernia. Six developed a hernia during observation, none of whom had any episode of incarceration. Only 2 patients with apparent resolution subsequently had recurrence with a hernia. Age at presentation and gestational age at birth showed no effect on resolution. The hydroceles of 64 boys had improved in size after a mean follow-up of 13.9 months when last seen.
Conclusions: Many infant hydroceles that are communicating by history or examination do resolve clinically without surgery and deserve observation. Progression to hernia was rare in our experience and did not result in incarceration. Consequently, little risk is taken by initial observation.

Editorial Comment

The authors of this retrospective study propose that observation of communicating hydroceles in young infants is warranted given a fairly high resolution rate and low rate of progression to a true hernia in their series. In addition, there were no episodes of incarceration of these hernias. Because the natural tendency of most pediatric urologists and pediatric surgeons is to repair communicating hydroceles near the time of presentation, we have previously had little data to demonstrate the natural history of these patients. This series provides nice data for us and suggests that observation may be reasonable for many of these patients. I suspect that over a 9-year period of time, there were more than 174 patients younger than 18 months who presented to their institution with a communicating hydrocele. It would be interesting to know what criteria were used to determine which patients should be followed and which patients should be repaired without observation.

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Later toilet training is associated with urge incontinence in children

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J Pediatr Urol. 2009; 5: 458-61

Objective: The objective of this study was to determine if later toilet training is associated with urge incontinence in children.
Methods: We used a case-control study design to yield level 2 evidence.
Results: Initiation of toilet training after 32 months of age was associated with urge incontinence (P=0.02).
Conclusion: For children who display signs of toilet-training readiness, training should be initiated prior to 32 months of age to reduce the risk for urge incontinence.

Editorial Comment

This was a case controlled study matching 58 patients who presented to a pediatric urology office with urge incontinence and 157 controls from a general pediatric practice. The patient ages range between 4 and 12 years. Parents were given a questionnaire that included demographics, socioeconomic status, urinary symptoms, and age at the initiation of toilet training. Parents also indicated whether they used a child or parent-oriented approach. The investigators found that the only statistically significant difference between cases and controls

was the mean age at toilet training. Patients with urge incontinence were trained at a mean age of almost 32 months whereas the control group was trained at just under 29 months. There was no difference in the type of toilet training method utilized.

Parents are often interested in getting advice on when and how to potty train their children. The study shows an association between urge incontinence and potty training at a later age. The authors point out that there is data to suggest that potty training at an age less than 27 months might not be helpful either. Certainly, each child and family must be looked at individually; however, we now have more evidence to suggest that there may be an "ideal time" to initiate potty training.

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Video
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Retrourethral Transobturator Sling AdVance® for the Treatment of Male SUI after Radical Prostatectomy

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ABSTRACT

Introduction: Despite improved surgical techniques, exist an increasing number of patients suffering post-prostatectomy stress urinary incontinence (SUI). Some 2-5% of the patients with incontinence after radical prostatectomy exhibit a persistent incontinence for >1 yr postoperatively despite conservative therapy attempts. For these patients surgical treatment is recommended and the artificial urinary sphincter is still the gold standard. The retro-urethral transobturator sling (AdVance®) represents a non-obstructive, functional therapeutic approach.
Methods: A 64-year-old male had an elevated PSA level of 6 ng/ml. The DRE findings were negative for palpable nodules and subsequent TRUS-guided needle biopsy of the prostate showed right-sided prostatic adenocarcinoma, Gleason score 7(3+4). The patient underwent a transperitoneal LRP, the tumor was confined to the prostate with negative surgical margins (stage T1cNxMx). The follow-up PSA level was undetectable and 14 months later presented moderate SUI (3-4 pads/daily), despite conservative therapy. A previous urethrocystoscopy was performed to evaluate the sphincter function and the mobility of the posterior urethra (changes achieved by perineal pressure). The AdVance® sling (American Medical Systems) was placed for the treatment of SUI according to the Rehder and Gozzi method, with a total operative time of 40 min and estimated blood loss of 70 mL. The hospital course was uneventful and the patient was discharged on the first post-interventional day. This video demonstrates the surgical technique (step-by-step).
Results: After 1 year, complete continence (no pads) was achieved and quality-of-life score improved significantly.
Conclusions: The AdVance® represents an effective, safe and minimally invasive treatment option for mild-to-moderate SUI post-radical prostatectomy.

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Available at: www.brazjurol.com.br/videos/july_august_2010/Rijo_518_519video.htm

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EDITORIAL COMMENT

The surgical video by Rijo et al. provides an excellent depiction on the use of a retrourethral transobturator sling in the surgical management of stress urinary incontinence (SUI) post-prostatectomy. As the editor of the video section, I am thrilled in publishing surgical videos of such high-quality both in content and in technical design. As our video section continues to grow exponentially, the emphasis remains on promoting surgical excellence and novel techniques. Surgical videos such as this have elevated the bar and set a new standard for both our contributors and readership. In addition, the authors have provided a very insightful minimally invasive approach to the surgical management of moderate to severe SUI in this patient cohort. Although long-term data is required before we can truly assess the merit of this technique as compared to the artificial urinary sphincter, it nevertheless remains that at centers of excellence such as this, it offers a minimally invasive approach to the management of SUI post-prostatectomy hence it should at the very least be a consideration.

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