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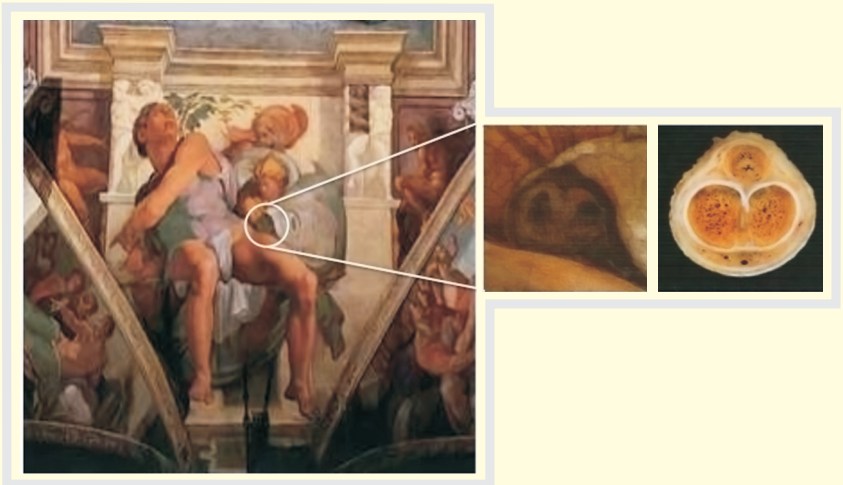

The Figure of the Prophet Jonah on the ceiling of Sistine Chapel, by Michelangelo Buonarroti, highlighting a detail of the scene of the prophet Jonah and his correlation to a design of a cross section of the base of a penis, with the two cavernous bodies with the septum between them and the spongy body. (Page 319)

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I can not understand!

The United States Preventative Services Task Force (USPSTF) made a final recommendation on May 21, 2012. The recommendation is against prostate-specific antigen (PSA) based screening for healthy men, asserting that there is "moderate or high certainty that the service has no benefit or that the harms outweigh the benefits," discouraged the use of the test by issuing it a Grade D rating. The D rating applies to men of all ages but does not apply to the use of PSA testing for monitoring patients after a prostate cancer diagnosis or treatment.

It is hard to understand the basis of this recommendation. The reaction of the American urological community was strong; the American Urological Association (AUA) called the USPSTF recommendation as inappropriate and irresponsible.

If we banish PSA testing we would go back 25 years ago when most of patients with prostate cancer had an advanced disease and the possible treatments were only palliative.

Furthermore there are many evidences that early diagnosis brings a benefit to prostate cancer patients in terms of mortality reduction. The European Randomized Study for the Screening of Prostate Cancer update, just presented last month at the AUA meeting, showed a 21% risk reduction in prostate cancer death rate because of the screening.

At this point every urologist agrees that are many prostate cancer patients who do not need to be diagnosed and treated but this is the reason to deny an early diagnosis to that men who would be saved by a radical treatment?

Interestingly, the Göteborg Trial showed 44 percent relative risk reduction in prostate cancer mortality occurring in men 50-64 years of age after a median of 14 years. In this area many patients are not aggressively treated for prostate cancer, indicating that a good clinical practice can prevent unnecessary treatments (1).

Maybe the reason for the tentative of discouraging PSA testing can be economical. The financial burden of thousands of biopsies, robot assisted or not surgeries, radiotherapy sessions probably has been too heavy for the American Healthcare System. If you link to http://csn.cancer.org/node/184022 the website Cancer Survivor Network of American Cancer Society you will find the statement of a man (number 142) who had been submitted to a robot-assisted radical prostatectomy; he detailed all the steps he went through and said that the cost of everything was $66,157.30 USD. In this basis to treat 50% of newly diagnosed prostate cancer/year in the USA the healthcare system would spend 7 billion USD!

There are still many problems with the treatment of prostate cancer. We still do not have markers with adequate sensitivity and specificity for this cancer; we are probably

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overtreating many patients and we are spending too much in the diagnosis and in the treatment, mainly in new technologies with no clear benefits to the patients. But this is not enough reason to banish the only powerful tool we have for an early diagnosis of prostate cancer and probably save many lives.

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Dr. Sidney Glina

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REVIEW ARTICLE

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Flexible Ureteroscopy and Holmium:YAG laser lithotripsy for stone disease in patients with bleeding diathesis: a systematic review of the literature

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ABSTRACT

Introduction and Objectives: The management of urolithiasis in patients on anticoagulants presents a challenge to the endourologist. Due to multiple comorbidities, it may be impossible to safely discontinue the anticoagulant treatment. Other modalities such as shock wave lithotripsy and PCNL are contraindicated in these patients, so ureteroscopic treatment may be the only option. We conducted a systematic review of the literature to look at the safety and efficacy of ureteroscopic management in these patients.

Methods: Systematic review and quantitative meta-analysis was performed using studies identified by a systematic electronic literature search from January 1990 to August 2011. All articles reporting on treatment for stones in patients with a bleeding diathesis using ureteroscopy and a Holmium:YAG laser were included. Two reviewers independently extracted the data from each study. The data was included into a meta-analysis and discussed.

Results: Three studies were identified reporting on 70 patients (73 procedures). All patients had stone fragmentation using Holmium laser. The mean stone size was 13.2mm with a range of 5-35mm. The quality of the included studies was modest. Stone free status was achieved in sixty-four patients (87.7%). There were no major complications and only 11% of the patients developed minor complications with only 4% rate of minor bleeding.

Conclusions: Retrograde stone treatment using ureteroscopy and holmium laser lithotripsy can be safely performed in patients with bleeding diathesis with a low complication rate.

ARTICLE INFO

Key words:
Ureteroscopy; Laser Therapy; Lithotripsy; Bleeding time; Blood Coagulation Disorders; Urinary Calculi

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INTRODUCTION

Nephrolithiasis is a common condition affecting the population with a peak incidence around the third to fourth decade of life (1). The lifetime risk of urolithiasis in the general population is 13% in men and 7% in women (2).

The preferred treatment modalities for ureteric calculi include shock wave lithotripsy (SWL) or ureteroscopy (URS) (3,4). With the advancement in technology of fibre optics and the production of smaller calibre ureteroscopes, ureteroscopic extraction has led to a higher stone free rate than SWL and is recommended as first line management for ureteric calculi (5-8).

However, despite the advancements made in the instrumentation, urologists have always opted to correct coagulopathy before undertak-

ing endourological procedures (7). This poses a controversial question concerning the management of patients who are anticoagulated or have a coagulopathy (9). SWL and percutaneous nephrolithotomy are contraindicated in these patients and correction of coagulopathy is recommended before endoscopic procedures (9,10). However, despite the use of low molecular weight heparin for thromboembolic protection, patients can still develop organ or life threatening clots (10). Conversely, if coagulopathy was not reversed, the procedures run the risk of causing continual bleeding or haematoma formation (10).

In view of all these facts, we aimed to conduct a systematic review to assess the safety and efficacy of ureteroscopic procedures in patients with bleeding diathesis.

MATERIALS AND METHODS

Search strategy and study selection

The systematic review was performed according to the Cochrane diagnostic accuracy reviews guidelines. The search strategy was conducted to find relevant studies from MEDLINE (1990- March 2011), EMBASE (1990- March 2011), Cochrane Central Register of Controlled Trials - CENTRAL (in The Cochrane Library - Issue 1, 2011), CINAHL (1990- March 2011), Clinicaltrials.gov, Google Scholar and Individual urological journals.

Terms used included: 'ureteroscopy', 'coagulopathy', 'anticoagulant', 'warfarin', 'bleeding', 'urolithiasis', 'aspirin', 'coumarin', 'clopidogrel', 'thrombocytopenia', and 'calculi'.

Mesh phrases included: ("Ureteroscopy"[Mesh]) AND "Blood Coagulation Disorders"[Mesh], ("Anticoagulants"[Mesh]) AND "Ureteroscopy"[Mesh], ("Ureteroscopy"[Mesh]) AND "Hemorrhage"[Mesh], ("Anticoagulants"[Mesh]) AND ("Lasers"[Mesh] OR "Laser Therapy"[Mesh]), ("Lasers"[Mesh]) AND "Calculi"[Mesh]) AND "Anticoagulants"[Mesh], ("Anticoagulants"[Mesh]) AND "Calculi"[Mesh], ("Ureteroscopy"[Mesh]) AND "Aspirin"[Mesh], ("Ureteroscopy"[Mesh]) AND "clopidogrel" [Supplementary Concept], ("Ureteroscopy"[Mesh]) AND "Coumarins"[Mesh], and ("Ureteroscopy"[Mesh]) AND "Thrombocytopenia"[Mesh], ("Kidney Calculi"[Mesh] OR "Ureteral Calculi"[Mesh]) AND "Aspirin"[Mesh], ("Coumarins"[Mesh]) AND ("Kidney Calculi"[Mesh] OR "Ureteral Calculi"[Mesh]), ("Kidney Calculi"[Mesh] AND "Ureteral Calculi"[Mesh]) AND "Coumarins"[Mesh], and ("Thrombocytopenia"[Mesh]) AND ("Kidney Calculi"[Mesh] OR "Ureteral Calculi"[Mesh]).

Reference lists of previous reviews and previous trials were included; papers in languages other than English were included, references of searched papers were evaluated for potential inclusion, and recently published versions were included if the publication was duplicated. Authors of the included studies were contacted whenever the data was not available or not clear.

Two reviewers (OA and BS) identified all studies that appeared to fit the inclusion criteria for full review. Each reviewer independently selected studies for inclusion in the review. Disagreement between the two extracting authors was resolved by consensus. If consensus between the two reviewers could not be reached, a third author (MM) was deferred to for arbitration and consensus.

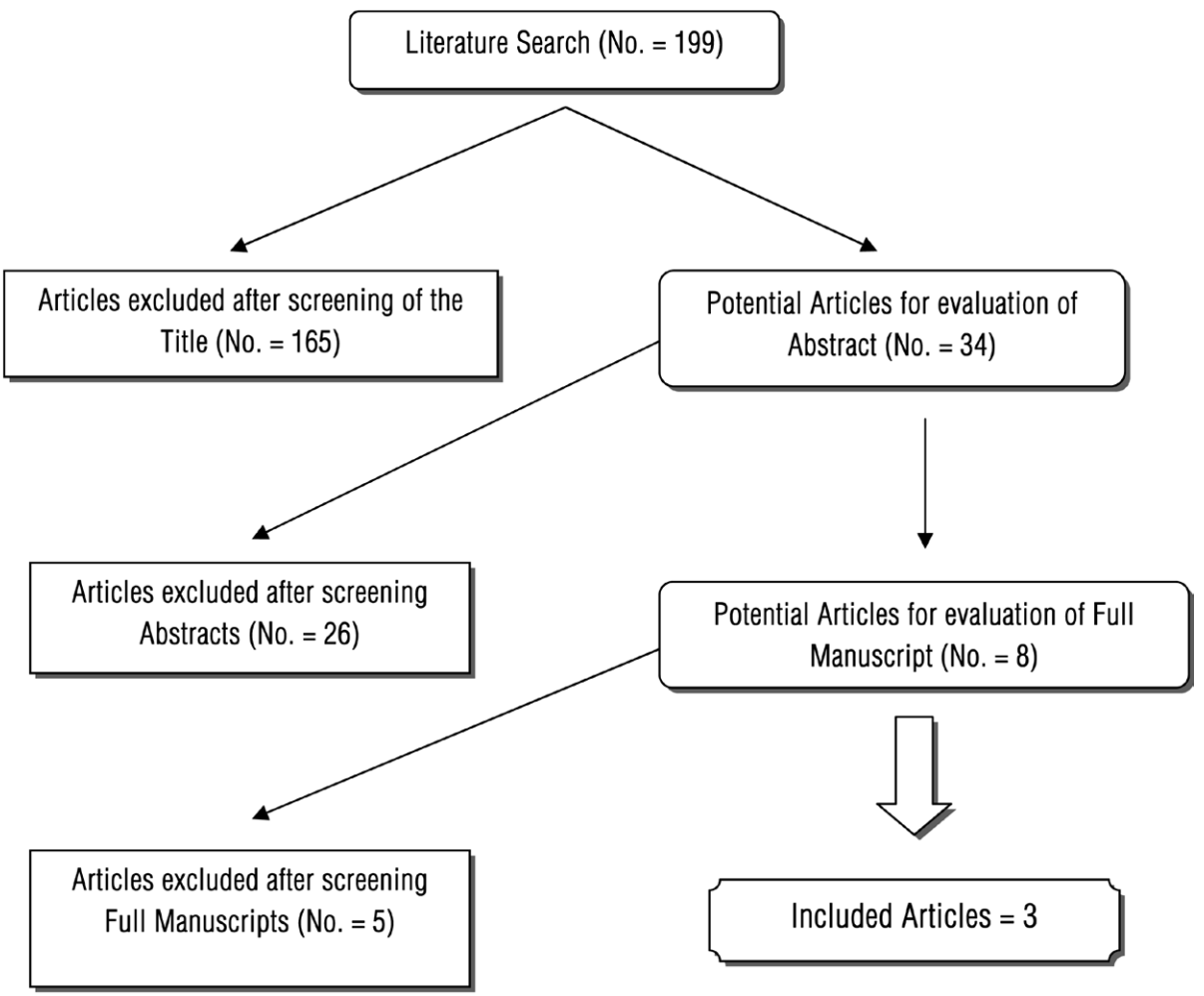
Data extraction and analysis

Studies reporting on the treatment of patients with a bleeding diathesis with flexible ureteroscopy and laser lithotripsy were included. Patients included were adults with a bleeding diathesis who had urinary stones. The following variables were extracted from each study: period of the study; country of origin of the study; study population demographics; type of anticoagulant used or coagulopathy; stent insertion; stone free rates; follow up; and complications. The data of each study was grouped into a meta-analysis to allow a numerical representation of the results. A quality assessment of harms using the McHarm scale was conducted for each included study (11). We used Review Manager (RevMan 5.0.23) to plot the quality assessment of harms tables.

RESULTS

The literature search yielded 199 studies, of which 165 were excluded by title or abstract for non-relevance to the aims of this review or not reporting on ureteroscopy treatment of patients with a bleeding diathesis (Figure-1). Eight

Figure 1 - Flowchart for article selection process of the review.



studies were then retrieved for further assessment, of which three were included in the review (7,8,12). All the included studies were published between 1998 and as recent as 2008, reflecting the continued debate of how to treat stones in patients with bleeding diathesis.

All 3 studies were retrospective studies; however, Turna et al. also compared the anticoagulated group to a similar group of patients as a control group. All the studies reported on the variables indicated in the 'data extraction section' and were plotted into Table-1. Wherever data was not available in the reports or there was not enough clarification, lead authors were contacted to get the raw data.

Five articles were excluded after reading the full manuscript. One study was not included since the authors looked at all treatment modalities for urinary stones and though mentioned that 8 patients were ureteroscopically treated only 2 were holmium laser treated (10). Furthermore, the authors had not provided demographic, coagulopathy, or stone details separately for these patients and therefore could not be extracted. Attempts at contacting the author were unsuccessful. The remaining four studies did not look

Table 1 - Table of included studies.

Name	Journal	Year	Period	Pla-ce	No.	Age mean (range)	Bleeding Diathesis mean (range)	Mode	Stone Size/ Location (mm) mean (range)	Stent	Stone Free Rate	Complications
Kuo	Urology	1998	1997-1998	USA	8	58.3 (42-74)	Coumadin (INR: 2.1 (1.6-2.9)): 5 Thrombocytopaenia: 2 vWd: 1	Flexi Hol	(3-15) Ureteric (proxi-mal: 1; middle: 1; distal: 1) Renal: 5	All	6/7 (one 2mm residual frag (1 patient had no stone on URS)	2 – (epistaxis: 1; post-op urinary retention: 1)
Watterson	Journal of Urology	2002	1996-2001	USA	25 (28)	(42-84)	Warfarin (INR 2.3): 17 Thrombocytopaenia: 4 vWd: 1 Liver dysfunction: 3	Flexi Hol	11.9 (6-25) Ureteric (proxi-mal: 9; middle: 3; distal: 7) Renal: 9	87% (26/30)	96% (27/28) 2 patients had electrohydrau-lic lithotripsy treatment	2 – (renal colic: 1; AF: 1)
Turna	Journal of Urology	2008	2001-2007	USA	37	58.2 (35-86)	Coumadin (INR 1.8 (1.1-3.3)): 14 Clopidogrel: 5 Low dose (81mg) Aspirin: 13 High dose (325 mg) Aspirin: 5	Flexi Hol	13.2 (5-35) Ureteric: 8 Renal: 29	All	81.1% (30/37)	4 – (transient macroscopic haematuria: 3; UTI: 1)

AF: Atrial Fibrillation; Flexi: Flexible Ureteroscopy; Hol: Holmium Laser; INR: International Normalisation Ratio; mm: millimetre; URS: Ureteroscopy; vWd: von Willebrand disease

at patients with bleeding diathesis and therefore were excluded (2,4,9,13).

Characteristics of the included studies

All the studies were conducted in the USA (Table-1). Seventy patients who underwent 73 procedures were included in this review. The study population was composed of patients with 35 to 86 years old. All patients had some sort of coagulopathy including 36 patients on warfarin, 6 patients with thrombocytopaenia, 2 with von Willebrand disease, 3 had liver dysfunction, 5 on clopidogrel, 13 on low dose aspirin, and 5 patients on a high dose aspirin. None of the patients had their coagulopathy reversed, except for 2 patients who had thrombocytopaenia and had recently had chemotherapy; both were given 2 units of platelets for fear of the platelet count dropping further (7,12). The mean international normalization ratio (INR) for the patients on warfarin was 2.1 with a range of 1.1-3.3. Turna et al. had included patients on coumadin; however, their INR was 1.1, and there was no mention of how many patients with subtherapeutic INR levels were included. All patients were treated with a flexible ureteroscopy and a holmium:YAG laser. The stone sizes ranged from 3-35mm with 43 renal stones and 30 ureteric, of which 10 were proximal, 4 middle and 8 distal ureteric. Turna et al. had not mentioned the location of the ureteric stones.

Two studies routinely stented their patients after ureteroscopy and holmium:YAG laser fragmentation. However, the third study (by Watterson et al.) did not differentiate between the patients who had holmium treatment and those that had electrohydraulic lithotripsy and stent insertion, therefore their data was not included.

With regards to stone free rate, 87.7% (64/73) of the patients were stone free. In this review, none of the patients developed any major complications and 11% (8/73) of the patients developed minor complications; however, five of the patients had complications unrelated to their coagulopathy. This brought the complication rate that could be attributed to an anticoagulated state, i.e. bleeding, to 4.1% (3/73). These three patients developed transient macroscopic haematuria for at least 3 days but did not require continuous bladder irrigations, secondary procedures or blood transfusions (8). The five other complications included one patient who developed a post-operative urinary retention, one patient developed worsening renal colic attributed to stone passage, another developed atrial fibrillation, another developed a urinary tract infection and the last developed an epistaxis. The epistaxis was attributed to ketorolac; however, there was no mention of how they were certain that ketorolac was the cause rather than the coagulopathy. All patients were routinely followed up, however each study varied in the length of follow up. Kuo et al. followed up their patients for 4-6 weeks, while Turna et al. followed up for 4 weeks, and Watterson et al. for 1-2 weeks only. All the patients were stone free and complication free after follow up discharge.

Methodological quality assessment of the included studies

Overall, the quality of the reported studies was modest as two of the studies were reported as retrospective while one was unclear; however it seemed to be retrospective from the methodology. All the included studies might have been subjected to bias as their method of recruitment of patients consisted of recruiting patients from databases; this could lead to selection as well as reporting bias. None of the studies were randomized, blinded (7,12), and only one study had a control group (8). However, the study group (coagulopathy patients) was significantly older than the control group, which poses the question to whether or not these groups could be compared. Furthermore, there was no mention on how the control group patients were selected from the authors' database of 692 patients. This again could be construed as selection bias.

The quality assessment of harms indicates that the studies generally have a low risk of bias concerning reporting the harms that could potentially be caused by the procedure (Figures 2 and 3).

DISCUSSION

Normalizing coagulopathy pre-operatively is the mainstay of patients' management before surgical procedures. This usually leads to the combined consult and co-ordinated efforts of the sur-

Figure 2 - Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies.

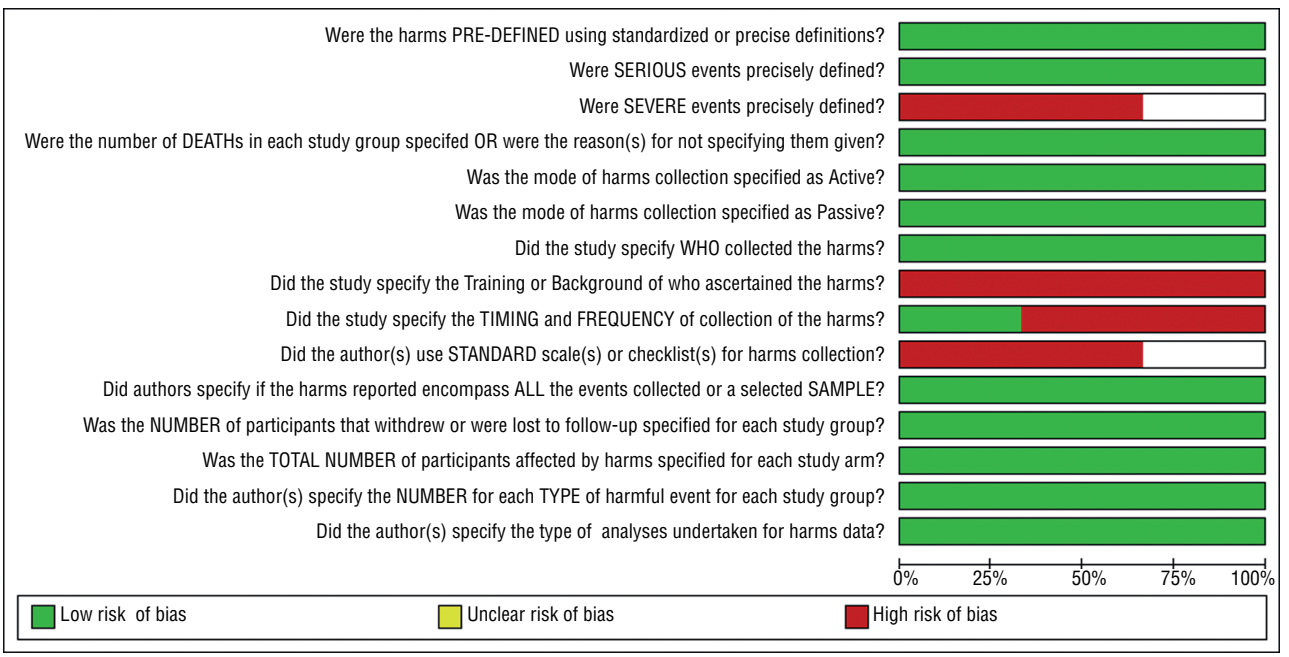
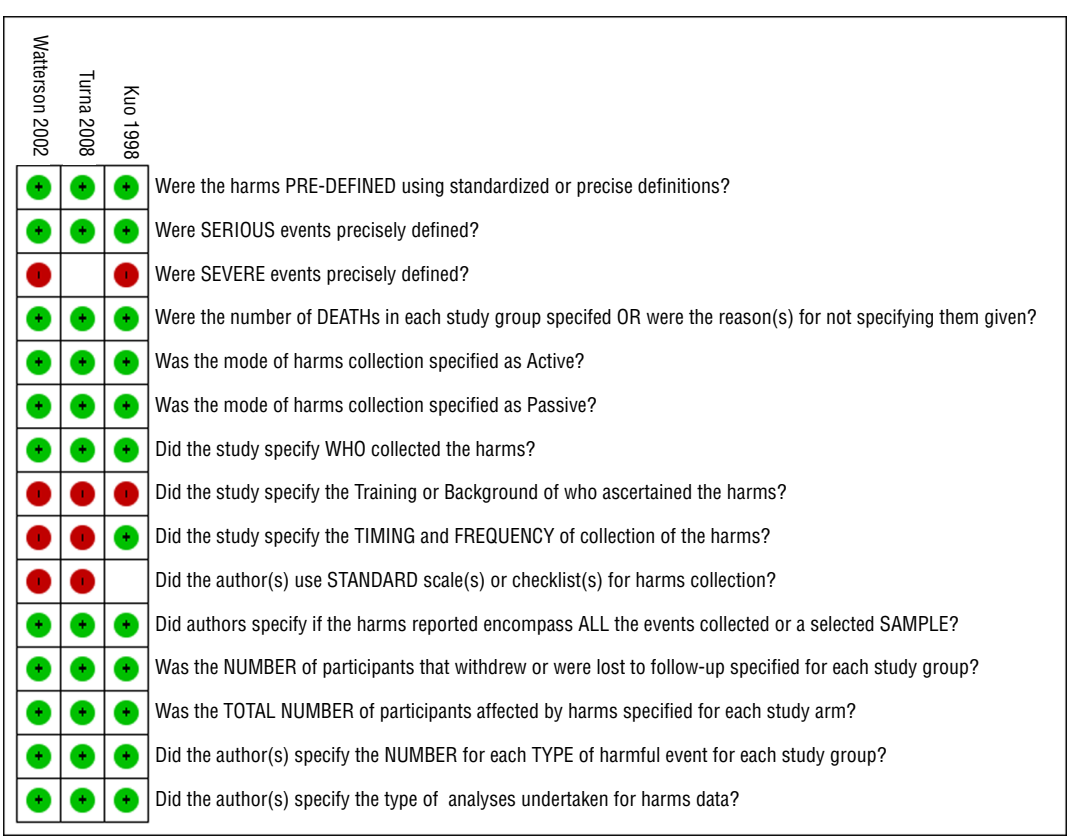


Figure 3 - Risk of bias summary: review authors' judgements about each risk of bias item for each included study.



geons with the haematologists and anaesthetists (12). However, the risk of thromboembolic events during perioperative bridging with heparin is 1-2% (14). Furthermore, treating the coagulopathy is significantly more costly when compared to patients without coagulopathy undergoing similar procedures (10).

Though other modalities exist for the treatment of large urinary stones, such as SWL, PCNL, and open or laparoscopic surgery, these are contraindicated if the bleeding diathesis is not corrected (8,10). This only leaves ureteroscopic management for these patients (8).

Advancements in endoscope engineering and laser technologies allow an operator to visualise and treat stones in the whole upper urinary system, including the renal calyxes with a reported long-term complication rate of less than 1% (12,15,16). Holmium lasers provides effective and efficient intracorporeal lithotripsy for even hard stones such as cysteine and calcium oxalate monohydrate stones, and can also be used to ablate upper urinary tract tumours (7,12). Furthermore, holmium lasers offer haemostatic capabilities during the procedure, which gives an additive benefit to patients with bleeding diathesis (12). Lastly, holmium laser energy is rapidly absorbed by water, leading to a minimal risk of ureteric injury if the laser fibre is at least 0.5mm away from the ureter and no risk of ureteric perforation if the distance is more than 1mm (12).

This review found that the use of flexible ureteroscopes and holmium lasers on patients with bleeding diathesis is not only safe but also efficient, with an overall stone free rate of 87.7%, a minor complication rate of 11%, but only a 4% rate of minor bleeding, and a major complication rate of 0%.

The validity of the results of systematic review depends on the quality of included studies including selection of participants and inclusion criteria. The studies included seemed to all be retrospective reports of a larger database. Therefore at most this review has a level 2a Levels of Evidence according to CEBM (17). No study evaluated cost analyses.

The other limitation of this review is related to the patient population; the majority of patients were on warfarin. However, the remaining had various other causes for coagulopathy, whether the heterogeneity of the study sample would impact outcomes is not known. However, we aimed at reviewing all patients with coagulopathy and did not target one group. Furthermore, due to the limited number of patients, we did not see a need of conducting sub-groups analysis which would have reduced the cohort even further.

Furthermore, though the level of evidence is considered a 2a, this review has a small cohort of patients (70) from case series basing this evidence on. In addition, no trial or study was found in the literature. This reflects the need for further larger participant studies to further explore the safety and efficacy of ureteroscopy in these patients.

Despite the limitation, grouping of the data was possible and revealed the safety and efficacy of the combined studies. Furthermore, this review opens possibility for further research into the question.

This review has shown that it is not only safe but also efficient to treat patients suffering with urinary stones and afflicted with a bleeding diathesis with ureteroscopy and holmium laser. This can have cost benefits in practice as patients on anticoagulants need not undergo reversal and most patients with coagulopathy need further management to support their coagulation system.

Future research efforts should be concentrated on higher quality, more rigorous evaluation of ureteroscopic treatment in these groups of patients. Studies should be multi-institutional and protocol driven, preferably peer reviewed before the start. Studies should be prospectively evaluated and include a control group of patients who are not anticoagulated for comparison. A detailed evaluation of the different types of bleeding diathesis such as patients on warfarin, clopidogrel, thrombocytopenia or haemophilia should be analyzed individually rather than as a whole. Furthermore, health economic outcome measures should be analyzed.

CONCLUSIONS

The use of ureteroscopy with the holmium laser is a safe and efficient modality for treating patients with urinary tract calculi who also have a bleeding diathesis or are anticoagulated. Further-

more, these patients do not need their coagulopathy reversed, which leads to reduction the risk of thromboembolism with very minimal short-term complications and no long term consequence.

CONFLICT OF INTEREST

None declared.

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EDITORIAL COMMENT

It has been recently published that urolithiasis is an entity associated with metabolic syndrome, which is characterized by hypertension, obesity, diabetes and abnormal lipid levels (1).

As the world drags its way towards obesity, urologists of all around the globe have noticed that, not only kidney stones have become more frequent, but also those patients who present them have more often other co-morbidities. One particular instance is the drug-induced blood diathesis, which is characterized by the use of "blood thinners" for cardiovascular protection.

These phenomena (obesity, metabolic syndrome, kidney stones, blood thinners) have brought upon the endourologist a current and challenging topic that every kidney stone specialist needs to be up-to-date on: Stone treatment versus bleeding diathesis.

The present study reports on what has been published in the literature that could serve as foundation to our decision making process while counseling a stone patient with any kind of bleeding diathesis. Surprisingly, the authors very well presented the lack of prospective (high evidence levels) studies on this matter; however, based on what has been judiciously selected in the literature, stone free and complication rates of flexible ureteroscopy with holmium: YAG laser lithotripsy for patients with blood diathesis are similar to healthy individuals.

It is important to emphasize that if one considers doing a retrograde endoscopic stone treatment in a patient with bleeding diathesis, it is strongly advised, based on evidence level 2a, following analogous surgical technique to what has been described in the selected studies of this systematic review:

A) Energy/lithotripsy - there is no scientific support for using other energy than holmium:YAG laser;

B) Scopes - flexible ureteroscopes were used in all cases;

C) Double J - stenting seems to be a wise routine.

D) General anesthesia might be safer (routine in USA), given the obvious risks of spinal puncture in such patients.

In conclusion, due to a pandemy of obesity and its metabolic consequences, kidney stone patients will more often present co-morbidities and also some kind of bleeding diathesis (aspirin, warfarin, clopidogrel), thus, they must be informed that flexible ureteroscopy and holmium:YAG laser lithotripsy is safe and efficient for treating their ureteral/renal stones.

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REVIEW ARTICLE

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Current Controversies in reconstructive surgery of the anterior urethra: a clinical overview

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ABSTRACT

We performed an overview of the surgical techniques suggested for the treatment of anterior urethral strictures using MEDLINE. In applying the MEDLINE search, we used the ''MeSH'' (Medical Subject Heading) and ''free text'' protocols. The MeSH search was conducted by combining the following terms: ''urethral stricture'', ''flap'', ''graft'', "oral mucosa", "urethroplasty", "urethrotomy" and "failed hypospadias". Multiple ''free text'' searches were performed individually applying the following terms through all fields of the records: ''reconstructive urethral surgery'', "end-to-end anastomosis'', ''one-stage'', ''two stage''. Descriptive statistics of the articles were provided. Meta-analyses were not employed. Seventy-eight articles were determined to be germane in this review. Six main topics were identified as controversial in anterior urethra surgery: the use of oral mucosa vs penile skin; the use of free grafts vs pedicled flaps in penile urethroplasty; the use of grafts vs anastomotic repair in bulbar urethral strictures; the use of dorsal vs ventral placement of the graft in bulbar urethroplasty; the use of definitive perineal urethrostomy vs one-stage repair in complex urethral strictures; the surgical options for patients with failed hypospadias repair. Different points of view are documented and presented in the literature by various authors from different countries. The aim of this clinical overview is to survey the main controversial issues in surgical reconstruction of the anterior urethra focusing on the use of flap or graft, substitute material, type of surgery and challenging situations, such as failed hypospadias or complex urethral stricture repair.

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INTRODUCTION

Various surgical techniques are currently being used to repair anterior urethral strictures with the goal of reducing morbidity and obtaining the best outcome with few complications; however, the superiority of one surgical technique over another has not yet been clearly defined (1-3). The urologist is thus requested to be familiar with the use of various surgical techniques to deal with any condition of the urethra that might emerge at the time of surgery. Urethral reconstruction is a continuing challenge and excellent results can be obtained with today's techniques, with single-stage repairs on the increase and continued improvements in patient outcome, but a significant advantage is to be gained by having tissue engineered material available for urethral reconstruction in the not so distant future (4,5). In developed countries,

strictures of the anterior urethra are commonly caused by external trauma, catheterization and transurethral surgery, whereas lichen sclerosus is reported to be the most frequent cause of distal penile urethral strictures (6,7). In less developed countries, pelvic trauma or explosive blast and gunshot injury are the most prevalent causes of urethral injury and disease mainly involving the posterior tract (8). Treatment options for urethral strictures include dilation, urethrotomy and reconstructive surgical techniques, and no one technique is appropriate for all stricture diseases (9). Dilation and urethrotomy continue to be the most commonly used approach despite many patients progressing to eventually requiring surgical repair (9). Some authors suggest that endoscopic treatment of urethral strictures using dilation or urethrotomy exacerbates scar formation, thus adding to stricture length and severity, complicating subsequent open repair (10). Open urethroplasty is regarded as the gold standard treatment of urethral strictures and this surgical therapy should not be withheld solely on the basis of age, as older men tolerate urethroplasty well and without complications (11,12). Minimizing time in surgery, maximizing adjuvant pain therapy and decreasing the incidence and severity of side effects have permitted most patients to leave the hospital within four hours of surgery (13). Finally, some authors suggest that urethrotomy and dilation work less than we believe and patients with urethral stricture disease should be referred to a center of expertise for the best available treatment options (14,15).

The aim of this overview is to survey the main issues in surgical reconstruction of the anterior urethra, focusing on the use of flap or graft, substitute material, type of surgery and challenging situations, such as failed hypospadias and panurethral stricture.

MATERIALS AND METHODS

Although the aim of the paper was not to conduct a systematic review or meta-analysis (the reason for which the manuscript does not comply with The PRISMA Statement), an overview of the literature was performed using MEDLINE. In applying the MEDLINE search we used ''MeSH'' (Medical Subject Heading) and ''free text'' protocols. Specifically, the MeSH search was conducted by combining the following terms retrieved from the MeSH browser provided by MEDLINE: ''urethral stricture'', ''flap'', ''graft'', "oral mucosa", "urethroplasty", "urethrotomy" and "failed hypospadias". Multiple ''free text'' searches were performed by applying the following terms individually through all fields of the records: ''reconstructive urethral surgery'', "end-to-end anastomosis'', ''one-stage'', ''two stage''. The search was restricted to the English language.

The results of our research were divided according to different topics regarding reconstructive urethral surgery which are currently under discussion, as points of view on these topics vary in the literature and at urological meetings.

Six main topics were identified as current controversies in anterior urethra surgery: the use of oral mucosa vs. penile skin; the use of free grafts vs. pedicled flaps in penile urethroplasty; the use of grafts vs. anastomotic repair in bulbar urethral strictures; the use of dorsal vs. ventral placement of the graft in bulbar urethroplasty; the use of definitive perineal urethrostomy vs. one-stage repair in complex urethral strictures; the surgical options for patients with failed hypospadias repair.

Substitute material: oral mucosa vs skin

In 1953, Presman and Greenfield first reported the reconstruction of the bulbar urethra with satisfactory result using a free full-thickness skin graft from the prepuce (16). In 1956, Peyton and Headstream, following Presman and Greenfield's suggestions, reported the construction of the bulbar urethra using a split thickness skin graft from the prepuce (17). In 1961, Devine and Horton fully described the use of preputial skin to repair hypospadias using a one-stage technique (18). In 1963, Devine et al., after successfully using preputial skin in one-stage hypospadias repair, popularized the use of the free skin graft in the repair of urethral strictures (19). From 1973 to 1985, beginning

with these preliminary studies, several authors reported the use of preputial skin grafts in urethral reconstruction, suggesting a range of different surgical techniques (20-31).

In 1993, El-Kasaby et al. first suggested the use of oral mucosal graft from the lip in the management of penile and bulbar urethral strictures (32). From 1966 to 2006, a total of 1,267 studies were reported in the literature on the use of oral mucosa in reconstructive surgery. These included 1,353 cases involving oral mucosa-based urethroplasty for the repair of defects associated with urethral strictures and hypospadias / epispadias (33). In these reports, 724 (53.5%) urethroplasties were performed for urethral strictures, while 629 (46.5%) were performed for hypospadias / epispadias repair (33). Reconstruction for urethral stricture and hypospadias / epispadias was successful in 418 (66.5%) and 553 (76.4%) cases, respectively (33).

Is oral mucosa really superior to skin as a substitute material in urethroplasty?

In 2005, Alsikafı et al. compared the outcome of 95 oral graft and 24 penile skin graft urethroplasty in an effort to answer whether oral mucosa is really superior to the skin (34). The overall success rate of skin urethroplasty was 84% with a mean follow-up of 201 months, while the success rate of oral urethroplasty was 87% with a mean follow-up of 48 months (34). These authors concluded that penile skin and oral mucosa are both excellent materials for substitution urethroplasty, with a comparable success rate, though penile skin appears to have a longer follow-up (34). Nevertheless, these authors do not report, in the Abstract of their paper, which type of urethroplasty is better (penile vs. bulbar, one-stage vs. two-stage), but instead compare the skin graft to the oral mucosa graft, creating a great limitation of these studies (34). In 2008, Barbagli et al. reviewed a large series, 375 patients, who underwent one-stage bulbar urethroplasty using either penile skin or oral mucosal grafts. The authors concluded that oral mucosa is superior to skin for one-stage bulbar urethroplasty, showing an overall success rate of 82.8% compared to 59.6% (35).

Markiewicz et al. documented the main biological and clinical characteristics of oral mucosa that justify why oral mucosa has received increased attention and popularity in the field of urological surgery (33,36). Oral mucosa is hairless, is readily available in all patients and is easily harvested from the cheek with low postoperative oral morbidity and high patient satisfaction (37). In patients requiring a long graft, oral mucosa is easily harvested from both cheeks (37). Oral mucosa is easy to handle because it has a thick elastin-rich epithelium, promoting its use as a graft employing original inlay or onlay techniques in one- or two-stage steps (33,36). Oral mucosa has a thin and highly vascular lamina propria that facilitates inosculation and imbibition (33,36). Oral mucosa avoids cosmetic consequences caused by the use of genital or extragenital skin because it leaves a concealed donor site scar (33,36). Oral mucosa is resistant to infection. Because it hosts a number of microorganisms, the tissue's inflammatory response to the organism is minimal (33,36). There are multiple immunological processes intrinsic to the oral mucosa that makes it impervious to native flora colonization (33,36). Histological studies have demonstrated that oral mucosa is highly compatible with the urethral recipient site, at times being indistinguishable from surrounding tissues (33,36). The structural integrity of oral mucosa remains intact following transplantation to a distant site (33,36). Oral mucosa is elastic and resilient, and when exposed to compression, stretching and shearing forces, it is highly resilient, due to its particular lamina propria-oral epithelium interface (33,36). Oral mucosa is easy to adapt to any type of urethroplasty and it is rarely affected by lichen sclerosus disease (33,36).

Penile urethroplasty: graft vs. flap

Penile urethroplasty can be a simple procedure in patients with a normal penis, but it can be a difficult challenge in men with strictures associated with failed hypospadias repair or genital lichen sclerosus in which the penis is fully involved by the disease. Basically, the choice of a surgical procedure for repair of penile urethral strictures is based on the etiology

of the disease (38). When the penile skin, urethral plate, corpus spongiosum and dartos fascia are available for urethral reconstruction, one-stage repair is the surgery of choice worldwide. In patients who have experienced failed hypospadias repair, where the penile skin, urethral plate and dartos fascia are not fully usable for urethral reconstruction, staged urethroplasty is suggested (38). In patients with lichen sclerosus, the use of oral mucosa is mandatory since lichen sclerosus is a skin disease and any skin that would be used for the repair is already or may become diseased (39-41). The surgical technique for penile urethral reconstruction must also be based on the proper anatomic characteristics of the penile tissues to ensure flap or graft take and survival. The use of flaps or grafts should not compromise penile length or cause penile chordee, and certainly should not untowardly affect penile appearance.

In 1968, Orandi first reported the reconstruction of the anterior urethra using a pedicled skin flap (42). In 1993, McAninch first described the reconstruction of extensive urethral strictures using a circular fasciocutaneous penile flap (43). The surgical technique described by McAninch still represents the most important and advanced evolution of Orandi's flap. The use of circular fasciocutaneous penile flap for anterior urethroplasty, and specifically penile urethroplasty, renders a durable 5- and 10-year estimated stricture-free survival rate of 84% and 79%, respectively, in properly selected patients (44).

Penile urethroplasty using a graft was greatly improved in 1999 when Hayes and Malone suggested an evolution of Snodgrass's longitudinal incision of the urethral plate, laying an oral mucosal graft into the incised urethral plate (45). A striking evolution of Hayes and Malone's technique appeared in 2001, when Asopa et al. popularized a similar technique for penile stricture repair (46). Asopa's technique represents the beginning of a new era in reconstructive urethral surgery with the use of free graft techniques, mainly in the repair of penile urethral strictures (44). In our experience, Asopa's penile urethroplasty presents numerous advantages compared to the use of extensive penile skin flap; it maintains great respect for the penile shaft components (44).

This technique does not require extensive training in reconstructive tissue transfer procedures and may be done using either oral mucosa or preputial skin graft with a minimally invasive approach (circumcision or simple perineal incision) (44). This technique should also be used in selected patients with failed hypospadias repair or lichen sclerosus, but is not suggested in patients with a narrow and fibrous urethral plate (44). In our experience using Asopa's technique, oral mucosa was better than skin graft material, but the difference (82% vs. 78%) does not justify the use of oral mucosa as a first choice (47). The choice of substitute material (oral mucosa vs. preputial skin) should be based primarily on surgeon preference and background.

The controversy over the best means of reconstructing the penile urethra, using flap or graft, is still under debate (44). The current literature, however, does not clearly support the use of one technique over the other, and some prospective randomized studies on the use of graft versus flap are not useful because they compare a non-homogeneous series of patients and stricture disease (48). At present, we are uncertain about the proper anatomic characteristics that the penis should have to ensure that the free graft takes, as penile spongiosum tissue and dartos fascia do not ensure sufficient support for the graft in all patients. In which patients will the use of a vascularized pedicled flap have a better chance of success than a free graft? In the future, more homogeneous and larger series of patients with adequate follow-up might clarify whether the use of a free graft is preferable to the use of a vascularized flap.

Bulbar urethroplasty: graft vs end-to-end anastomotic repair

The current literature suggests that the surgical technique for the repair of the bulbar urethral stricture should be selected according to stricture length (9,38). Primary end-to-end anastomosis is suggested for 1-2 cm strictures, augmented roof-strip anastomosis is suggested for 3-5 cm strictures, and substitution urethro-

plasty is suggested for longer strictures (9,38). In patients with strictures associated with local adverse conditions, staged urethroplasty is preferred (9,38). Since the 2009 American Urological Association (AUA) Meeting, the controversy over the use of end-to-end anastomosis or augmented roof strip anastomosis in non-traumatic bulbar urethral strictures has been open to debate (49). Transecting the urethra to perform an end-to-end anastomosis or augmented roof strip anastomosis allows complete removal of the scarred tissue, but may cause vascular and neuronal damage to the urethra and penis, thus promoting post-operative sexual dysfunction (49). Not transecting the urethra is a vascular and neuronal sparing procedure, but it does not allow removal of the scarred tissue (49). In strictures following blunt perineal trauma and bulbar urethral injury, removal of the traumatic scarred tissues and performance of a direct anastomosis between the two healthy urethral edges is mandatory, as not removing this tissue is the cause of stricture recurrence over time. In non-traumatic urethral strictures, is it mandatory to transect the urethra and remove the tissues or is it sufficient to open the urethra and perform only an augmentation of the original urethral plate?

Traumatic (following blunt perineal trauma and urethral injury) short bulbar strictures are generally amenable to scar excision and direct anastomosis using a simple perineal approach. This technique has a 90-95% success rate, as reported by some authors (50,51). Guralnick and Webster suggested that end-to-end anastomosis is appropriate only for bulbar strictures of 1 cm or less because excision of a 1 cm urethral segment with opposing 1 cm proximal and distal spatulations results in a 2 cm urethral shortening, which may be adequately accommodated by the elasticity of the mobilized bulbar urethra without chordee (52). These authors emphasize that longer excision risks penile shortening or chordee, even using lengthening maneuvers (52). On the contrary, Morey and Kizer also suggested the use of an extended anastomotic approach in patients with proximal bulbar urethral strictures longer than 2.5 cm (53). Al-Qudah and Santucci reported post-operative sexual dysfunctions (chordee and erectile dysfunction) in 18% of patients who underwent anastomotic urethroplasty, and concluded that oral mucosal urethroplasty had a superior success rate and fewer complications than anastomotic urethroplasty, even for short strictures (54). Barbagli et al. investigated, using a non-validated questionnaire, 60 patients who underwent bulbar end-to-end anastomosis, and reported that 23.3% of patients experienced ejaculatory dysfunction, 18.3% had decreased glans sensitivity, 11.6% had a glans that was neither full nor swollen during erection, and 1.6% had a cold glans during erection (51). No patient complained of penile chordee or impotence (51). Petersen and Webster suggested that for bulbar urethral strictures ranging from 2 to 4 cm, the best option is augmented anastomotic urethroplasty (9). During this procedure, the worst section of the stricture is removed and the urethra is reanastomosed and dorsally augmented with a free graft (9).

Other authors also suggest the use of this procedure in patients undergoing urethroplasty for strictures that contain a particularly narrow or dense area of 1-2 cm (55). However, none of these authors, who also suggest transection of the urethra in non-traumatic bulbar urethral strictures, report any investigation on the incidence of post-operative sexual complications in these patients, a factor that greatly limits these studies (9,52-55). In the future, large and homogeneous series of patients with adequate follow-up are necessary to investigate the incidence of post-operative sexual dysfunction in patients who have undergone full transection of the bulbar urethra for anastomotic repair in non-traumatic strictures.

Bulbar urethroplasty: dorsal vs ventral graft

One-stage oral mucosal graft urethroplasty represents the most widespread method for the repair of bulbar urethral strictures due to its highly vascular spongiosum tissue. The location of the free graft on the dorsal or ventral urethral surface has become a contentious issue since Barbagli et al. described, in 1996, the technique of dorsal onlay graft urethroplasty (56-58). Wessells suggests a list of the techni-

cal advantages of bulbar ventral onlay urethroplasty (59). Armenakas emphasizes that ventral graft placement, requiring less urethral dissection and mobilization, is technically easier (60). Success with bulbar oral mucosal grafts has been high with a dorsal (57,58,61-63) or ventral (59,60,64,65) graft location, and the different graft positions have shown no difference in success rate (35,66,67). Barbagli et al. showed that placement of the grafts on the ventral, dorsal or lateral surface of the bulbar urethra provided the same success rates (83% to 85%) and stricture recurrence was uniformly distributed in all patients (66). In our daily clinical practice, we currently use the ventral graft location in all patients with non-traumatic urethral strictures located in the proximal bulbar urethra (68). In patients with non-traumatic urethral strictures located in the distal bulbar urethra, we prefer the dorsal graft location (69).

Complex urethral strictures: definitive perineal urethrostomy vs one-stage repair

Complex anterior urethral strictures include strictures simultaneously involving the penile and bulbar urethra (panurethral stricture) and strictures in patients who had undergone numerous prior failed urethroplasties. Lichen sclerosus is the most frequent cause of pan-urethral strictures (70) and failed hypospadias repair is the most frequent cause of complex anterior urethral strictures (71,72). How to treat patients with complex urethral stricture disease is still a difficult and controversial issue in the field of reconstructive urethral surgery. In these patients, the use of one-stage techniques requires careful preoperative patient evaluation and selection and represents a complex and challenging surgery (73). Breyer et al. reported that stricture length (greater than 4 cm) or previous failed urethroplasty are predictive of failure after urethroplasty (74). Some authors suggest that heroic measures or one-stage repair may not always be justified in extensive urethral strictures associated with a high risk of failure (75,76). Strictures associated with local adverse conditions, such as fistula, false passage, abscess, cancer or repeated failed urethroplasty are best treated with staged procedures (75,76). Perineal urethrostomy can be a temporary or definitive solution to the complex urethral stricture. As some patients choose not to have the urethra reconstructed in a second or third step and continue to void through a perineal urethrostomy, the first stage becomes a definitive procedure (75,76). Some patients (mean age 53 years) having undergone failed hypospadias repair (mean previous operations 4.2) or repeated failed urethroplasty (mean previous operations 4.1) or other conditions requiring periodic dilation or urethrotomy to avoid urinary retention informed us "I underwent innumerable prior failed operations. I am tired." (76). These patients could not accept the possibility of another complete urethroplasty failure (76). In other patients with aggressive stricture recurrence following repeated urethroplasties, some urologists have said "I don't know which kind of urethroplasty is best to perform because I do not know the pathological status of your urethra" (76). In these patients, it is the status of the urethral tissues that conditions the surgical approach and not the technical expertise of the surgeon (76). Likely, the experienced urethral surgeon is able to perform one-stage repair in a majority of urethral strictures, but is it the correct approach? Is it always correct to transplant an oral graft in ischemic or scarred urethral tissue? Is it correct to once again transect the urethra in patients who have already undergone a prior end-to-end or augmented roof strip anastomosis, showing urethral shortening? Is it correct to do one-stage urethroplasty in patients with multiple failed treatments of urethral strictures that began 20-30 years ago and showing multiple bladder diverticula or detrusor acontractility? (76).

An important question regards the patient's satisfaction and acceptance of the urinary diversion through a perineal urethrostomy. One-stage repair provides restoration of micturition through normal standing position and avoids patient discomfort caused by perineal urinary diversion that may not be accepted by the patient for religious, hygienic, cultural or psychological reasons; although, in our experience, many patients in this population are already accustomed to seated voiding because of age and prior void-

ing difficulties. In contrast to complex one-stage reconstruction, perineal urethrostomy is a minor surgical intervention that can be performed on an outpatient basis, providing an early return to normal activities. (75,76) In older patients or patients with multiple failed repairs, serious comorbidity, histologically severe disease or a severely scarred urethral plate, we discussed the possibility of performing a temporary or definitive perineal urethrostomy with the patient. However, the final choice was selected on the basis of patient decision alone (75,76).

Surgical options in patients with failed hypospadias repair

How to treat patients with failed hypospadias repair represents a difficult issue in the field of reconstructive urethral surgery, and the current literature does not provide any sure guideline.

Recently, two studies on the largest series of patients (1,176 cases) with failed hypospadias repair to date were published in the literature, and the suggestions coming from these two studies were interesting enough to be here presented and discussed (77,78). The authors reported that, from 1988 to 2007, 1,176 patients with a mean age of 31 years (range 1 to 76 years) were treated for complications after initial hypospadias repair, consisting of 953 patients in Serbia and 223 in Italy (78). Patients were stratified into four different groups according to the involvement of a single or multiple anatomical compartment(s) of the male genitalia at the time of surgery. Group 1 included patients who underwent only urethral surgery; group 2 included patients who underwent only corpora cavernosa surgery; group 3 included patients who underwent urethral and corpora cavernosa surgery; group 4 included patients who underwent complex reconstructive surgery including urethral, corpora cavernosa, glans and penile skin resurfacing (78). It is interesting to note that only 301 patients (25.5%) required only urethral surgery for meatal, penile or bulbar stricture, retrusive meatus, fistula or diverticulum (78). Out of the 1,176 cases, 60 (5.2%) required only corpora cavernosa surgery for residual penile curvature, corpora cavernosa deformity, penile shortening or torsion, and 8 (13.4%) patients in this group required corpora cavernosa resurfacing using grafting material as indicated in surgery for Peyronie's disease (78). In 166 cases (14.1%), surgery involved both the urethra and corpora cavernosa functions for stricture, fistula or diverticulum associated with some degree of residual glans/penile curvature (78). But it is very interesting to note that in this study, 55.2% of patients (649 cases) required complex reconstructive surgery for complications fully involving the genitalia with glans dehiscence, partial glans necrosis, glans torsion or curvature, loss of penile or scrotal skin, midline septum, penile skin torsion, abnormal peno-scrotal or peno-pubic junction, buried penis, trapped penis, other problems (78). The final message from this study is that failed hypospadias repair is not a problem for the pediatric urologists to solve as the mean age of the patients was 31 years, or for the urethral surgeon, as the surgery was restricted to the urethra in only 25.5% of the cases (78). To greatly improve anatomical and functional outcome in patients with failed hypospadias repair, it is essential to create centers specially dedicated to the treatment of these patients. Only full collaboration between the urethral surgeon and the surgeon widely skilled in reconstructive surgery of the corpora cavernosa (penile prosthesis implantation, surgery for Peyronie's disease, surgery for male to female transition) can ensure the best cosmetic and functional outcome (78). Shouldn't patients with complex failed hypospadias repair be referred to a centre of expertise? Medically and ethically speaking, it is the right thing to do (78).

CONCLUSIONS

Reconstructive surgery of the anterior urethra is a continually evolving process and new controversies must be discussed and resolved so that the patient receives the highest possible standard of care. This objective will become possible by increasing the use of minimally invasive techniques, developing new research and translating basic scientific results into daily clinical practice.

CONFLICT OF INTEREST

None declared.

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EDITORIAL COMMENT

The manuscript entitled "Current Controversies in Reconstructive Surgery of the Anterior Urethra" is a review article on surgical treatment of anterior urethral strictures and hypospadias.

The authors have comprehensively reviewed this issue and organized the text according to six main controversial topics:

1. The use of oral mucosa vs penile skin in anterior urethra surgery
2. The use of free grafts vs pedicled flaps in penile urethroplasty
3. The use of grafts vs anastomotic repair in bulbar urethral strictures
4. The use of dorsal vs ventral placement of the graft in bulbar urethroplasty
5. The use of definitive perineal urethrostomy vs onestage repair in complex urethral stricture
6. The surgical options for patients with failed hypospadias repair

Reconstruction of the urethra is a challenging surgery for the urologists. Due to some circumstances that may occur at the time of surgery, the surgeon must be familiar with various surgical techniques.

In regards to the controversial topics raised by the authors, it is important to emphasize that well-design randomized trials are lacking in the literature. The few randomized studies compare non-homogeneous series of patients and stricture disease. So, it is an open-field for research.

Nevertheless, the review brings some interesting aspects to be pointed out:

- Oral mucosa and the penile skin are both excellent materials for substitution urethroplasty. A large series of patients presents better outcomes for oral mucosa. However, superiority of oral mucosa is to be proven with randomized studies.
- It is not possible to make evidence-based recommendations on the use of grafts versus pedicled flaps in penile urethroplasty. Nevertheless, in patients with lichen sclerosus the use of oral mucosa is mandatory (primary skin disease).
- Stricture length is the main factor to be analyzed before indicating graft versus end-to-end anastomosis in bulbar urethral strictures. Primary end-to-end anastomosis is suggested for 1-2 cm strictures.
- Case series suggest that different graft positions (ventral vs dorsal) have shown no difference in success rates for bulbar urethroplasty.
- It is important to listen to the patients' expectations before considering the use of a definitive perineal urethrostomy. It can be a less invasive option for complex urethral stricture.
- Patients with failed hypospadias repair should be treated in reference centers for urethral reconstructive surgery in order to greatly improve anatomical and functional outcomes.

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ORIGINAL ARTICLE

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The interpretation of the figure of the prophet Jonah by Michelangelo on the ceiling of the sistine chapel: anatomical urological vision

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ABSTRACT

Purpose: A detailed analysis in the iconography and pictorial appearance of the scene of the "Prophet Jonah" painted by the artist Michelangelo Buonarroti (1475-1564) on the ceiling of the Sistine Chapel between the years 1508 and 1512.
Materials and Methods: Literature review on the Italian Renaissance period and the life of Michelangelo Buonarroti and analysis of historical aspects of the evolution of studies of human anatomy in this period and the works of the artist.
Results: A comparative analysis of the representation of the figure of the fish on the left thigh of "Jonah" with a cross section of penis shows a curious similarity. The pictorial and iconographic analysis reveals an intensity of light on the pubic area and the position of the prophet with the legs spread apart and left hand placed on this region. A tube-shaped cloth covers the region and the angel at the side seems to be looking at this anatomical region of "Jonah". In fact, sets of iconographic and pictorial relate to the deciphered code.
Conclusions: This description helps to confirm the relationship of the Renaissance art with the human anatomy; science has been much studied in this period. The design of a cross section of the penis is revealed with the two cavernous bodies with the septum between them and the spongy body. Considering the circumstances in which Michelangelo had painted, subjectivity was fundamental due to religious motivations added to the vigorous implications of a limited scientific knowledge typical of that era.

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INTRODUCTION

Michelangelo Buonarroti (1475-1564) is a famous Italian Renaissance artist. In addition to famous sculptures as "Pieta" and "David", the artist painted the ceiling of the Sistine Chapel between the years 1508 and 1512 in Rome.

Michelangelo had a life-long interest in anatomy that began with his participation in public dissections in his early teens, when he joined the court of Lorenzo de' Medici and was exposed to its physician-philosopher members (1-4). These anatomic studies aided him in creating extremely accurate depictions of the human figure in his sculptures and paintings, notably the statue of "David" in Florence and paintings of "God" and other figures from the Book of Genesis in the Vatican's Sistine Chapel in Rome.

The link of anatomy with several artists of this period is confirmed by binding studies of anatomy at the School of Fine Arts in Florence during the Renaissance. Scholars of this period believed that knowing the minutiae of the human anatomy could help artists to reproduce the human form in sculptures and paintings.

Sistine Chapel frescoes are considered one of the monumental achievements of Renaissance art. In the winter of 1511, Michelangelo entered the final stages of the Sistine Chapel project and painted 4 frescoes along the longitudinal apex of the vault, which completed a series of 9 central panels depicting scenes from the Book of Genesis. In 1990, Frank Lynn Meshberger reported that Michelangelo concealed an image of the brain in the first of these last 4 panels, namely, the "Creation of Adam", showing a sagittal section of the brain as the bottom of the figure of the creator (5). The Creation of Adam on the ceiling of the Sistine Chapel has long been recognized as one of the world's great art treasures.

Others researchers reported that Michelangelo appears to have hidden an image of the brainstem and spinal cord in a depiction of God in the final panel of this series, the Separation of Light From Darkness (6). These findings by a neurosurgeon and a medical illustrator, published in Neurosurgery, may explain long controversial and unusual features of one of the frescoes' figures. Although the vast majority of subjects in this painting are considered anatomically correct, art historians and scholars have long debated the meaning of some anatomical peculiarities seen on God's neck in the part of this painting.

The Separation of Light From Darkness is an important panel in the Sistine Chapel iconography because it depicts the beginning of Creation and is located directly above the altar. The authors propose that Michelangelo, a deeply religious man and an accomplished anatomist, intended to enhance the meaning of this iconographically critical panel and possibly document his anatomic accomplishments by concealing this sophisticated neuroanatomic rendering within the image of God (6).

An American renal physician reckoned to have found convincing evidence that Michelangelo was familiar with the anatomy and function of the kidneys (7). According to Eknoyan, the artist's interest in the kidney started when he became afflicted with urolithiasis and sought help from the most prominent physician in Rome, Realdo Colombo (8). In the painting "The Separation of Land and Water" on the ceiling of the Sistine Chapel, the mantle of the creator resembles a bisected right kidney (7).

Another panel in ceiling of Sistine Chapel is "The Figure of the Prophet Jonah". According to the Old Testament, "Jonah" stayed inside the belly of a whale (some translations call it a fish) for three days and three nights (Jonah, 1:17 - Bible), thus escaping death by drowning. The scene is located at one end of the vaulted ceiling of the Sistine Chapel. The prophet is depicted leaning back and turned slightly to the right, and he thus creates an unusual effect by "contradicting" the architecture of the ceiling. "Thanks to the strength of art, the vault, which is in fact curving toward the front, seems to be pushed back", described Vasari (1). We made a detailed analysis of the scene of the "Prophet Jonah" on the ceiling of the Sistine Chapel in the iconography and pictorial appearance with a cross section of the penis.

MATERIALS AND METHODS

Literature review on the Italian Renaissance period and historical aspects of the evolution of studies of human anatomy in this period and the life and works of the Michelangelo Buonarroti were analyzed. A detailed analysis of the iconographic and pictorial clues of the scene of the "Prophet Jonah" was made, with special attention to position of the figures, the direction the characters are looking at, the "movement"/position of the hands and the region of the body that is painted in lighter shades.

A detailed analysis of the scene of the "Prophet Jonah" on the ceiling of the Sistine Chapel in its iconography and pictorial appearance and its correlation to a design of a cross section of the penis was done.

RESULTS

A comparative analysis of the representation of the figure of the fish on the left thigh of "Jonah" with a cross section of penis shows a curi-

ous similarity (Figure-1). The pictorial and iconographic analysis reveals an intensity of light on "Jonah's" pubic region; the prophet was positioned with his legs spread apart, and a tube-shaped cloth covers the region; the angel at the side seems to be looking at this anatomical region of "Jonah".

The fish painted next to "Jonah's" left thigh has an unusual aspect (the front part does not look like a mouth). Comparing this front part of the fish with a cross-section of the base of a penis, the two cavernous bodies can be seen with the septum between them and the spongy body. All these structures can be found in this detail of the painting of the fish.

Add to these evidences, a study of the Prophet Jonah by Michelangelo Buonarroti reveals that the artist portraited the prophet with the right hand over the genital area. A draft for the ceiling of Sistine Chapel not maintained on the original painting confirms the genital focus of the scene (Figure-2).

DISCUSSION

The art of the Renaissance, not satisfied with copying the nudes of antiquity, encouraged its contributors into anatomical dissection to better reproduce the body in their art. With time, traditional courses of instruction for aspiring artists actually included a study of human anatomy, not only for its external features, but also for that of its supporting structures. In fact, the Florentine Academy of Art was the first to institute an obligatory course in anatomy, in which aspiring artists copied directly from cadavers and skeletons (4,9-11). In Florence, according to the Statuta universitatis et studdi florentini de 1387, the painters and sculptors were accepted into the Consorteria dei Medici e degli Speziali in the year 1303. The artists were given the bodies of people who died of natural causes in Santa Maria Novella Hospital or in other hospitals of the city, while to doctors only two cadavers of criminals hanged of both sexes were supplied per year for the study of anatomy.

The reason for this uneven distribution was that while the artists were concerned only with the surface of cadavers, the doctors dissected them and destroyed all their parts. The church, of course, objected on principle to the desecration of the dead, but did allow for dissection of the cadavers of condemned criminals and even facilitated it. Cadavers were either stolen or made available through the

Figure 1 - The Figure of the Prophet Jonah on the ceiling of Sistine Chapel, by Michelangelo Buonarroti, highlighting a detail of the scene of the prophet Jonah and his correlation to a design of a cross section of the base of a penis, with the two cavernous bodies with the septum between them and the spongy body.

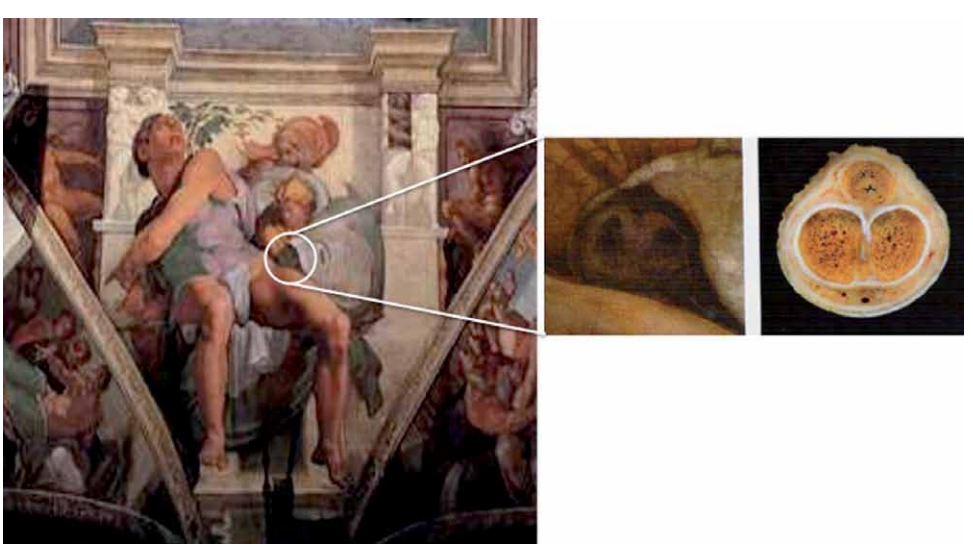
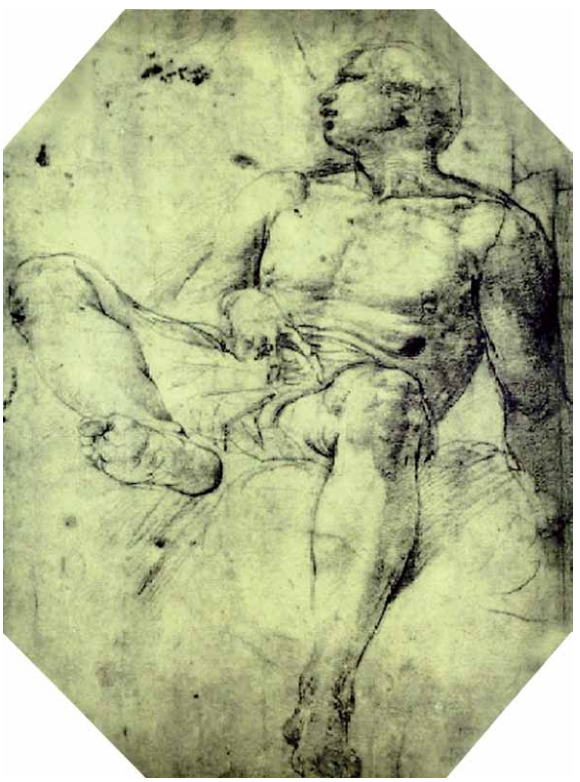


Figure 2 - Michelangelo Buonarroti reveals the genital focus in a draft of the scene not maintained on the original painting of the ceiling of Sistine Chapel. The study shows the Prophet Jonah with the right hand over the genital area.



church. Permission to dissect corpses, provided the remains were buried decently, had been granted by Pope Sixtus IV, who had been a student at the Medical School of Bologna (9-15). While some of the more daring artists performed actual dissections, most participated in public anatomies conducted by physicians versed in the art of dissection and accompanied by reading and interpretation of medical texts by the physician-anatomist (4,9-11).

Michelangelo probably participated in public dissection early in his youth, probably conducted by Elia del Medigo, a physician-philosopher who was a member of Lorenzo de Medici's circle, which Michelangelo joined in his mid teens (2-4). Michelangelo began to perform his own dissections and demonstrations, as recorded by his two biographers, Vasari and Condivi (1,8,16). He is said to have made molds of muscles to experiment in their shapes and forms during various body positions, which he was to render so masterfully in his subsequent sculpture and painting. This is clearly evident in the 20 nude slaves ("ignudi"), seated on blocks above the thrones of the "Sibyls" and "Prophets", that decorate the small panels of the Sistine Ceiling and in more than 300 figures that he painted in the "Last Judgment", which, according to Vasari, was intended to represent "the most perfect and well-proportioned composition of the human body in its most varied positions" (1,17).

Beginning in 1492, Michelangelo did most of his dissections at the Monastery of Santo Spirito to whose prior, Fra Niccolo Bichiellini, he made the gift of a wooden crucifix (7). According to both Vasari and Condivi, one of the foremost anatomists of the Renaissance, Matteo Realdo Colombo (1516-1559), was a close Michelangelo's acquaintance. Condivi stated that at one point, Colombo even sent a corpse to Santa Agata for Michelangelo to dissect. We can only guess as to the depth of Michelangelo's anatomic knowledge because he did not publish any detailed drawings of his cadaver dissections. It is reported that he probably destroyed his numerous anatomic sketches and notes (18). Only a few sketches of his anatomic studies have survived, and these are limited to depictions of musculoskeletal or topographic anatomy (19).

Barring the discovery of additional literary documentation, it is impossible to know exactly how much knowledge of anatomy Michelangelo had (19). Robert Beverly Hale has remarked that anyone today can know as much anatomy as Michelangelo did by studying it for several years and doing a few dissections. Any textbook will quickly provide information that Michelangelo had to work years to get (20). In this context, it is imputed to Michelangelo the phrase: "To paint a fish, buy a fish, to paint a man, dissect a man".

About Michelangelo's achievement there are doubts whether we ascribe the forms in his figures to knowledge of dissection or (what is even less tenable) to expressive and free distortion and invention. Where Michelangelo excelled beyond what we can do was in a quality so simple it escapes the sieves of scholarship: the ability to observe. Some authors believe that the anatomic accuracy of his figures is not due to dissection but to observation, and in fact the observation is so penetrating that it blocks attempts to isolate the

other sources of forms, dissection and the Antique. His works exhibit anatomical details that are unobservable upon dissection, like the very important class of cases in which surface contours are produced by active muscles. Since the forms are forms of life, visible on the living body, they are not a closed subject for specialists, but are open to anyone as a new way to approach and appreciate his achievement. Attempts to show how much anatomy Michelangelo knew by analyzing individual unlabeled works simply reinforces the conclusion expressed above that observation, not dissection, is responsible for most surface contours (19).

Inspired in the publications of Meshberger (5) and Eknoyan (7), two authors initiated a detailed analysis of each scene of Sistine Chapel Ceiling looking for anatomical figures hidden in the main image (21). The authors believe that Michelangelo uses a number of resources to make this intention clear. The code – or set of iconographic and pictorial clues – is present not only inside the scene, but also in its adornments and they indicate some aspects that must be observed inside the scenes: the position of the figures, which often indicates the part of the body that Michelangelo "camouflaged" at some other point (in most cases, in the folds of the garments); the direction they are looking to; the "movement" of the hands, which often points to the hidden anatomical part; the region of the body that is painted in lighter shades. The deciphered code is present in each of the thirty-two scenes analyzed by authors (21). Specifically in the scene of Prophet Jonah, although not maintained on the original painting, a draft reveals the genital focus (Figure-2).

Some critiques believe that the references to the studies of anatomy of Michelangelo, emphasized by the authors, although needed for the argument, are not enough to sustain it (22). Besides, there is not a characterization of the period in question that supports the interpretations offered in order to approximate the culture of the Renaissance to documents as texts and drawings from Michelangelo's contemporary artists. This approximation becomes necessary to be able to find the cognitive style of an epoch. As the Michelangelo's frescoes in Sistine Chapel are anatomical tridimensional forms, many, according to the angle which are seen, serve both to interpretations of the authors as to many others, according to good will and creativity of the viewer, which in some cases, have to be extraordinarily strong. Thus, according to critiques, there would be no reason to think that in a series of frescoes with a distinctly theological plane, Michelangelo represented anatomical forms (22).

Nephrologists, of course, may be especially apt to see kidney shapes. Similarly, neurologists tend to see the shape of a human brain (5). In summary, it seems that Michelangelo's creative depictions of human body allow for physicians from varied specialties to identify with different aspects of his work.

Regarding scientific and subjective criteria adopted in this study rational, considering the circumstances in which Michelangelo have painted the ceiling of the Sistine Chapel, subjectivity was fundamental due to religious motivations added to the vigorous implications of a limited scientific knowledge typical of that era.

On the other hand, if this is the best one can do when studying art and history in a reality far away along the time line, it is not a strong enough motivation to ignore or to do not reflect over the important creations of the remote past. Furthermore, noting Santayana's sentiments "those who forget history are condemned to repeat it". And given the Michelangelo reputation and the importance of his works we are privileged to be able to access lots of credible documents.

The study does not have the pretension to definitively prove our hypotheses, which may be impossible considering the age and circumstances of the facts discussed. However, we consider the presented manuscript a hypothesis generating. In this context, hopefully, astute observations (even historical) can add to our current understandings; they exercise shrewd observation skills and a curious analytical mind to understand unexplained features of a given circumstance, providing the bases and rational for future researches that will lead to "intelligent design" and evidence-based behavior.

It is clear that this study is beyond the science and aggregates art and history and since art interpretation is subjective, the quest will doubtless

continue (23). As mentioned by Suk and Tamargo (6) at the conclusion of their article "In art history, there are few opinions that stand undisputed, and most are sustained by either circumstantial evidence or simply by the cumulative analyses of observers, because artists do not issue their works with an explanatory text".

CONCLUSIONS

This description helps to confirm the relationship of the Renaissance artist and his relationship with the human anatomy; science has been much studied in this period. We reveal the presence of the design of a cross section of the penis with the two cavernous bodies with the septum between them and the spongy body, in the ceiling of the Sistine Chapel painted by Michelangelo Buonarroti between the years 1508 and 1512.

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CONFLICT OF INTEREST

None declared.

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EDITORIAL COMMENT

It is well documented that there have always been a close and subjective relationship between science, medicine and art, especially during the Renaissance. This can be evidenced in many of Michelangelo's frescoes, such as in "Creation of Adam" and "Separation of Light From Darkness", all of them painted on the ceiling of the Sistine Chapel.

Analyzing "human history archives" retrospectively, it is evident that medical sciences did not become an object of devotion in a short period of time. Approximately 2.500 years have certainly passed until Medicine becomes a recognized and respectful science, as it is seen nowadays. Not surprisingly, many individuals from different ethnic origins and characteristics have had a relevant, irrelevant and even a detrimental role in developing the knowledge we see in actual medical textbooks. It is also important to highlight that many individuals have sacrificed their own lives towards this knowledge.

During this period, Hippocrates, in Ancient Greece, Galeno, during Roman Empire and Ibn Sina (Avicena) during Middle Ages, have developed the understanding of human physiological functions based only on morphological aspects. In Ancient Times, surgery and dissections were considered a drastic intervention and they were only limited to human surfaces. The advent of Scientific Revolution, commonly dated from Renaissance or 17th century, has certainly played an important role in developing more rational human thoughts against the magical and miraculous ones, although these thoughts were still limited to the existing knowledge. It is interesting to observe that it is exactly in this period of history that the objective and discussion of this article is based on.

The authors' detailed analysis of a possible design of a cross-section of the penis in "Prophet Jonah" fresco on the Sistine Chapel, created by the Italian Renaissance artist Michelangelo Buonarroti, is obviously a subjective and hypothetical interpretation. However, the nature of art interpretation and creativity is definitely subjective and maybe this is the exact point that turns art into an admirable and astonishing subject. In this regard, one of the ways that best summarizes the interpretation link between art and science subjectivity, as can be evidently observed in the current paper, is this famous Hippocrates quote "Life is short, the art long, opportunity fleeting, experiment treacherous, and judgment difficult".

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ORIGINAL ARTICLE

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Robotic-assisted laparoscopic radical cystectomy: Surgical and oncological outcomes

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ABSTRACT

Purpose: Our first 91 consecutive cases undergoing a robotic assisted cystectomy were analyzed regarding perioperative outcomes, pathological stages and surgical complications.
Materials and Methods: Between 2007 and 2010 a total of 91 patients (76 male and 15 female), 86 with clinically localized bladder cancer and 5 with non-urothelial tumors underwent a radical robotic assisted cystectomy. We analyzed the perioperative factors, length of hospital stay, pathological outcomes and complication rates.
Results: Mean age was 65.6 years (range 28 to 82). Among the 91 patients, 68 were submitted to an ileal conduit and 23 to a neobladder procedure for urinary diversion. Mean operating time was 412 min (range: 243-618 min.) and mean blood loss was 294 mL (range: 50-2000 mL). In 29% of the cases with urothelial carcinoma the T-stage was pT1 or less, 38% were pT2; 26% and 7% were classified as pT3 and pT4, respectively. 14% of cases had lymph node positive disease. Mean number of lymph nodes removed was 15 (range 4 to 33). Positive surgical margins occurred in 2 cases (2.1%). Mean days to flatus were 2.13, bowel movement 2.88 and inpatient stay 18.8 (range: 10-33). There were 45 postoperative complications with 11% major (Clavien grade 3 or higher). At a mean follow-up of 15 months 10 patients had disease recurrence and 6 died of the disease.
Conclusions: Our experience demonstrates that robotic assisted radical cystectomies for the treatment of bladder cancers seems to be very promising regarding surgical and oncological outcomes.

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Robotics; cystectomy; bladder; carcinoma

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INTRODUCTION

Robotic-assisted laparoscopic radical cystectomy has become a surgical option for patients with bladder cancer, providing the benefits of minimal invasive surgery with lower blood loss, early return of bowel function and more rapid patient recovery, while apparently maintaining functional and oncological outcomes (1-6). The surgical and perioperative outcomes of initial reports appear to be comparable to the open approach. However, larger experiences are required to adequately evaluate and validate this procedure as an appropriate surgical and oncological method for patients with bladder cancer. We report our initial experience with robotic assisted laparoscopic radical cystectomy, evaluating the perioperative and pathological outcomes of this procedure.

MATERIALS AND METHODS

All patients underwent preoperative laboratory studies and imaging (chest x-ray and abdominal/pelvic cross-sectional imaging). In all urothelial carcinoma cases a transurethral bladder tumor resection was performed preoperatively. No patients received preoperative neoadjuvant chemotherapy. Table-1 lists patient characteristics.

Table 1 - Patient characteristics.

Mean Age (range)	65.6 (28-82)
Nº gender	
Male	76
Female	15
Mean body mass index (kg/m^2)	26.7
Mean American Society of Anesthesiologists score	2.21
Nº clinical stage	
cT1 or less	37
cT2	46
cT3-T4	3
Other tumors	5

All patients underwent robotic assisted laparoscopic radical cystectomy using the da Vinci® surgical system. Three surgeons, versed in robotic assisted laparoscopic radical prostatectomies, but neither experienced in laparoscopy nor trained in robotic assisted laparoscopy for radical cystectomies, performed the operations. The robotic-assisted laparoscopic approach was used for the cystoprostatectomy and bilateral pelvic lymph node dissection portions of the procedure and for pre-placing urethral anastomotic stitches in orthotopic neobladder cases. After completing the extirpative portion of the procedure the robot was undocked and urinary diversion (ileal conduit or orthotopic ileal neobladder) was performed via a 5 to 8 cm lower midline incision. The decision as to which urinary diversion would be performed was taken beforehand and individualized, depending on patient age and preferences. The nerve sparing procedure was performed with titanium clips and cold scissor dissection in a manner similar to that used for robotic prostatectomy, as previously described by Menon et al. (7). The robotic assisted laparoscopic pelvic lymphadenectomy involved lymph nodes up to the level of the common iliac vessels (8).

After surgery, all patients were taken to the urology intermediate care unit and underwent routine postoperative support, which included nasogastric tube removal immediately after surgery, prokinetic agents, nonnarcotic analgesics and early diet advancement.

In this series, which presents the learning curve at our center, we evaluated operative variables such as total surgical time (cystectomy, bilateral pelvic lymphadenectomy and urinary diversion) and estimated blood loss. We included aspects of hospital recovery such as time to flatus, bowel movement and hospital discharge including the 30-day complication rate. Furthermore, we analyzed pathological outcomes such as pathological stage, margin status, bladder entry and the number of lymph nodes removed.

Complications were measured using the Clavien classification system (9). This system is well known in the general surgery and urological literature, and helps to report complications across different institutions (10-11).

RESULTS

The mean age of patients undergoing the robotic procedure was 65.6 years (range: 28-82 years) with a male to female ratio of 5:1. The mean American Society of Anesthesiologists score (ASA) was 2.21. The majority of cases (46) were clinical stage T2, followed by cT1 or less (37) and finally cT3 or cT4 (3). The mean clinical follow-up was 15 months (range: 3-44).

In terms of perioperative outcome mean estimated blood loss was 294 mL (range: 50-2000 mL) and mean overall operating time was 265 min. (range: 243-618 min).

Mean time to flatus and bowel movement was 2.13 days (range: 1-6 days) and 2.88 days (range: 1-7 days), respectively. Pertaining to the date of discharge, 27 patients (29.6%) were discharged between the 9th and 13th postoperative day (POD), 28 (30.8%) were discharged between the 14th and 18th POD, 17 (18.8%) between the 18th and 22nd POD and 19 (20.8%) were discharged after the 22nd POD. The mean inpatient stay was 18.8 days (range: 10-33). In total, 60% of patients were discharged less than 18 days after surgery. This extended time of stay can be explained by the fact that, as with conventional open cystectomy patients, these patients were only discharged after all catheters, including ureteral stents, had been removed. This usually took place on the 10th and 11th POD. Therefore, the earliest possible discharge of patients with an ileal conduit was on the 12th POD. Patients with an orthotopic neobladder remained in hospital until removal of the indwelling catheter, which usually took place on the 21st POD. Most patients were also given the option of staying in hospital for a few extra days if they had a strong preference of being discharged directly into a rehabilitation program without prior return to home. As so few postoperative complications were seen in the first patients, the current trend in our center is to decrease the inpatient hospital stay. Table-2 shows the above discussed parameters.

Table-3 lists pathological outcomes. An inadvertent bladder entry did not occur at all. Organ confined disease was found in 67% of patients. In 29% of the cases with urothelial carcinoma, the T-stage was pT1 or less, 38% were pT2, and 26% and 7% were classified as pT3 and pT4, respectively. Two patients (2.1%) had positive surgical margins. In one case, the positive surgical margin was discovered postoperatively in the urethra after an intraoperative frozen section described it to be negative. The other patient had a known pT4 prostate cancer that was previously been operated, without successful removal of the primary tumor.

Lymph node positive disease was diagnosed in 14% of the cases. A mean of 15 lymph nodes (range 1 to 33) were removed with a standard dissection up to the bifurcation of the common iliac vessels.

In the early postoperative period (less than 30 days after surgery) 45 patients had complications, including postoperative bleeding, rehospitalization for nausea/ vomiting, ileus, febrile urinary tract infections, deep vein thrombosis, cardiopulmonary complications and occlusion of the neobladder. However, only 10 patients (11%) had major complications (Clavien Grade III or higher) that needed invasive treatment. One of those patients died a few days after surgery due to an uncontrollable epileptic attack, complicated by cardiac failure.

The 30 day-readmission rate for this case series was 10.9% (10 of 91 patients).

Table 2 - Surgical and pathological outcomes.

Nº diversion type (%)	
Conduit	68 (74.7)
Neobladder	23 (25.3)
Mean time of surgery (min) (range)	265 (243-618)
Pathological findings (n (%))	
(Urothelial carcinoma n = 86)	
pT1 or less	25 (29)
pT2	33 (38)
pT3	22 (26)
pT4	6 (7)
Nº removed lymph nodes (range)	14.48 (1-33)
Nº positive lymph nodes (%)	13 (14)
Nº positive surgical margins (%)	2 (2.1)
Mean days to flatus (range)	2.13 (1-6)
Mean days to bowel movement (range)	2.88 (1-7)
Mean days to discharge home (range)	18.8 (10-33)

Table 3 - Postoperative Clavien complications.

Nº patient with postoperative complications (%)	45 (49.4)
Nº patient with major complications (Clavien 3 or higher) (%)	10 (10.9)
Complications after Clavien classification	
I	14 (15.3)
II	21 (23.1)
III	6 (6.6)
IV	3 (3.3)
V	1 (1.1)

There were 7 patients (7.6%) with evidence of recurrence of the disease.

During follow-up 5 patients died of advanced urothelial carcinoma and 1 died of other causes, thus making the overall survival rate of 93.4% and a disease specific survival rate of 94%.

DISCUSSION

Our first experiences in the robotic approach for laparoscopic radical cystectomies demonstrate that the pathological results and desired oncological outcomes could be achieved. It has been postulated that lymph node count and positive soft tissue margins might serve as measures of surgical quality for cystectomy. Experts have recommended that a lymph node yield greater than 10 and a positive surgical margin rate of less than 10% (some even say less than 5%) are relevant indices of quality and adequacy (1,12-14). In our study, a mean number of 15 lymph nodes were removed and two patients (2.1%) had positive surgical margins. In a recent comparison of open and robotic cystectomy by Ng et al., the robotic surgery was associated with decreased blood loss, equal mean operative time, and an equal positive surgical margin rate and lymph node yield (7.2% and 17.9 nodes, respectively) (15). Haber and Gill reported a 5% positive surgical margin rate, a mean lymph node yield of 14 nodes and 92% 5-year cancer specific survival in 37 patients undergoing laparoscopic radical cystectomy (16). As such, the current report adds to a growing body of literature suggesting that in appropriately selected patients minimally invasive radical cystectomy offers equivalent operative and pathological outcomes, and an acceptable intermediate term oncological efficacy.

With regards to perioperative outcomes a mean robotic operating time of 265 min is comparable with the results published in other studies (1-6,17-20).

Similarly, we described a low surgical blood loss that contributes to a faster postsurgical recovery as was demonstrated in other robotic cystectomy reports (1-6,17-20).

In addition, postoperative outcomes, including time to flatus and to bowel movement are also favorable in our experience. Our study is limited with regards to the time period till discharge. As previously discussed, this has to do with the fact that patients are discharged after the removal of all the catheters and that the patients can remain in hospital until they are admitted to a rehabilitation center. The longer hospital stay can therefore not be used as a benchmark of the patients' general postoperative health or status.

We used the Clavien system of complication assessment and observed an overall complication rate of 49.4%, with 11% of patients having a major complication (i.e. Clavien grade 3 or higher). Nevertheless, the rates and magnitude of these complications where on a par with those in well established open cystectomy literature (21-23).

This report has several important and noteworthy limitations. It fails to answer the question of whether robotic surgery is superior to conventional open surgery. In addition, this study does not evaluate long-term cancer related outcomes, e.g. 2-year and preferably 5-year results that remain the true benchmarks of oncological efficacy. Despite this, it is a relatively large case series examining the perioperative and pathological results of robotic surgery.

CONCLUSIONS

Our experience with robotic-assisted laparoscopic radical cystectomy shows acceptable operative, pathological and short-term clinical outcomes. This suggests that robotic radical cystectomy has similar short-term cancer control and complication rates, less operative time and blood loss and earlier return to bowel function than laparoscopic or open radical cystectomy.

Certainly larger series are required to adequately evaluate and validate this procedure as an appropriate surgical and oncological option for patients with bladder cancer. Attention should however be drawn to the fact that these were the first cases ever performed at our center and certainly do include a form of learning curve. Considering this fact, the future of robotic assisted laparoscopic radical cystectomies seems extremely promising.

CONFLICT OF INTEREST

None declared.

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Laparoscopic Radical Cystectomy: a 5-year review of a single institute's operative data and complications and a systematic review of the literature

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ABSTRACT

Objective: We aim to evaluate our experience and results with laparoscopic radical cystectomy and conduct a systematic review of studies reporting on 50 or more procedures.
Materials and Methods: Between February 2006 and March 2011, a prospective study in a single institute on patients with bladder cancer who underwent laparoscopic radical cystectomy was conducted.
A search of the Cochrane Library, PubMed, Medline, and Scopus databases was conducted for studies reporting on 50 or more laparoscopic radical cystectomy procedures to compare with our results.
Results: Sixty men and five women underwent laparoscopic radical cystectomy during the 5-year study period. Thirty-nine patients were submitted to ileal conduits, 24 to neobladders, and two patients to ureterocutaneostomies. The mean operative time was 294 ± 27 minutes, the mean blood loss was 249.69 ± 95.59 millilitres, the mean length of hospital stay was 9.42 ± 2 days, the mean morphine requirement was 3.69 ± 0.8 days.
The overall complication rate was 44.6% (29/65). However, the majority of the patients with complications (90% (26/29)) had minor complications treated conservatively with no further surgical intervention needed.
The literature search found seven studies, which reported on their institutions' laparoscopic radical cystectomy results of 50 or more patients. Generally, our results were similar to other reported studies of the same calibre.
Conclusion: Laparoscopic radical cystectomy is a safe and efficient modality of treatment of bladder cancer. However, it comes with a steep learning curve, once overcome, can provide an alternative to open radical cystectomy.

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INTRODUCTION

The gold standard for treatment of localized muscle-invasive, bladder cancer is open radical cystectomy (ORC) (1,2). However, in the turn of the century, numerous centres have adopted a more minimally invasive approach (1-3). In 1993, de Badajoz et al. performed the first laparoscopic radical cystectomy (LRC) which is thought to lead to a faster recovery, shorter hospital stay, decreased

morbidity, and more rapid return to daily activities, in addition to maintaining the same functional and oncological outcomes as ORC (1,2,4). However, despite the success numerous centres have had with LRC, it remains a matter of debate as there is no head to head trial comparing the long term outcomes and oncological results between the two modalities (1-3,5).

As it stands, almost 50% of patients who undergo ORC will have a tumour recurrence, which lead to the mortality of many of these patients, while the 5-year tumour recurrence survival rates ranges between 73 and 89% in node negative organ confined disease, 45-55% with extravesical disease, and 25-35% with nodal involvement (1-4). To try to improve the survival, different centres have adopted an extended pelvic lymph node dissection (PLND) approach (1). This has also found its way in to the laparoscopic procedure; however, no long-term results are available.

The complication rate of ORC is in the range of 40-65% with a transfusion requirement of around 66%, while the major complication rates range between 10-12% and a mortality of 2-3% (1,4). LRC can also provide an advantage of less blood loss, analgesic requirement, reduced scarring, and less complications (2,6). Despite these advantages, LRC is a technically challenging procedure that requires a high level of laparoscopic skills and has a long learning curve (1,2). However, numerous reports have emerged showing success with the procedure in addition to success with urinary diversion methods (2,5). Centres have attempted both intra and extracorporal urine diversion with good results (2,5,7).

To this end, we report our 5-year experience of a single surgeon's LRC with PLND and urinary diversion operative results and complications and compare it to those of similar published data.

MATERIALS AND METHODS

Patients

Between February 2006 and March 2011, all patients with pathologically confirmed bladder cancer who underwent laparoscopic radical cystectomy and pelvic lymph node dissection and urinary diversion were included. The indications for radical cystectomy were patients who had muscle invasive disease, high-grade disease, or recurrent non-muscle invasive disease, i.e. CIS. Patients who refused laparoscopic cystectomy, patients with severe cardio-respiratory disease, and patients with metastatic disease were excluded. An experienced laparoscopic surgeon performed the LRC procedure. The data for all the patients were inputted prospectively into a database. All patients had a post-transurethral resection of bladder tumour (TURBT) pathologically proven and a staging computed tomography (CT) scan proven organ confined T2N0M0 bladder cancer disease.

Operative Procedure

The LRC procedure is started by establishing a pneumoperitoneum and the insertion of two 5 mm and three 10 mm trocars. We use the ligadure system, harmonic scalpel, bipolar scissors, metal and plastic clips to dissect the tissue and ligation of vessels. After identifying the anatomy, we start with dissection of the seminal vesicles and posterior surface of the prostate. After dissection of the Retzius space, we incise of the pelvic fascia, dissect the prostate apex and dissect the urethra. Obturator, external, internal, common iliac, presacral, para-aortic and paracaval lymph nodes are all dissected for pathological analysis. The specimen is removed in a silicon bag. In females, the procedure is started by dissection of the uterus ligaments and peritoneum in the Douglas cavity. Bladder with the urethra, uterus, adnexa with anterior vaginal wall and lymph nodes are removed transvaginally. Urinary diversions are performed via a minilaparotomy technique with the left ureter carried on to the right side under the sigmoid colon mesentery. Ileal neobladders are formed according to the technique described by Studer et al. (8). A 14F drain is left in the abdominal cavity after surgery.

Outcome Measures and Analysis

The outcome measures evaluated the patients' demographics, cystectomy pathology grading, operative time, conversion rate, blood loss, transfusion rate, urinary diversion method, morphine analgesic requirement, length of hospital stay, and complication rates.

The complications were classified according to the Clavien classification (CC) of surgical complications (9). We considered CC I and II as minor complications and CC III and above as major.

All CT scans were reported by experienced uro-radiologists and all specimens were analysed by experienced pathologists.

Follow-up

Patients were seen in clinic one month postoperatively, then every three months for the first year, then every six months for the next year, then on a yearly basis. Follow-up investigations consisted of transabdominal ultrasound, CT and LAB Tests.

Learning Curve

To analyse the learning curve for the procedure, a comparison was conducted between the first half and the second half of the cohort. The parameters compared were the demographics, operative time, blood loss, conversion rate, length of hospital stay, and the complications. For dichotomous data, a Mantel-Haenszel Chi square was used for statistical analysis and for continuous data, an Inverse variance analysis was conducted. A P value of < 0.05 was considered statistically significant.

Review of the Literature

A literature search for publications reporting on LRC was conducted in January 2012. The Cochrane Library, PubMed, Medline, and Scopus databases were searched. Terms used included 'laparoscopic radical cystectomy', 'laparoscopic cystectomy', 'radical cystectomy', and 'cystectomy'. Only studies reporting on 50 or more cases were included; furthermore, studies comparing LRC to other procedures were not included to allow 'like-for-like' comparison of the literature.

RESULTS

Patient Data

During the 5-year period, 60 men and 5 women were included, with a mean age of 59 (± 7.89) years (Table-1). The 91% of the patients were smokers; with an average body mass index (BMI) of 27.45, and 17% had previous operations (Table-1). The majority of the patients (84.6%) had a TURBT pathology result of G2pT2, while the remaining had G3pT2 bladder cancers all of which were N0M0.

Postoperative Data

The mean operating time was 294 minutes, with four patients requiring open conversions due to technical problems due to the high BMI, in addition to no significant progress in the surgery because of clinical underestimation of the stage. Seven patients required blood transfusions and each received two units. The mean intra-operative blood loss was 249 mL. All patients had extended lymph node dissection with an average lymph node yield of 18. The mean length of hospital stay was 9 days with an average morphine requirement of 3.7 days.

Sixty percent of the patients underwent an ileal conduit diversion, while 36.9% were submitted to neobladders , and 3.1% to ureterocutaneostomies. All patients with neobladders remained continent with a median of 18 months (range 1-48 months) post-operative follow-up.

Complications

There were 9 intra-operative complications which were managed during the procedure and did not have any effect on the post-operative recovery (Table-2). In total, 44.6% (29/65) of the patients developed post-operative complications. Among them, the majority were CC I (22/29), while 4 were CC II, and 3 were CC V. In total, 40% (26/65) developed minor complications and 4.6% (3/65) developed major complications.

Pathological Data

The pathological staging post cystectomy is on Table-1. There were no distant metastases in any of the patients. However, 14 patients had positive lymph nodes and received adjuvant chemotherapy with gemzar and cisplatinum (Table-1). All the specimens had negative margins.

Nerve-sparing Data

Only three patients underwent nerve-sparing surgery, of which two had full erections within 3 months of their surgery and the third had full erection 6 months post-operatively. However, all 3 needed the use of tadalafil initially.

Table 1 - Patient Demographics and Operative Parameters.

Parameter		Median (range)
Sex (M:F)	60:5	
Previous Operations	16.9% (11/65)	
BMI (mean ± SD)	27.45 ± 2.2	28 (23-32)
Smokers	90.8% (59/65)	
TURBT Path:		
G2	84.6% (55/65)	
G3	15.4% (10/65)	
Operative Time (minutes (mean ± SD))	294 ± 27	290 (240-340)
Blood Loss (mL (mean ± SD))	249.69 ± 95.59	210 (170-500)
Extended LND	65	
LN yield	17.97 ± 3.49	
Urinary Diversion:		
Ileal Conduit	39	
Neobladder	24	
Ureterocutaneostomy	2	
Length of hospital stay	9.42 ± 2	9 (7-18)
Morphine Requirement (days (mean ±))	3.69 ± 0.8	4 (2-6)
Cystectomy Path:		
G2pT2b	38	
G2pT3a	8	
G2pT3a N1	1	
G2pT3b N1	6	
G3pT3a	5	
G3pT2b N1	1	
G3pT3a N1	4	
G3pT3b N1	1	
G3pT4a N1	1	

Table 2 - Complications.

Intraoperative injuries:	
Bowel	4
Rectal	3
Vascular	2
Postoperative complications:	
Sepsis	3
Urine Retention	1
Bleeding	6
Thromboembolism	4
Neurologic	3
Muscular	1
Ileus	10
Cardiac	1
Ureteral stenosis	12
Urine Leak	3
Lymph leak	3
C-Classification per Patient:	
I	22
II	4
IIIa	0
IIIb	0 (3)
IVa	0
IVb	0
V	3

Learning Curve

Table-3 depicts the comparison of group 1 and 2, which represent the first half and the second half of the cohort respectively. There was no difference between the two groups regarding sex, age, BMI, or previous operations. Group 2 had a significantly reduced operative time (p = 0.002), however no statistical significance was found regarding blood loss, conversion rates, length of hospital stay, or complication rates (Table-3).

Literature Search

Figure-1 depicts the flowchart of the article selection process. All articles excluded were due to either non-relevance to the aim of this review or reported on less than 50 LRC. Seven articles were included, their results are depicted on Table-4 (10-17). No studies were found describing the learning curve for laparoscopic cystectomy procedures.

DISCUSSION

Laparoscopic surgery has advanced considerably in the last decade, extending its use even to difficult prolonged procedures such as radical cystectomies. Since the first LRC was described, numerous centres have published their centres experience with the procedure (18). However, only a handful of centres have published data on more than 50 procedures (Table-4). However, ORC remains the gold standard of treatment for select bladder cancer cases and LRC is quickly emerging as a viable and safe alternative.

We present our data on our 5 year experience with LRC all performed by one surgeon. Our data suggests that localized muscle invasive bladder cancer can be successfully treated laparoscopicly; however, there does seem to be a steep learning curve. We were able to achieve operating times, intra-operative blood loss, and length of hospital stays in par with the largest published series and better results with others. Despite this, we had a high overall complication rate of 44.6% (29/65). However, if it is take into consideration the severity of the complications, about 90% (26/29) of the patients that developed complications were treated conservatively with no further surgical intervention required and were classified as Clavien classification I and II. The remaining three patients, representing 10% of those that developed complications and 4.6% of the overall patient cohort, required re-operations. Two of these patients were re-operated due to ileal leakage due to tight anastomosis and one due to mechanical

Table 3 - Comparison between the initial and final cohort of patients.

Parameter	Group 1	Group 2	P value
Sex (M:F)	36:1	34:4	P = 0.21
Age	59 ± 8.67	59 ± 7.2	P = 0.99
BMI	27.25 ± 1.88	27.64 ± 2.49	P = 0.44
Previous Operations	6/37	5/38	P = 0.71
Operative Time (minutes (mean ± SD))	303 ± 28	285 ± 22.93	P = 0.002
Blood Loss (mL (mean ± SD))	259.69 ± 102.2	240 ± 89.2	P = 0.37
Conversions	4/37	0/38	P = 0.12
Length of hospital stay	9.38 ± 20.9	9.45 ± 1.99	P = 0.88
Complications	15/37	14/38	P = 0.74
Clavien I & II	13/37	13/38	P = 0.93
Clavien > III	2/37	1/38	P = 0.55

obstruction. All three patients developed severe sepsis and died.

Although no statistical significance was found regarding most of the outcomes (Table-3), Figure-2 depicts the complications that occurred with a comparison to the number of procedures conducted in each year. The figure clearly shows that the number of procedures is on an upwards increasing slope, while the complications are relatively plateauing. Furthermore, it is worth mentioning that the two patients that had tight anastomosis and died were amongst the first quarter of the patient cohort operated on. This analysis shows that complications decreases as the number of patients increase.

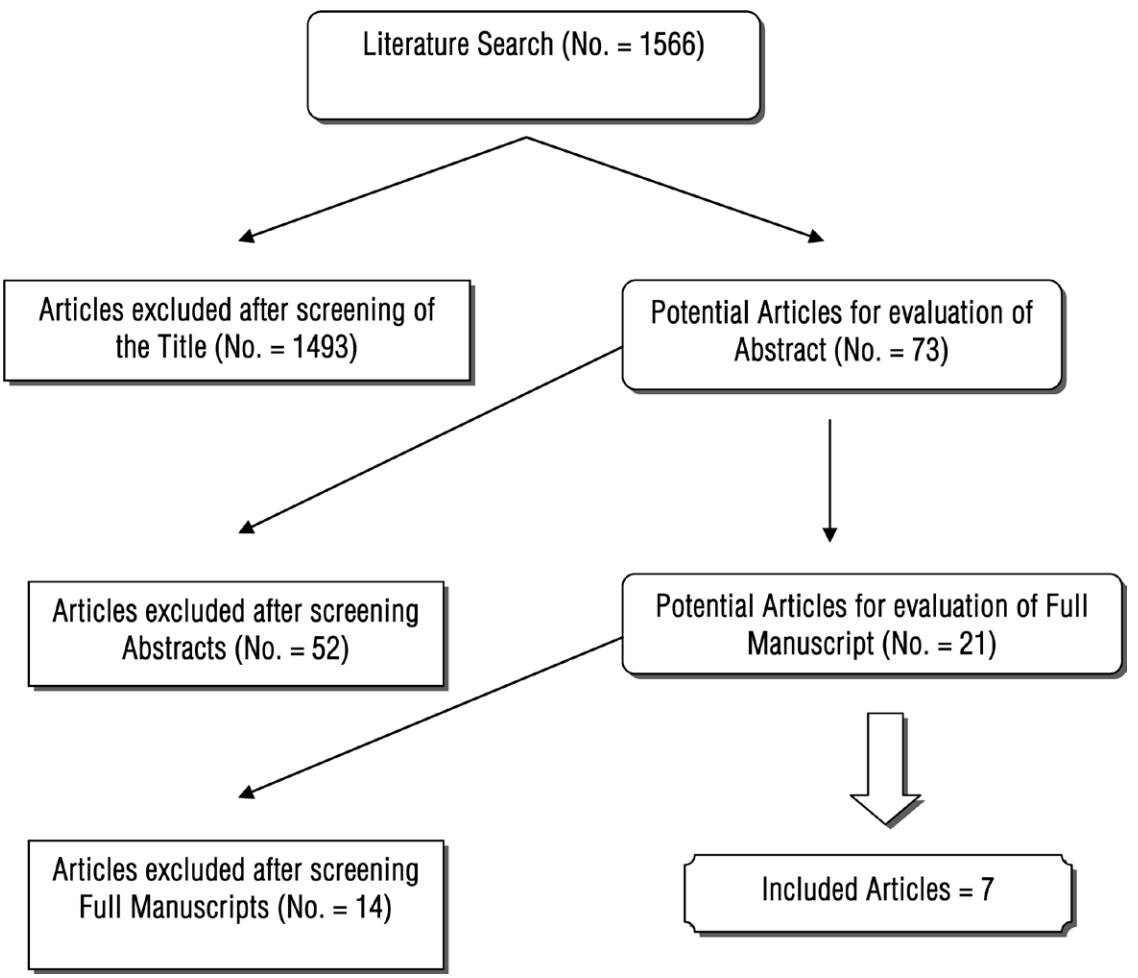
Despite this, the major complication rate of 4.6% in our study is comparable to previous studies and remains less than that of ORC procedures (Table-4) (1,4,17,19). In a comparative analysis of 50 patients in each the LRC and ORC groups, Haber et al. reported that LRC had a minor complication rate of 18% while ORC had 22%. Furthermore, they reported that LRC had a major complication rate of 8% compared to 6% of that of ORC. However, neither comparison reached statistical significance.

Huang et al. reported the largest series to date, where 171 patients underwent LRC. Their median operative time was 325 minutes with a median blood loss of 270 mL. Their study was the only other study to classify their complications into the Clavien classification. They reported a total complication rate of 39.2% (67/171), with 6.4% (11/171) CC I complications, 19.3% (33/171) CC II complications, and 13.5% (23/171) CC III complications.

Interestingly, these results matched the same author's initial results of 85 patients, where their overall complication rate was 14.1% as opposed to their larger report with 39.2% complication rate, with similar operative times, blood loos, and post-operative hospital stay.

Castello et al. data from their initial report and their follow-up report with 59 and 85 patients respectively showed that there seems to be an improvement in their operative time and a decrease in the complication rates (Table-1). However, all

Figure 1 - Flowchart for literature review.



studies reported more complications in patients who had neobladders, as compared to those with ileal conduits. This abode the same for our cohort, as more complications were noted in patients who were submitted to neobladders as opposed to those with conduits. This would explain why Huang et al. have a higher complication rate than other studies, as they only performed neobladder procedures.

It is evident that there is a variation between the studies regarding operative times and blood loss, as well as complication rates. This not only portrays the varying expertise of the surgeons and centres, but also emphasizes that there is no standardize operative procedure and techniques; furthermore, there is no consensus on which urinary diversion technique is best suited for laparoscopic surgery.

Though Castello et al. started their study in 1997 and published their work up until 2005, this period remains short with inadequate data to be able to compare between the oncological results of LRC to those of the open counterpart. A systematic review conducted by Chade et al., which included 19 studies comparing ORC to LRC as well as robotic

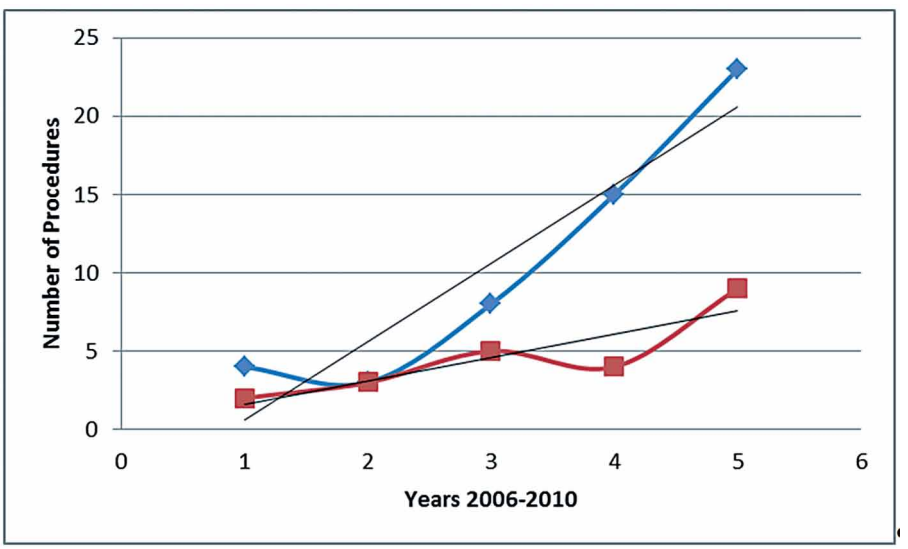
Table 4 - Literature Search.

Study	Number of Patients	Sex M:F	Operative Time	Blood Loss	Urinary Diversion	Conversion	LoHS	Morphine use	Complications
Cathelineau 2005 (14)	84	71:13	median 280 minutes (215-330)	median 550 (150-2000)	Ileal Conduit: 33 Neobladder: 51	0	median 12 days (8-31)	58% required for 1 day	UTI: 1 Haematoma: 3 Fistula: 2 PE: 1 Pyelonephritis: 1 Death: 0 Complication rate: 18%
Castillo 2006 (16)	59	46:13	mean 337 (150-600)	mean 488 (50-1500)	Ileal Conduit: 30 Neobladder: 25 Indiana Pouch: 4	1	NA	NA	Intraoperative: 7 (vascular injury) UTI: 3 Pneumonia: 1 Wound infection: 1 Ileus: 5 Lymph leak: 2 Fistula: 3 Hernias: 2 Bowel obstruction: 1 Death: 2 Complication rate: 42%
Sighinolfi 2007 (13)	83		mean 520 (264-760)	mean (376 (200-600)	Ileal Conduit: 43 Neobladder: 26 Ureterocutaneos-tomy: 14	2	mean 13.2 (8-19)	NA	Urine leakage: 1 Fistula: 1 Atrium Rupture: 1 Death: 1
Huang 2008 (11)	85	77:8	median 320 (210-605)	median 280 (50-1000)	Neobladder: 85	0	median 14	NA	Strictures: 4 Fistula: 3 Ileus: 3 Pneumonia: 1 Pyelonephritis: 1 Complication rate: 14.1%
Haber 2008 (17)	50	NA	mean 6.3 ± 0.26	mean 363 ± 259	NA	NA	mean 8 ± 3.2	NA	Minor complications 18% Major complications 8%

Castillo 2009 (15)	85	57:19	mean 279 (180-375)	mean 436 (50-1500)	Ileal Conduit: 24 Neobladder: 42 Indiana Pouches: 10 Mainz II Pouches: 9	0	Mean 8.8 (4-28)	0	Peri-operative: (5 vascular, 2 evisceration, 2 sepsis) Late: UTI: 3 Stenosis: 1 Reservoir rupture: 2 Mesenteric Ischemia: 1 Death: 1 Complication Rate: 20%
Huang 2010 (12)	171	151:20	median 325 (210-605)	median 270 (50-1000)	Neobladder: 171	0	mean 13.1 (7-46)	NA	Early (23%): Incisional haematoma: 1 Wound infection: 2 Ileus: 5 DVT: 1 Deliruim and agitation: 2 Lymph Leak: 11 Pneumonia: 1 Pyelonephritis: 1 Vesico-urethral leak: 7 Pouch-vaginal Fistula: 2 Uretero-pouch stricture: 2 Colon-pouch fistula: 1 Ileal anastomotic leak: 1 Ileo-pouch fistula: 2 Mesenteric vein thrombosis: 1 Late (15.2%): Adhesive Ileus: 3 Urine Retention: 4 Pouchitis: 5 Pouch stone: 2 Uretero pouch stricture: 9 Vesico-urethral stricture: 3

LoHS: Length of Hospital Stay, DVT: deep vein thrombosis, NA: not available, PE: Pulmonary Embolism, UTI: Urinary tract infection.

Figure 2 - Yearly depiction of number of procedures.



radical cystectomy (RRC), was not able to show superiority of one modality to the other (3). Furthermore, they stipulate that despite the select cohort of patients that were included in the LRC and RRC groups compared to the non-selective ORC group, they were unable to see a clear impact regarding oncological outcomes. However, they do mention that the LRC and RRC results are encouraging and that continual follow-up of these patients will allow an improved statistical analysis. Haber et al. reported that though LRC was significantly longer than ORC, it had significantly less blood loss and transfusion requirement, however no other difference was found between the two procedures (17).

Bladder cancer management has a narrow opportunity for effective cancer control, as the outcomes are highly dependent on the radical cystectomy, in addition to a lack of salvage procedures for recurrence, which emphasizes on the need for more adequate precise resection during the initial operation (3). What is evident is that with time and improved skills and experience of surgeons, LRC might replace ORC. With the emergence of robotics, this procedure is rapidly replacing laparoscopy due to its shorter learning curve and at least similar if not better operative and post-operative results. In a comparative analysis between LRC and RRC, Abraham et al. found that both procedures could be performed safely without compromising the oncological results. Furthermore, they found that RRC has a shorter learning curve, less blood loss, complications, and earlier return of bowel function (20). Furthermore, the only randomised trial found in the literature comparing robotic radical cystectomy to open radical cystectomy showed that RRC had significantly improved outcomes regarding blood loss, operative time, analgesic use, with no difference regarding complications, length of hospital stay or pathological outcome (21). Despite these studies, a larger multi-institutional analysis comparing laparoscopic, open, and robotic radical cystectomy is needed to establish superiority of one procedure over the others.

Therefore, time will tell how far minimally invasive procedures will progress; until then, ORC still remains gold standard, and in experienced hands can have a low blood loss as well as a short hospital stay in par with LRC or RRC.

CONCLUSION

LRC is safe and efficient modality of treatment of bladder cancer that can be add to the urologist's arsenal of treatment options. Though there comes with it, a steep learning curve, once overcome, can provide an alternative to ORC. LRC though comes with a prolonged operative time, can also benefit from minimal blood loss as well as a complication rate similar to ORC if not better. However, longer follow-ups and larger cohort studies are needed to further evaluate its precise benefits over ORC and LRC is finding its way into many centres and time will tell of its full potential.

CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Collagen I and III and metalloproteinase gene and protein expression in prostate cancer in relation to Gleason score

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ABSTRACT

Purpose: To evaluate if the expression of metalloproteinase, collagen I and III are related to Gleason score, preoperative PSA and pathological stage in prostate cancer.

Materials and Methods: Our study group included radical prostatectomy specimens of 33 patients with prostatic adenocarcinoma who underwent surgery from 2001 to 2009. Patients were divided into 3 groups: Gleason score=6 (13 patients), Gleason score=7 (10 patients), Gleason score≥8 (10 patients). The control group included prostates of patients submitted to cystoprostatectomy and benign prostatic tissues adjacent to the cancer area. Specific areas of tissues were selected under microscope and further processed for collagen I and III analysis by real time PCR. In addition, 10 deparaffined sections of each group were used to evaluate collagen I, III and metalloproteinase immune expression. The results were correlated with Gleason score, preoperative PSA and pathological stage.

Results: We found significant difference in both collagen I and III gene expression between benign and tumoral areas in the prostate samples from Gleason score=6 (collagen I=0.4±0.2 vs 5±2.4, $p<0.05$; collagen III=0.2±0.06 vs 0.7±0.1, $p<0.05$) and Gleason score≥8 (collagen I=8±3.4 vs 1.4±0.8, $p<0.07$; collagen III=1.8±0.5 vs 0.6±0.1, $p<0.05$). There was no correlation of collagen expression with Gleason score, preoperative PSA or pathological stage. There was a positive correlation between metalloproteinase expression and Gleason score ($r^2=0.47$).

Conclusions: The positive correlation between metalloproteinase expression and Gleason score suggests that metalloproteinase could be a promising factor to improve Gleason score evaluation. Its expression and regulation do not seem to be related with collagen degradation.

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Prostate cancer;
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INTRODUCTION

Prostate cancer is by far the most frequently diagnosed cancer in American males and the second leading cause of cancer deaths in that population. In Brazil, prostate cancer is also the second cause of death in males, being the most frequent cancer after skin tumors. Thus, the progression of prostate cancer from histologic cancer to clinically detectable and metastasizing cancer is of utmost importance.

Histologically, the prostatic acinus is lined with secretory (typically cuboidal to columnar) luminal epithelial cells and a discontinuous layer of basal cells and is embedded in a fibromuscular stroma (1).

Studies of human breast, colon, and prostatic cancer specimens have identified activated stromal cell phenotypes, modified extracellular matrix (ECM) composition, and increased microvessel density, exhibiting biological markers consistent with what happens in stroma at wound repair site (2-4). Reactive stroma in cancer is composed of fibroblasts, myofibroblasts, endothelial cells, and immune cells. It seems that stromal components play important role in the tumor progression by stimulating the angiogenesis and by promoting cancer cell survival, proliferation, and invasion (5).

All cells included in tumor stroma would potentially affect tumor genesis, however, myofibroblasts are of particular interest. They are activated stromal cells typically found at site of pathologic tissue remodeling (4,6,7). Myofibroblasts in reactive stroma synthesize ECM components such as collagen I, collagen III, fibronectin, tenascin, and versican (8). In addition, myofibroblasts express proteases, fibroblast activation protein (FAP), and matrix metalloproteinases (MMPs). These elements cause remodeling of ECM and basement membrane and can stimulate cancer cell growth and migration (9). Indeed, some MMPs such as type 1, 2 and 9 have been related to prostate cancer (10,11).

The concept that the microenvironment is crucial for the maintenance of cellular functions and tissue integrity suggests that a cancer-induced change in the stroma may contribute to cancer invasion (12). Based on this fact the aim of this study was to evaluate if the expression of metalloproteinase, collagen I and III could be related with Gleason score, PSA and pathological stage.

MATERIAL AND METHODS

Our study group included radical prostatectomy specimens of 33 patients with prostatic adenocarcinoma who underwent radical surgery from 2001 to 2009. Such patients were divided into 3 groups according to the Gleason score: Gleason score = 6 (13 patients), Gleason score = 7 (10 patients), Gleason score ≥ 8 (10 patients). Since the Gleason score is a combination of primary and secondary grades (or patterns) in biopsy or surgical specimen both grades areas were used in all analysis. The prostate from patients with bladder cancer submitted to cystoprostatectomy were used as a control group. Benign prostatic tissues adjacent to the cancer area in the different Gleason grades were also used as a second control group. The Gleason score classification was performed by a same group of pathologists with large experience in prostate cancer. The study was approved by the internal review board of the institution and all patients agreed to sign the informed consent.

The criterion of tumor recurrence was defined as PSA ≥ 0.2 ng/mL according to the AUA recommendation. The postoperative follow up period varied from 1 to 62 months. The mean follow up was 20.5 months and the median was 18.0 months. The age of patients with prostate cancer ranged from 45 to 75 years (median age 63.3), and the age of patients submitted to cystoprostatectomy ranged from between 58 to 75 years (median 67.7 years).

The paraffin-embedded blocks of the different groups were used to determine the expression of collagen I, III and metalloproteinase by immunohistochemistry. The adenocarcinoma and benign areas were selected from tissues under microscope analysis and further used to determine gene expression of collagen I and III by real time PCR.

RNA was extracted using the recoverall total nucleic acid isolation kit (Applied biosystems, Carlsbad, CA, USA) according to the manufacturer's protocol. All samples were treated with DNAase Free reagent (Invitrogen, CA, USA) following the manufacturer's protocol for elimination of residual DNA. Then, a hundred nanograms of RNA were used to evaluate the gene expression of collagen I and III by real time PCR using the Express one-step SYBR GreenER kit (Invitrogen, CA, USA). The reactions were performed in triplicate and normalized by β-actin gene expression. The primer sequences used are described below: Collagen I: sense: gtgctaaaggtgccaatggt; antisense: accaggttcaccgctgttac. Collagen III sense: ctggacctcagggacc; antisense: gttccccaggttttccat. β-actin: Sense: ccagctcaccatggatga; antisense: acgatggaggggaagac.

For immunohistochemistry analysis, 10 deparaffined sections of each group were hydrated, treated with buffer TRIS-EDTA (pH 9.0) overnight at 60°C (collagen I and III) or citrate buffer, pH6.0, for 20 min at 600C (metalloproteinase 13) for antigen retrieval, and then treated with 3% hydrogen peroxide solution in methanol for 10 min to block endogenous peroxidase activity. These steps were followed by washing the sections in PBS and subsequently incubated 10 min at room temperature with 10% goat serum to block unspecific binding. The sections were then incubated for 2h at room temperature with Collagen I and III primary antibodies (Abcam, Cambridge, MA, USA) or overnight at 4°C with metalloproteinase 13 antibody (Millipore, Billerica, MA, USA) diluted in PBS with 1% BSA. Sections were then washed in PBS and incubated at room temperature for 20 min with biotinylated secondary antibody followed by incubation at room temperature for 10 min with streptavidin-peroxidase conjugate (Histostain-Plus Kit, Invitrogen, CA, USA). Sections were washed in PBS, then revealed by treating with liquid diaminobenzidine (Histostain-Plus Kit, Invitrogen, CA, USA), and then counterstained with hematoxylin. The negative controls were processed by replacing the primary antibody with PBS and no indication of staining was observed.

Images were digitized using an Olympus DP70 (12.5 megapixels, Tokyo, Japan) video camera coupled to a BX51 Olympus light microscope (Tokyo, Japan), which transferred all images captured to a microcomputer. The quantitative analysis was performed at a final magnification of 200x using a software Image-Pro Plus (4.5.0.29, Media Cybernetics, Inc, Bethesda, MD, USA) and a technique based on color segmentation (13).

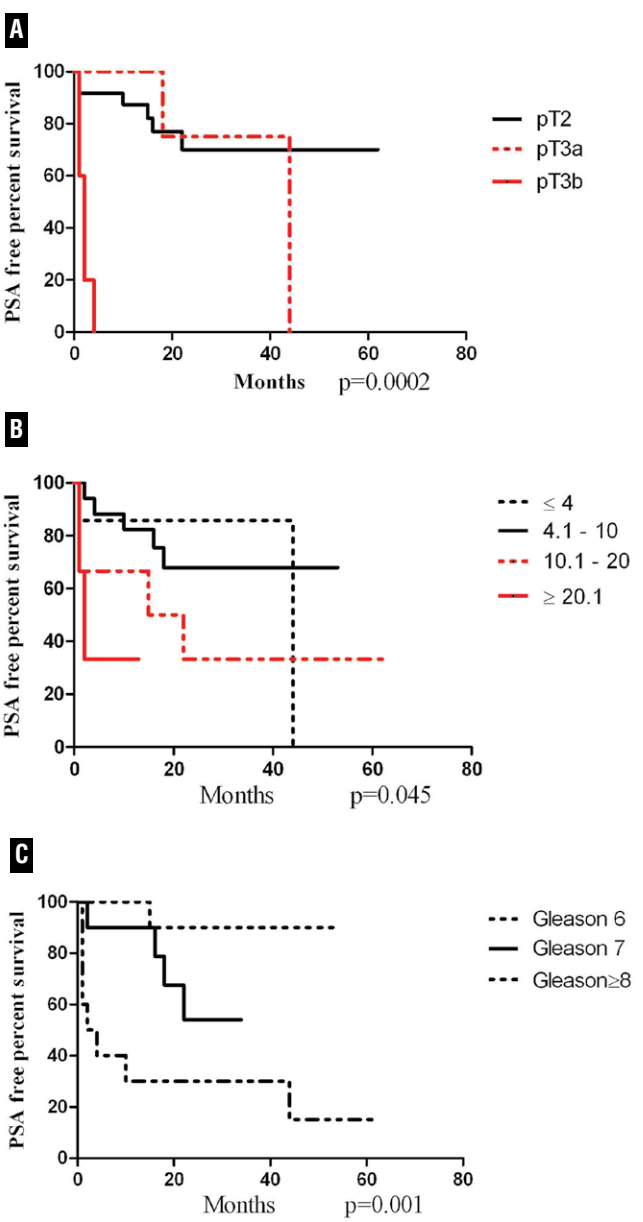
Statistical analysis

The tumor free survival time was determinate by Kaplan-Meier analysis. The odds ratio and its confidence interval of 95% were analyzed for each parameter studied (stage, PSA, Gleason). Statistical significance of gene expression and immunohistochemistry observations were determined by Student's-t-test. Statistical significance among cystoprostatectomy and benign areas from different Gleason scores was determined by ANOVA followed by Newman-Keuls test. All results were presented as mean ± SEM. The level of significance was set at $p<0.05$.

RESULTS

Figure-1 shows the results of PSA free percent survival in relation to pathological stage, preoperative PSA and Gleason score for 5 years.

Figure 1 - Kaplan-Meier disease-free survival curves. Pathological stage (A), PSA (B), Gleason scores (C).



The real time PCR analysis of both collagen I and III gene expression showed that there is a significant difference ($p<0.05$) between tumor and benign areas in the prostate samples from Gleason 6 and ≥ 8. The samples of Gleason 7 showed no statistical difference between the tumor and benign areas (Figure-2). We found no correlation between collagen gene expression and Gleason score in tumor areas (Figure-3).

The immunohistochemistry analysis by color segmentation technique showed that collagen I and III expression was significantly reduced in prostate

Figure 2 - Gene expression of collagen I and III in the benign (white bar) and tumor (black bar) areas of prostate of Gleason score=6 (A,B), Gleason score=7 (C,D) and Gleason score≥8 (E,F). β actin was used as an internal control. Data are represented as means ± SEM. Sample numbers were 13 for Gleason score=6; 10 for Gleason score=7 and 10 for Gleason score≥8. Different letters mean statistical significance evaluated by Student's-t-test.

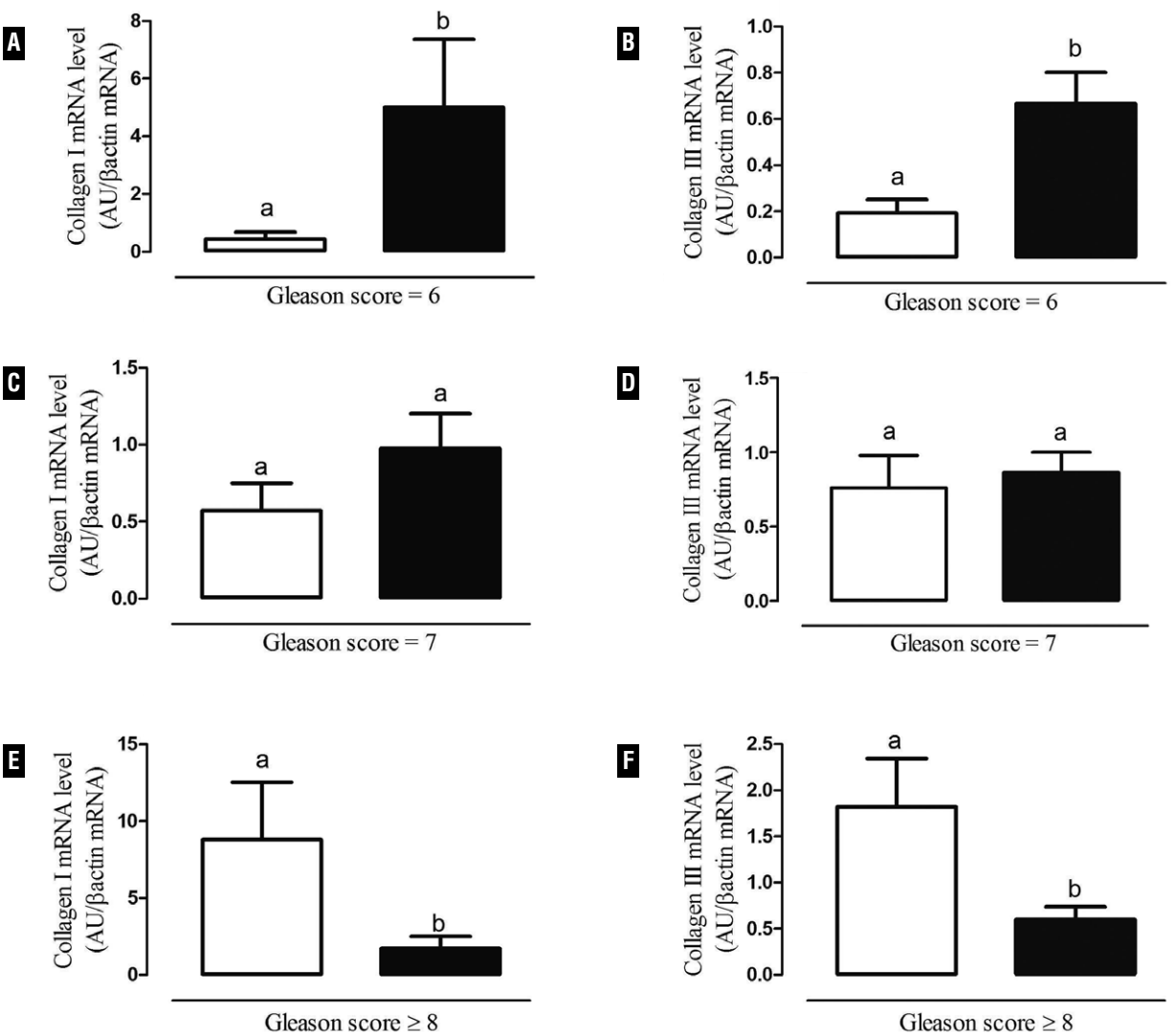
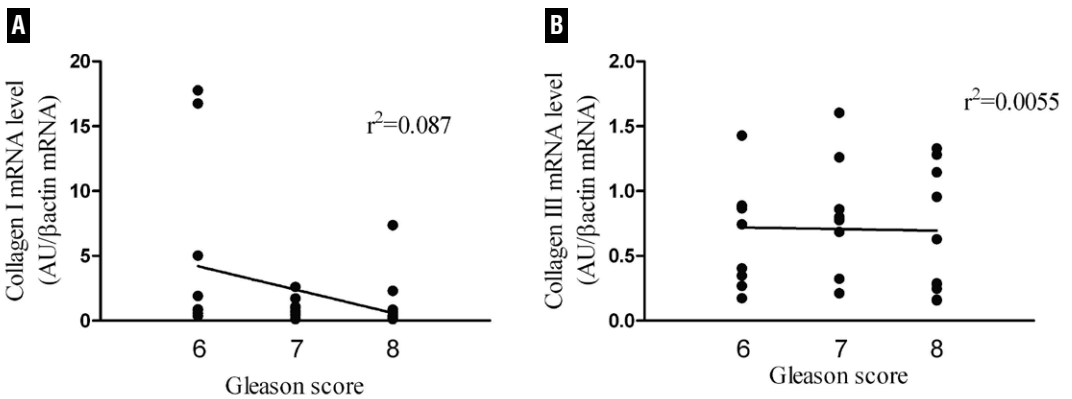


Figure 3 - Linear regression analysis between collagen expression and Gleason score. Collagen I (A) Collagen III (B).



cancer, in all Gleason scores, when compared to benign areas, while metalloproteinase expression was significantly increased (Figure-4). The histological sections are shown in Figures 5, 6 and 7.

There was no correlation on the immunohistochemistry results of collagen I and III in relation to Gleason score in tumor areas. However, there was a positive correlation between metalloproteinase expression and Gleason score (Figure-8).

There was no statistical difference in gene and protein expression of both collagen I and III, as well as in metalloproteinase expression between tissues obtained from benign prostates from cystoprostatectomy and from benign prostatic tissues adjacent to the tumor area (Figure-9). Figure-10 shows that there was no difference or correlation in collagen expression related to pathological stage or preoperative PSA.

DISCUSSION

Since its description in 1966 by Donald Gleason (14), the Gleason grading has remained a cornerstone in the diagnosis and management of prostate cancer. However, the Gleason grading system is certainly not without limitations. A major limitation of the system is poor agreement between Gleason score in biopsies and prostatectomy specimens. In only one-third of the cases such agreement typically exists, with another one-third having a prostatectomy specimen score that is ± 1 the score of a needle biopsy specimen. For the remaining one-third, the difference is 2 or more. Factors that contribute to such discrepancies include tumor heterogeneity, sampling errors, interchangeability and intrachangeability and interpretive errors. Surgical pathologists typically have a tendency to undergraduate the biopsy specimens (15-17). This fact raises the importance of development of new researches to evaluate other methods and factors that could improve the Gleason score diagnosis.

The concept that the microenvironment is crucial for the maintenance of cellular functions and tissue integrity suggests that a cancer-induced change in the stroma may contribute to cancer invasion (12).

It is known that MMPs such as type 1, 2 and 9 have been related to prostate cancer (10,11). However, we have failed to find any previously published results related to collagen gene expression and prostate cancer. Based on the fact that collagen is one of the most abundant and important ECM components (18), the aim of this paper was to evaluate if the expression of metalloproteinase 13, collagen I and III could be related with pathological stage, PSA and Gleason score.

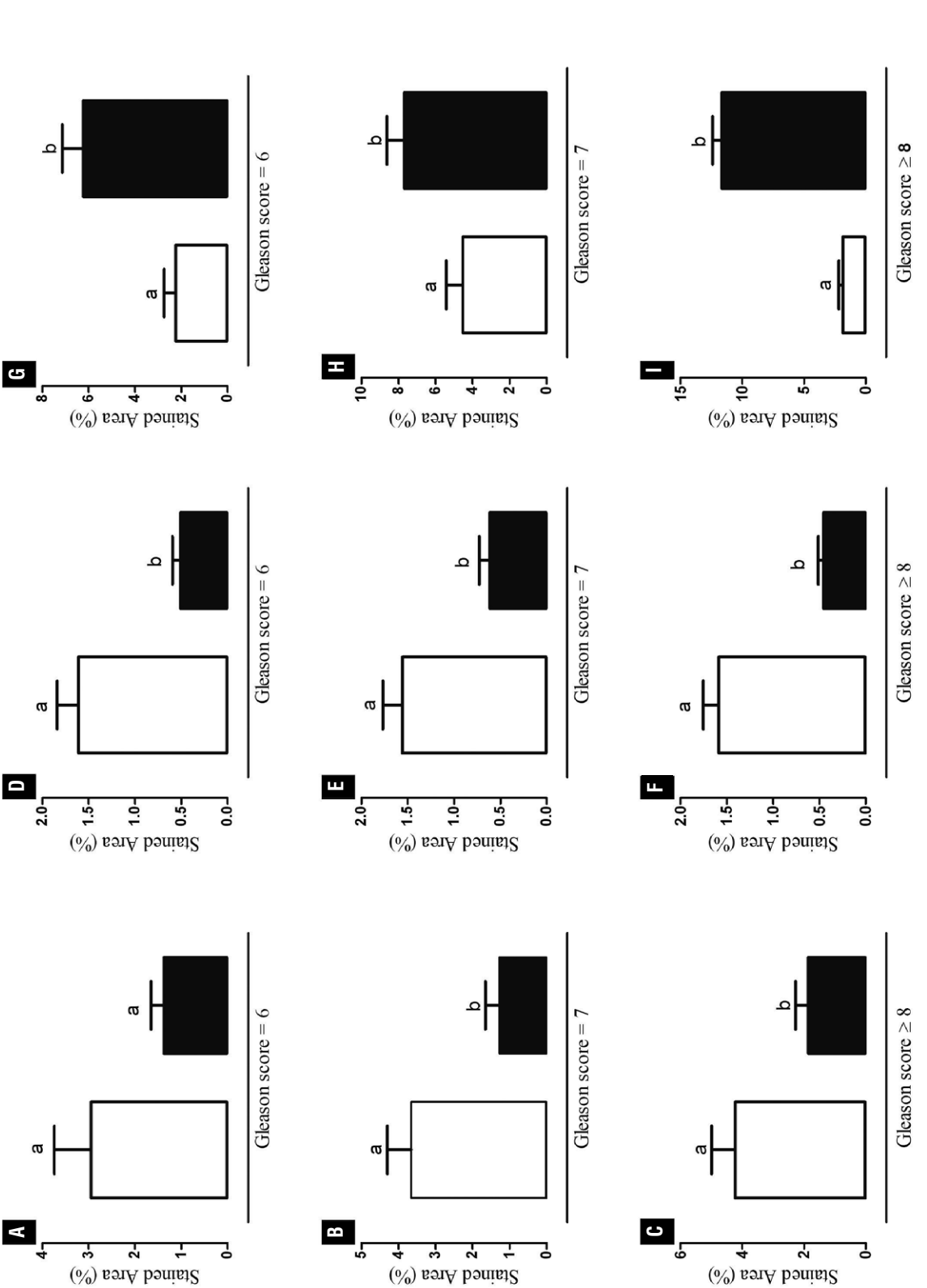


Figure 4 - Immunohistochemistry analysis of collagen I (A,B,C) and III (D,E,F) and metalloproteinase (G,H,I) in the benign (white bar) and tumor (black bar) areas of prostate of Gleason score = 6, 7 ≥ 8. Data are represented as means ± SEM of 10 samples for each group. Different letters mean statistical significance evaluated by Student's-t-test.

Figure 5 - Photomicrographs showing immunohistochemistry for benign and tumor areas for collagen I in prostate of Gleason score ≥ 8 (A,E), Gleason score =7 (B,F) and Gleason score =6 (C,G). Prostate from patients with bladder cancer submitted to cystoprostatectomy (D). Negative controls (H). Final magnification 200x.

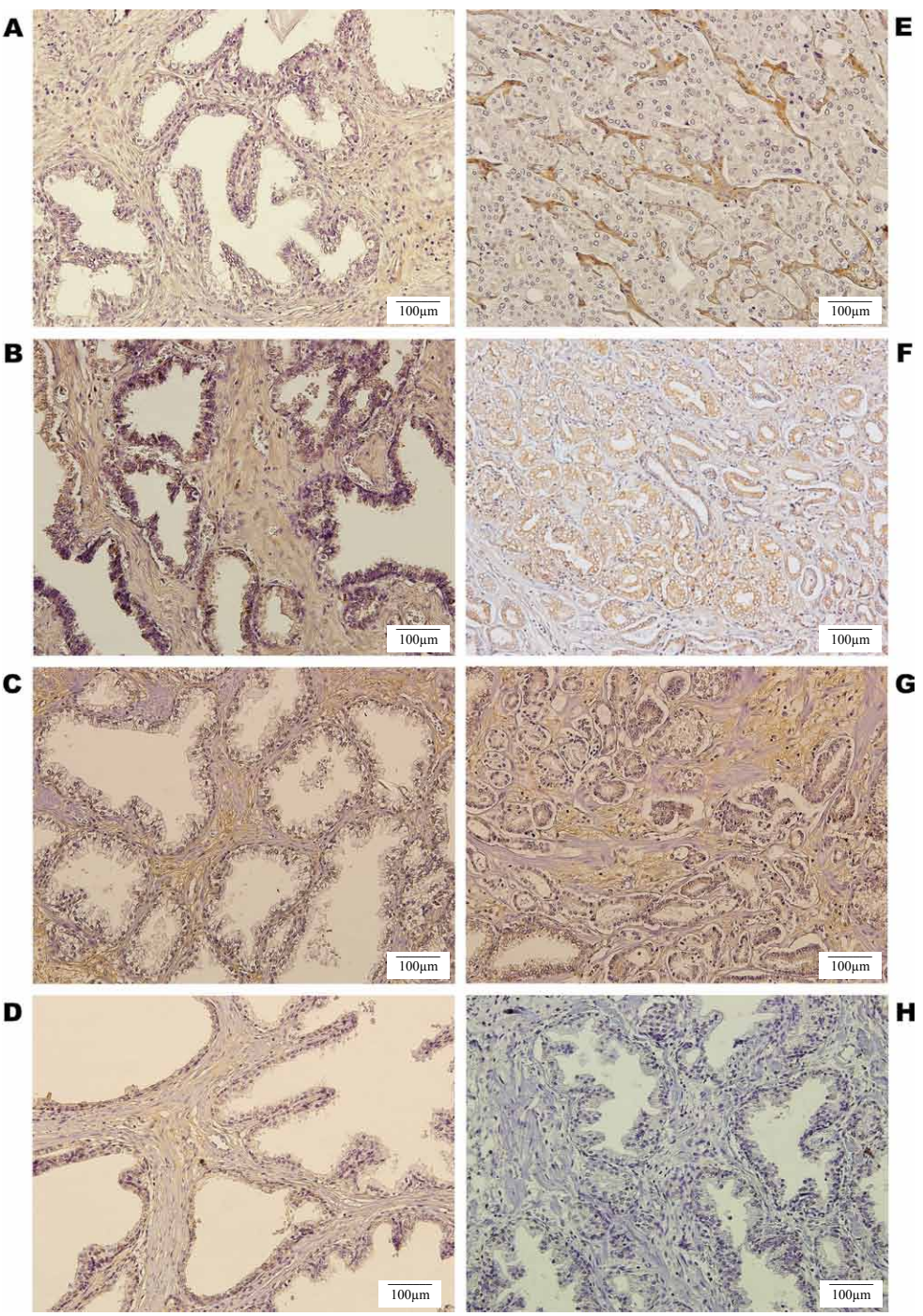


Figure 6 - Photomicrographs showing immunohistochemistry for benign and tumor areas for collagen III in prostate of Gleason score ≥ 8 (A,E), Gleason score =7 (B,F) and Gleason score =6 (C,G). Prostate from patients with bladder cancer submitted to cystoprostatectomy (D). Negative controls (H). Final magnification 200x.

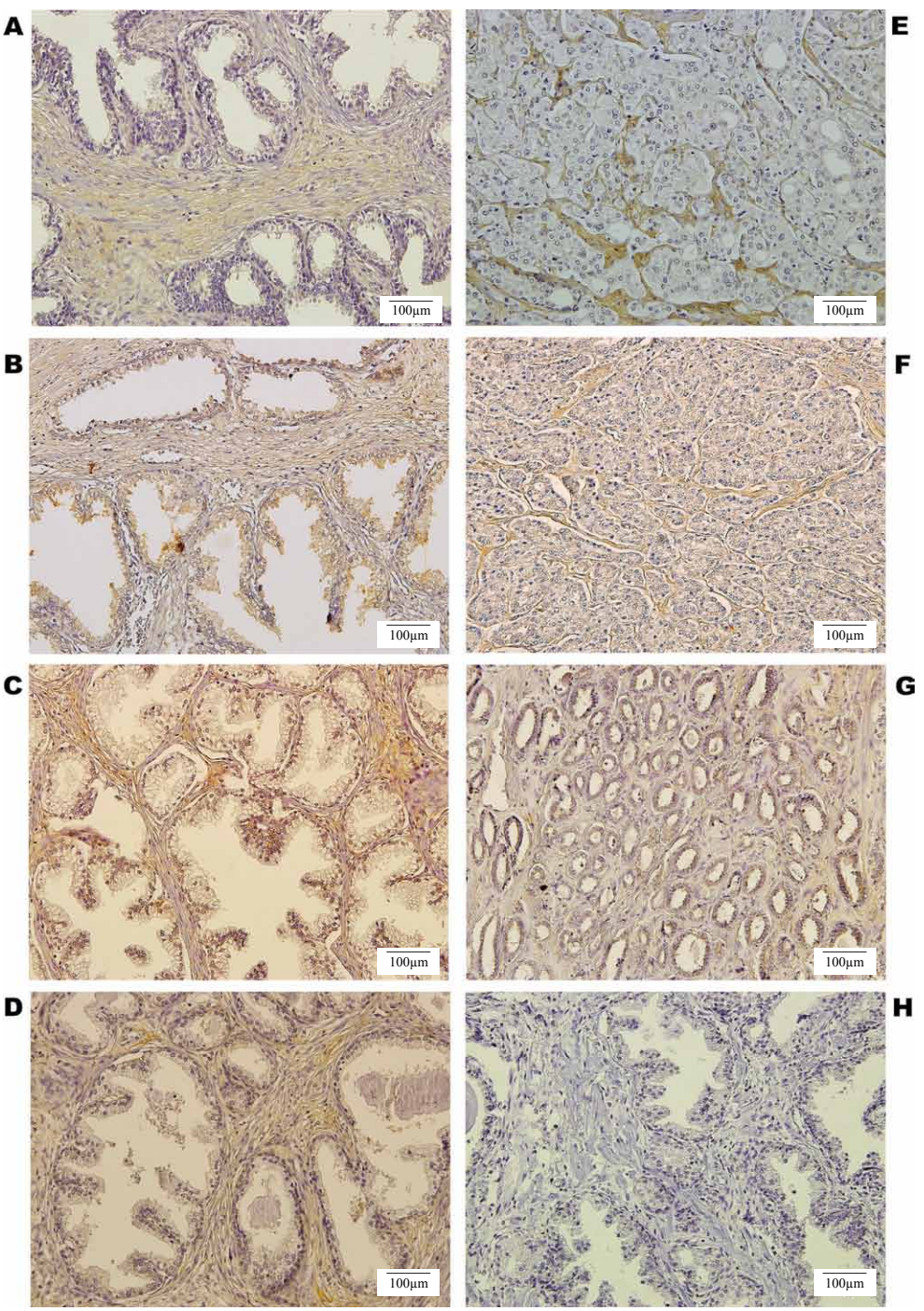


Figure 7 - Photomicrographs showing immunohistochemistry for benign and tumor areas for metalloproteinase in prostate of Gleason score ≥ 8 (A,E), Gleason score =7 (B,F) and Gleason score =6 (C,G). Prostate from patients with bladder cancer submitted to cystoprostatectomy (D). Negative controls (H). Final magnification 200x.

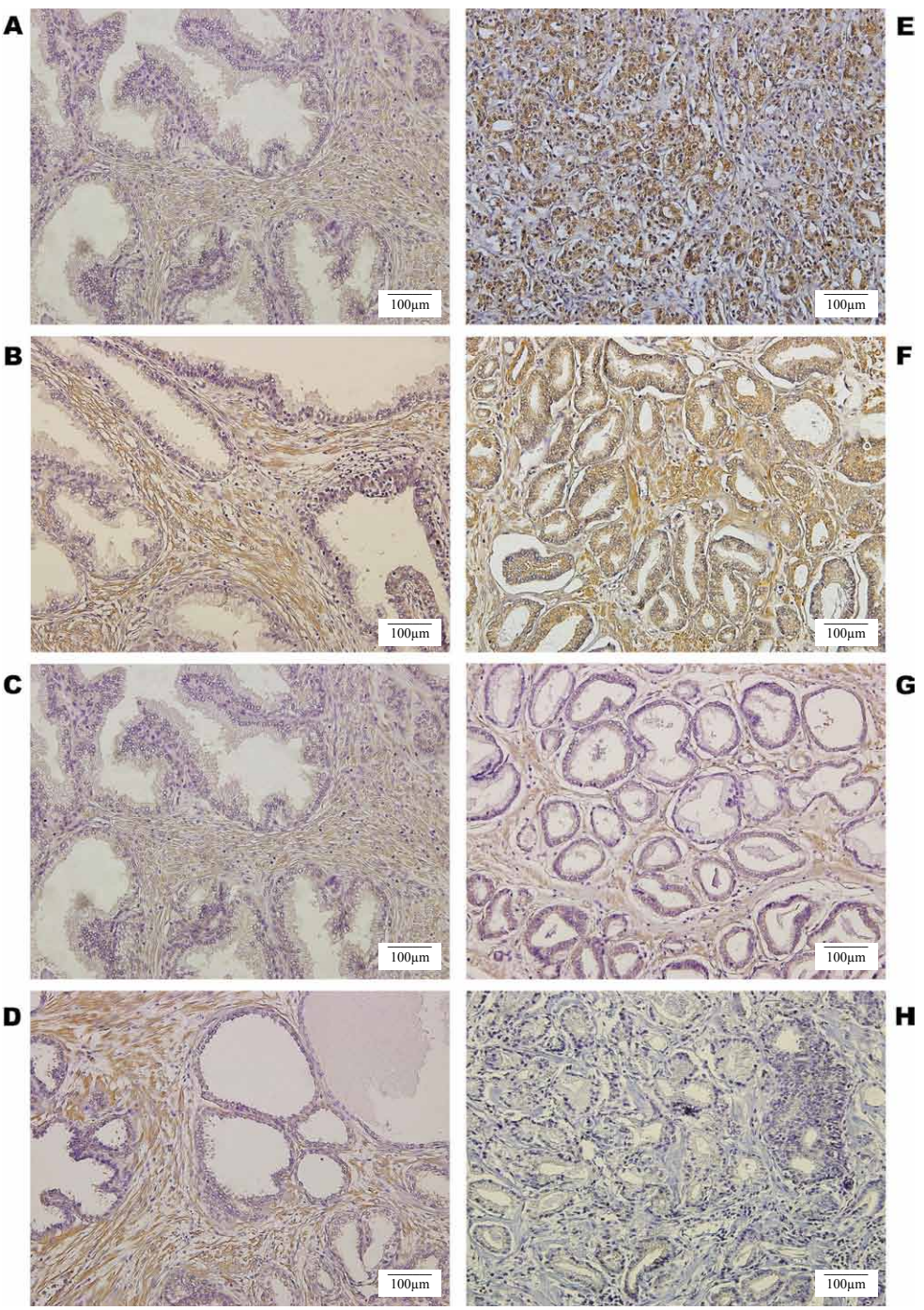


Figure 8 - Linear regression analysis between collagen I (A), III (B) and metalloproteinase (C) expression and Gleason score in tumor areas.

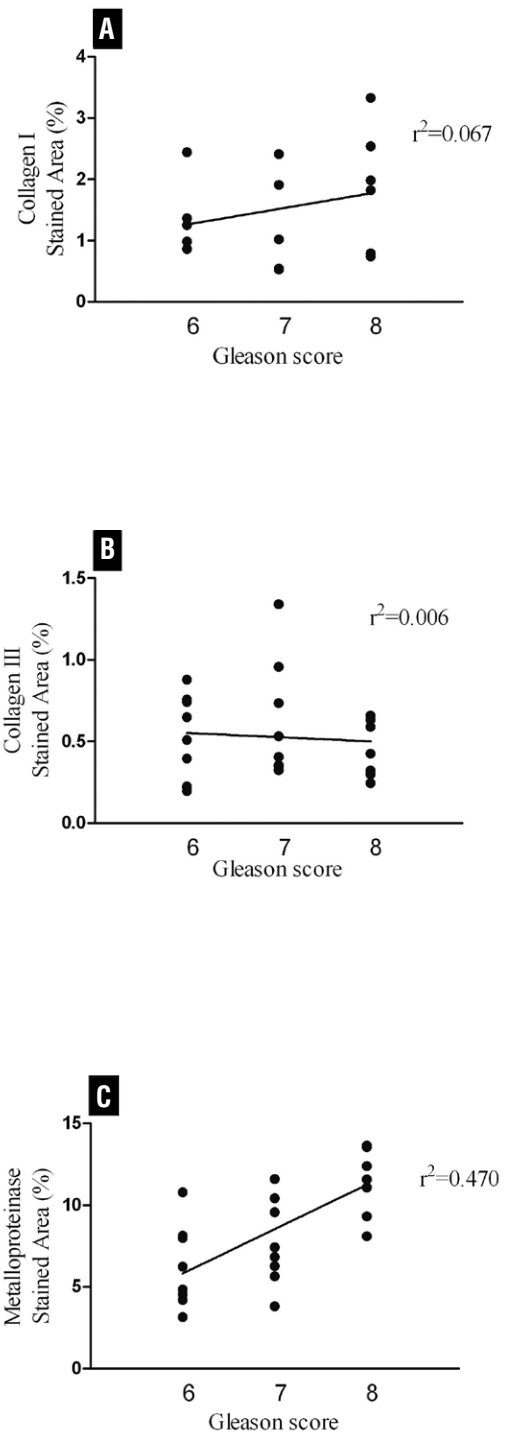


Figure 9 - Gene expression of Collagen I (A) and III (B) in the benign areas of cystoprostatectomy tissues and benign prostatic tissues adjacent to the tumor area in Gleason score=6 , 7, ≥ 8. β Actin was used as an internal control. Sample numbers were 13 for Gleason score=6; 10 for Gleason score=7 and 10 for Gleason score≥8. Protein expression of Collagen I (C), III (D) and Metalloproteinase (E) in the benign areas of cystoprostatectomy tissues and benign prostatic tissues adjacent to the tumor area in Gleason score=6 , 7, ≥ 8. Data are represented as means ± SEM. Statistical significance was determined by ANOVA followed by Newman-Keuls test.

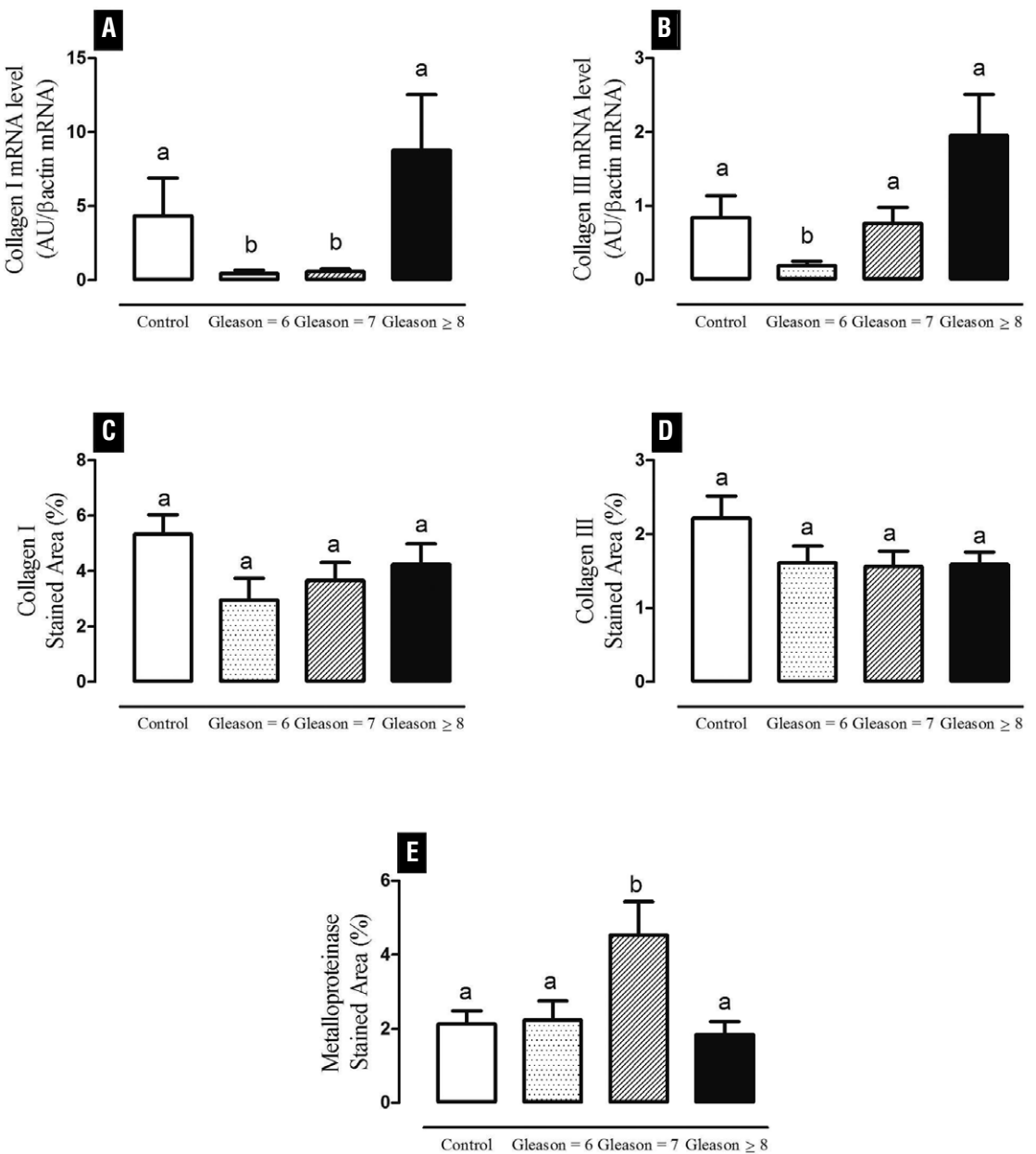
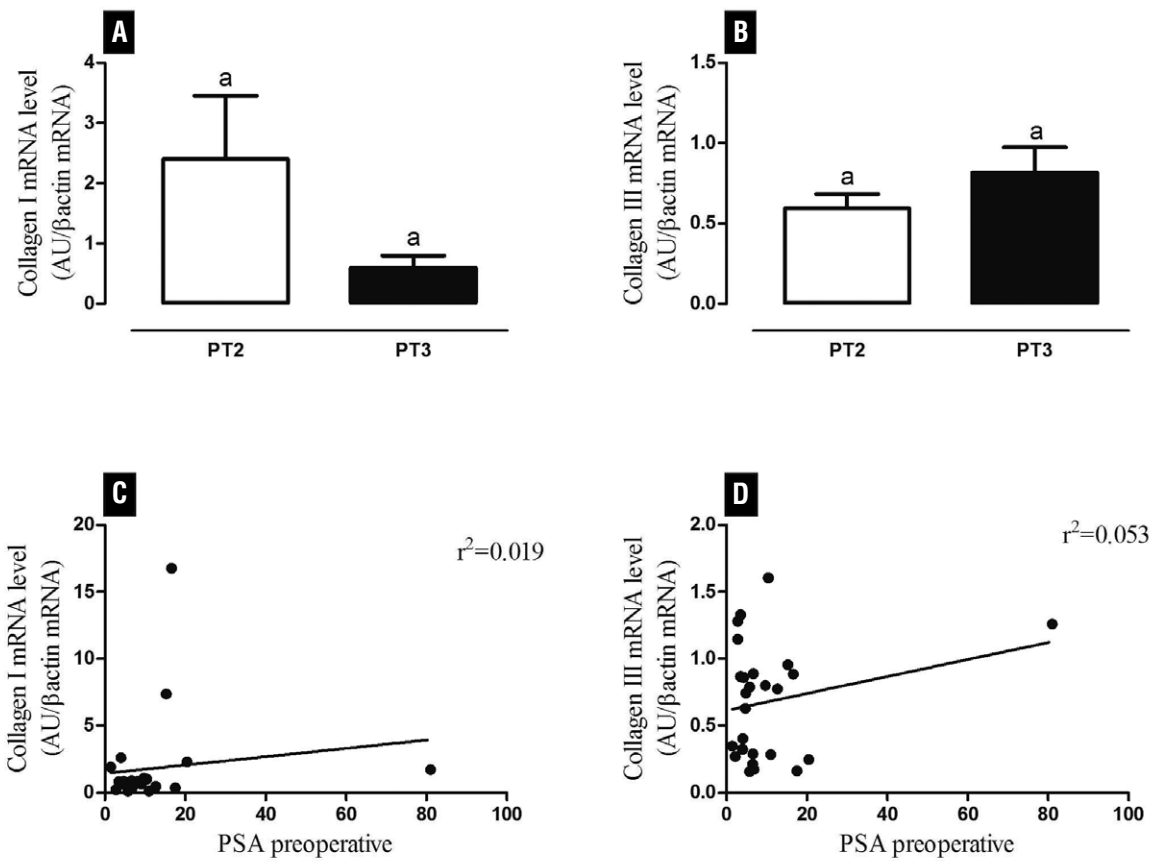


Figure 10 - Collagen I and III expression related to pathological stage (A,B) and Gleason score (C,D). Sample numbers were 13 for Gleason score=6; 10 for Gleason score=7 and 10 for Gleason score≥8. Data are represented as means ± SEM.



The results of long-term biochemical disease-free and cancer specific survival were evaluated in this study in relation to pathological stage, preoperative PSA and Gleason score. Despite the small number of patients, our sample is representative, since our results concerning patients' survival parameters are in agreement with another study from Johns Hopkins that evaluated 2409 patients from 1982 and 1999 (19).

The analysis of collagen I and III gene and protein expression showed different results. We can hypothesize that this discrepancy could be related to gene expression regulation mechanisms at RNA and protein levels. To try to clarify this hypothesis we evaluated the immune expression of metalloproteinase protein that is responsible for ECM remodeling. The opposite results between metalloproteinase and collagen immune expression suggest that the collagen reduction in the tumor area could result from a high metalloproteinase activity. Despite the fact that we failed to find studies that have evaluated the metalloproteinase 13, some papers have shown a high expression of metalloproteinase 1, 2 and 9 in prostate cancer (10, 11).

Another possibility is that since the protein half time life is longer than the gene, we could hypothesize that the results observed in this study for the gene expression more recently occurred in the cells than those of proteins.

Possibly a high metalloproteinase activity in tumor areas of all Gleason score was responsible for decreasing the collagen protein expression, in spite of a different gene regulation. Further experiments using cell culture are in progress to elucidate the gene regulation mechanisms in tumor areas.

Despite the lack of an explanation to the contradictory effects between gene and protein collagen expression, we failed to find other studies that have evaluated the collagen gene expression in prostate cancer with different Gleason scores, which, we believe, raises the importance of this study. We could also hypothesize that the difference in collagen expression between tumor and benign areas could have a functional relevance to the gland, the development and progression of the tumor.

Since there was no correlation between collagen expression and Gleason score at gene or protein level we can assume that collagen can not be used as a prognostic factor to improve Gleason score. However, the metalloproteinase immune expression showed a positive correlation with Gleason score. We strongly believe that the relation coefficient could be increased with the increment of samples. This data corroborate previous studies that evaluated different metalloproteinase proteins in prostate cancer (10,11).

The lack of correlation between collagen and preoperative PSA could be related to the fact that high PSA is not prostate cancer specific. This fact also explains the lack of correlation between collagen and pathological stage since the correlation between Gleason score and pathological stage is well documented in the literature (20).

There is a great disagreement in the literature about the perfect control group to prostate samples. Some papers believe that the control group should be obtained from benign areas from the same patient while others strongly believe that the best control sample should come from a different patient since the adjacent areas could be somehow genetically modified. So, in this paper we used 2 control groups and the results showed that it does not exist a collagen distribution pattern among the different areas. So, we can assume that both areas could be used as a control group at least in relation to collagen expression.

We can conclude that while there is a positive correlation between metalloproteinase expression and Gleason score, this correlation is missed for collagen expression. So, while metalloproteinase may be a prognostic factor due to the association with the Gleason score, its expression and regulation do not seem to be related with collagen degradation.

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CONFLICT OF INTEREST

None declared.

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EDITORIAL COMMENT

Several human cancers may induce a stromal reaction (desmoplasia) as a component of carcinoma progression. In cancers with stromal reaction, it seems that the response is similar, if not identical, to wound repair response. Prostate cancer may also be associated with wound repair type of reactive stroma composed of myofibroblasts and fibroblasts rather than normal prostate smooth muscle, which is displaced by the reactive stroma.

There is growing evidence that carcinogenesis is influenced and controlled by cellular interactions derived from a complex relationship between stromal, epithelial and extracellular matrix components. The neoplastic stromal environment is different from the stroma of the normal tissue and is characterized by modified extracellular matrix composition, increased microvessel density, inflammatory cells and myofibroblasts. Myofibroblasts in reactive stroma

synthesize extracellular matrix components such as collagen I, collagen III, fibronectin, tenascin, and versican. In addition, myofibroblasts express proteases, fibroblast activation protein, and matrix metalloproteinases.

The authors found a positive correlation between metalloproteinase expression and Gleason score. The Gleason grading system is one of the most important prognostic factors in prostate cancer and used all over the world. A revised version of the system was proposed by the International Society of Urological Pathology in 2005 (1). The results of the study favor that immunoexpression of metalloproteinase may be also a prognostic factor in prostate cancer due to the association with the Gleason score.

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ORIGINAL ARTICLE

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Anatrophic Nephrotomy as nephron-sparing approach for complete removal of intraparenchymal renal tumors

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ABSTRACT

Objective: Partial nephrectomy for small kidney tumors has increased in the last decades, and the approach to non-palpable endophytic tumors became a challenge, with larger chances of positive margins or complications. The aim of this study is to describe an alternative nephron-sparing approach for small endophytic kidney tumors through anatrophic nephrotomy.
Patients and Methods: A retrospective analysis of patients undergoing partial nephrectomy at our institution was performed and the subjects with endophytic tumors treated with anatrophic nephrotomy were identified. Patient demographics, perioperative outcomes and oncological results were evaluated.
Results: Among the partial nephrectomies performed for intraparenchymal tumors between 06/2006 and 06/2010, ten patients were submitted to anatrophic nephrotomy. The mean patient age was 42 yrs, and the mean tumor size was 2.3 cm. Mean warm ischemia time was 22.4 min and the histopathological analysis showed 80% of clear cell carcinomas. At a mean follow-up of 36 months, no significant creatinine changes or local or systemic recurrences were observed.
Conclusion: The operative technique described is a safe and effective nephron-sparing option for complete removal of endophytic renal tumors.

ARTICLE INFO

Key words:
Kidney neoplasms; nephrons; Urologic Surgical Procedures; Outcome Assessment (Health Care)

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INTRODUCTION

With the development of imaging diagnosis techniques, and the higher number of requested exams, there has been a growth of the incidence of small renal tumors, and an increase of the indication of nephron-sparing surgery, with excellent oncological and functional outcomes (1). In the last years, the incidental lesions are responsible for more than 60% of kidney tumors and 80% of these are in stages I and II (2).

Nephron-sparing renal surgery has been increasingly used for small renal mass (<4 cm) and, in selected cases, up to 7 cm renal tumors with similar oncological outcomes compared to radical nephrectomy, (3-5) with small complication ratio (6). The 5-year recurrence-free survival is approximately 96% for lesions smaller than 4 cm, and of 83% for lesions from 4 to 7 cm (7).

However, renal occult tumors diagnosed by imaging techniques represent greater technical difficulties for localization and resection, and may

increase the chances of complications (8) (Figures 1A-C). To precisely locate the tumor during surgical resection, intraoperative ultrasound during the operation has been used successfully (9).

The objective of this study is to present an alternative surgical approach for the complete enucleation of endophytic tumors through anatrophic nephrotomy, avoiding radical nephrectomy.

MATERIALS AND METHODS

The authors performed a retrospective analysis of patients undergoing partial nephrectomy at their institution and all subjects with endophytic tumors treated with anatrophic nephrotomy were identified (Figures 2A-D). Patient demographics, perioperative, functional and on-

Figura 1 - (A-C) Intraparenchymal tumors.

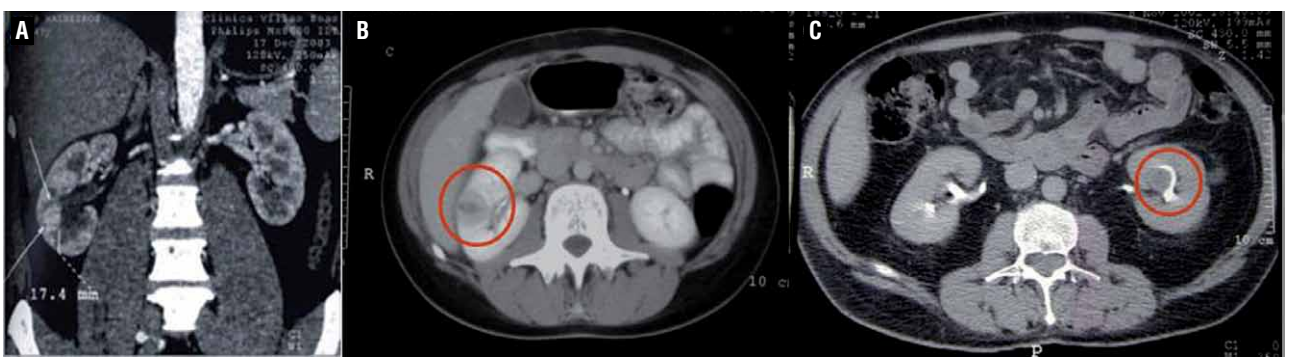
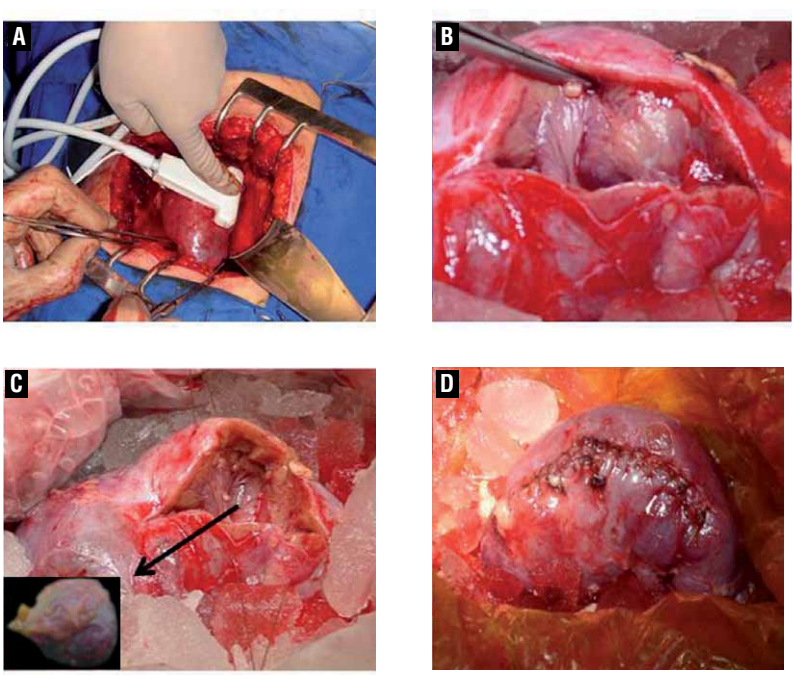


Figure 2 - (A) Identification of the tumor using ultrasound; (B) Brodel's vascular line incision and tumor identification; (C) Image after enucleation of the tumor ; (D) Renorrhaphy.



cologic results were evaluated. Among 554 renal tumors treated between 06/2006 and 06/2010, in 187 it was performed partial nephrectomy, and ten patients underwent partial nephrectomy through anatrophic nephrotomy.

The detailed operative technique is described below:

A) The access is made through either a flank or anterior subcostal incision; the kidney is dissected and exposed, and the renal artery and vein are repaired;

B) Ultrasound is used to identify the tumor borders and depth. Before renal artery clamping, it is infused intravenously 50 mL of mannitol 20% for nephron protection during warm ischemia. Ice is used to involve the kidney after clamping for 3 min before initiating the tumor resection, and in this case both artery and vein are clamped (Figure-2A);

C) Right after the renal artery clamping with a bulldog, the nephrotomy is performed along the Brodel's vascular line for the visualization of the tumor (Figure-2B). The tumor enucleation is then carried out using a combination of blunt and sharp dissection (Figure-2C). The surgical specimen is immediately sent to the histopathological laboratory in order to verify the margins during the renal reconstruction. The hemostasis is done through parenchymal stitches of 2.0-chromed catgut. Renorrhaphy is then performed with a running Vicryl® 0 suture (Figure-2D);

D) A suction drain is placed surrounding the closed Gerota's fascia in all cases and the incision is closed.

RESULTS

Ten patients were evaluated. The mean age was 42 yrs (30-59), and nine were male. The mean tumor size was 2.3 cm (1.5–3.5), compared with 3.5 + 2.4 cm of all partial nephrectomies. Mean warm ischemia time was 22.4 min (15-30) (Table 1). The mean pre-operative serum creatinine was 0.88 mg/dL, and the post-operative value was 0.94 mg/dL, measured two weeks after surgery.

Table 1 – Patient demographics, renal functional outcomes and warm ischemia time

Patient	Gender	Age (years)	Tumor size (cm)	Tumor location	Ischemia Time (min)
1	M	46	1.5	TM / RK	MP
2	M	34	1.7	UP / LK	25
3	M	49	1.5	MP / RK	30
4	M	30	2.7	LP /LK	24
5	M	49	1.7	MP / RK	19
6	M	59	2.0	MP / LK	25
7	M	51	3.0	LP / RK	22
8	F	39	3.5	MP / RK	28
9	M	30	2.5	LP / LK	16
10	M	32	3.0	UP / LK	15
Mean		42	2.3		22.4

UP= Upper Pole; MP = Medium Third; LP = Lower Pole
RK= Right kidney; LK= Left kidney

Table 2 – Pathological characteristics of kidney tumors

Patient	Histology*	Fuhrman grade	Vascular Invasion	Pre-operative Creatinine (mg/ml)	Post-operative Creatinine (mg/ml)
1	Clear Cell	2	No	1.0	0.9
2	Clear Cell	1	No	0.9	0.9
3	Papillary	1	No	0.6	0.8
4	Clear Cell	1	No	0.9	1.0
5	Chromophobic	1	No	0.8	0.8
6	Clear Cell	3	No	1.1	1.3
7	Oncocytoma	-	-	0.9	1.0
8	Oncocytoma	-	-	0.7	0.8
9	Clear Cell	1	No	1.1	1.1
10	Clear Cell	2	No	0.8	0.8
Mean				0.88	0.94

* Renal Cell Carcinoma; PS = Upper Pole; TM = Medium Third; PI = Lower Pole

On the pathological analysis, two lesions had benign etiology (oncocytoma), and among the renal cell carcinomas, six were of clear cells, one papillary, and one chromophobic. The majority of the lesions were of low Fuhrman grade, and no positive surgical margin has been observed in this series (Table 2).

No patient showed significant intraoperative bleeding, with no blood transfusion, as well as urinary fistulas or collections. There was no loss of the renal unit in any patient submitted to surgery. All patients were monitored using the same protocol during a mean follow-up of 36 months (6–72), with no local recurrence or systemic metastatic disease observed.

DISCUSSION

Anatrophic nephrotomy for handling solid and tumors completely endophytic represents a feasible approach for preservation of the kidney function in all cases, with adequate exposure and safety for tumor resection, as well as collector system closure and hemostasis.

Renal intraparenchymal (endophytic) tumors represent greater technical difficulties for localization and resection and may increase the chances of complications (8). Partial nephrectomy and/or enucleation represent 30% of the surgical procedures used for renal tumors, with long-term survival similar to radical nephrectomy for the initial stages, with cancer-specific and overall survival of 98% and 97% respectively (1,4,10). For tumors <4cm, there is no need to remove any additional surgical margin for optimal cancer control. The use of intraoperative ultrasound facilitates the precise identification of the lesion(s), its relation with intra-renal structures and the proximity of the major kidney vessels (9).

There are several definitions of central tumors, of which the best accepted are those of Black et al. (11), which consider that the lesion is completely surrounded by normal renal tissue, and that of Brown et al. (12) that define those with a distance shorter than 5 mm from the collection system or hilar vessels.

The anatrophic nephrectomy was developed for the treatment of staghorn lithiasis, with

the opening of the renal parenchyma in the posterior face of the kidney under cold ischemia. Surgery studies targeting surgery for calculi occupying the entire collecting system show an average cold ischemia time varying from 20 to 45 minutes without significant blood loss (13, 14). Regarding the loss of renal function, the literature shows that 55% of the kidney units have their function maintained and just 13% exhibit a discrete reduction (13). The study of the relative function with DMSA shows an average reduction of 4% (15). Nohara et al. (15) described their technique for anatrophic partial nephrectomy with selective arterial clamping of the area of the tumor, after an angiography study, with a minor increase of the serum creatinine in the postoperative follow-up.

Nephron-sparing surgery for central but exophytic tumors has shown safety and efficacy compared to peripheral tumors. In a study with 118 patients, the operative and ischemia time, need of the closure of the collection system, and blood transfusion did not exhibit statistically differences (8). However, estimated blood loss was higher in the central tumor group: 220 mL (20 – 3500 mL) against 50 (5 to 1500 mL) in peripheral tumors. This same study showed a trend towards a higher number of urinomas and urinary fistulas in the central tumor group (8).

The technique presented herein allows a reliable approach, with a clear view of the tumors and their anatomical relations, with low complication rates (8,6). Moreover, it is technically reproductible by the majority of urologists, because of their familiarity with the anatrophic nephrectomy technique used to remove large staghorn calculi. The mean ischemia time of 22.4 minutes is comparable to the time of the partial nephrectomy studies (8) and below the average of those of nephrotomies for treating calculi (13,14).

There is currently a growing use of minimally invasive ablative therapies for small kidney tumors, such as radiofrequency and cryotherapy, providing greater safety and expanding its indications. However, recent studies show a higher failure rate varying from 13% to 35% for RFA and 4% to 6% for cryoablation (16,17). The main factor for failures on procedures aided by laparoscopy is the endophytic nature of the lesions (8). Besides, there are no series for these types of treatments with patients that are so young such as those presented in this study, and with an adequate follow-up.

The limitations of the present study are represented by the small number of patients, with a median follow-up of 36 months and with no studies of the renal functions of the operated units other than serum creatinine. However, the lesions were safely enucleated and rendered negative margins, with acceptable warm ischemia time.

The nephron-sparing surgery for tumors completely endophytic through anatrophic nephrotomy was safe, with low complication rate and short warm ischemia time. It may be a reliable option when dealing with complex small kidney tumors.

Nowadays, the Urologists have the obligation to try to perform the nephron-sparing surgery in tumors <7cm, whenever possible (3-5). In particular, this study wants to reinforce the need for this strategy, since the partial open surgery is mandatory in these cases to avoid loss of renal unit in small (< 4 cm) and asymptomatic tumors.

CONFLICT OF INTEREST

None declared.

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Enucleation ratio efficacy might be a better predictor to assess learning curve of holmium laser enucleation of the prostate

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ABSTRACT

Purpose: To appraise the evaluation methods for learning curve and to analyze the non-mentor-aided learning curve and early complications following the holmium laser enucleation of the prostate.
Materials and Methods: One-hundred and forty (n=140) consecutive patients who underwent HoLEP from July 2008 to July 2010 by a single surgeon (SJO) were enrolled. Perioperative clinical variables, including enucleation time, morcellation time, enucleation ratio (enucleation weight/transitional zone volume), enucleation efficacy (enucleated weight/enucleation time), enucleation ratio efficacy (enucleation ratio/enucleation time), and early complication rate were analyzed.
Results: Mean prostate volume was 62.7 mL (range 21-162) and preoperative International Prostate Symptom Score (IPSS) was 19.0 (4-35). Mean enucleation time and morcellation time were 49.9±23.8 (S.D.) min and 11.0±9.7 min, respectively. Median duration of postoperative indwelling catheter was 1 (1-7) day and median hospital stay was 1 (1-6) day. There were a total of 31 surgery-related complications in 27 patients (19.3%), and all were manageable. There was an increasing trend of enucleation efficacy in the first 50 cases. However, enucleation efficacy was linearly correlated with the prostate size (correlation coefficients, R=0.701, p<0.001). But, enucleation ratio efficacy could eliminate the confounding effect of the prostate size (R=-0.101, p=0.233). The plateau of enucleation ratio efficacy was reached around the twenty-fifth case.
Conclusions: Our results demonstrated that the operative learning curve plateau is reached after about 25 cases. We propose that a more appropriate parameter for estimating the operative learning curve is enucleation ratio efficacy, rather than enucleation efficacy.

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Key words:
Prostatic Hyperplasia; Lasers; Solid-State; Prostatectomy; Learning curve

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INTRODUCTION

Benign prostatic hyperplasia (BPH) is a highly prevalent disease in elderly men. By the age of 60, almost 60% of the cohort in the Baltimore Longitudinal Study of Aging had some degree of clinical BPH (1). The gold standard surgical treatment for BPH is transurethral resection of the prostate (TURP) (2). Recent surgical techniques using lasers, such as photoselective vaporization of the prostate (PVP), holmium laser enucleation of the prostate (HoLEP) and thulium laser enucleation of the prostate (ThuLEP) have been popularized, with a number of reports document-

ing their merits. HoLEP can confidently remove the entire enlarged adenoma through the use of Holmium:YAG laser in the natural tissue plane of the surgical capsule.

After the first clinical report by Gilling et al. in 1996, several randomized control trials have been reported (3). Compared to TURP or open prostatectomy, HoLEP has similar clinical outcome, lower postoperative complication rate and requires shorter hospital stay (4-8). In addition, HoLEP appears to be a true endourological alternative to open prostatectomy, because it can effectively treat a very large prostate (9,10).

However, a steep operative learning curve may be the main hindrance to widespread use of HoLEP despite its advantages. Some researchers have argued that a surgeon needs between 20 and 30 cases of experience to attain competency (6,11), while others have argued that up to 50 cases are needed (12,13). This discrepancy comes mainly from the absence of an accurate way to evaluate the operative learning curve. Therefore, the purpose of this study was to evaluate a proper method to assess the learning curve to attain competency to perform enucleation of prostate using HoLEP.

MATERIALS AND METHODS

One-hundred and forty (n=140) consecutive patients who underwent HoLEP for the treatment of BPH from July 2008 to July 2010 were enrolled. All patients were treated by a single surgeon (SJO). Preoperatively, history taking, physical examination with digital rectal exam, International Prostate Symptom Score (IPSS), quality of life (QoL), frequency-volume chart, urinalysis, prostate-specific antigen, transrectal ultrasonography and pressure-flow study were performed on each patient.

Protocol for the HoLEP procedure is as follows. Each patient was placed in the lithotomy position under spinal anesthesia. The urethra was dilated with a 30 Fr metal catheter prior to introduction of a working sheath. We used a 80W holmium laser (VersaPulse® PowerSuite™, Lumenis, Yokneam, Israel), 550 µm end-firing laser fiber (SlimLine™, Lumenis, Yokneam, Israel), 26 Fr continuous-flow resectoscope with a dedicated inner sheath, and a 30º telescope (27040 XAL and 27005 BA, Karl Storz, Tuttlingen, Germany) for enucleation of adenomas. Enucleation was performed in 7 steps: 1. apical incision (lateral side to verumontanum), 2. bladder neck incision (5 and 7 o'clock positions) and conjoining transverse incision in front of verumontanum, 3. median lobe enucleation, 4. upward separation of lateral lobes, 5. longitudinal incision at 12 o'clock, 6. downward incision of lateral lobes (to 3 and 9 o'clock side), and 7. connecting mucosal incision of apical prostate and enucleation of lateral lobes. For morcellation, we changed the resectoscope to a 26 Fr nephroscope with an adapter (27293 AA and 27040 LB, Karl Storz, Tuttlingen, Germany). Enucleated tissue was morcellated using a VersaCut™ morcellator (Lumenis, Yokneam, Israel). After the procedure, a 22 Fr Foley catheter was placed. Postoperatively, continuous bladder irrigation was applied as the occasion demanded. Usually, on the first or second postoperative day, the patient was discharged after removal of the urethral Foley catheter and spontaneous urination was confirmed.

Perioperative clinical variables, including enucleation weight, enucleation time, morcellation time, enucleation ratio (enucleation weight/transitional zone volume), enucleation efficacy (enucleated weight/enucleation time), enucleation ratio efficacy (enucleation ratio/enucleation time), and early complications were recorded prospectively on a planned data registry form. These data were retrieved from the registered database, and then analyzed. This study was approved by the institutional review board (IRB) of Seoul National University Hospital.

Basic data were expressed by a descriptive method. Data for analysis of the learning curve were compared using the Student t-test, ANOVA and chi-square tests. To compare postoperative outcomes with preoperative outcomes, a paired samples t-test was used. To determine the relationship between enucleation efficacy and prostate volume, we performed linear regression analysis. All data were analyzed using SPSS version 17.0 (SPSS, Inc. Chicago, USA). For all statistical comparisons, significance was considered when $p < 0.05$.

RESULTS

Patients' mean age was 68.9±6.1 (S.D.) years, and mean preoperative prostatic specific antigen level was 3.5±3.1 (S.D.) ng/mL. Mean prostate volume was 62.7 mL (range 21-162) and preoperative International Prostate Symptom Score (IPSS) was 18.8±7.7 (S.D.). Mean preoperative maximum flow rate and postvoid residual were 9.6±4.7 (S.D) mL/sec and 86.5±124.6 (S.D.) mL, respectively. Various operative parameters related to learning curve were summarized in Table-1 along with the number of cases experienced. There was no TURP conversion. Median duration of postoperative catheter indwelling and hospital stay was equal to 1 day (mean 1.61, range 1-7 and mean 2.61, range 1-6, respectively). Preoperative and postoperative functional outcomes at 6 months are presented in Table-2.

A total of 31 surgery-related complications were recorded in 27 patients (19.3%) (Table-3). However, all complications were mild and transient, so they were easily and properly managed. No patient developed stress urinary incontinence persistent for more than 3 months.

Enucleation efficacy had a strong linear correlation with total prostate volume (correlation coefficient, $R=0.701$, $p<0.001$) and transitional zone volume ($R=0.740$, $p<0.001$) (Figure-1). Figure-2 summarizes the changes in operative parameters in the order of sequential cases. Enucleation efficacy was increased in the first 50 cases, however the enucleation ratio did not change significantly. The estimated prostate volume did not changed in the first 20 cases, compared to the next 20 cases (mean values 53.9 vs. 50.6 mL, $p=0.587$). However, there was significant increase in estimated prostate volume from case number 41 to 60, compared to the prior 20 cases (mean value 70.1 mL, $p=0.015$). Enucleation ratio efficacy, having eliminated the confounding effect of prostate size, reached a plateau around the twenty-fifth case. And this parameter agreed with the surgeon's confidence in his operative skills.

DISCUSSION

TURP and open prostatectomy have been the treatments of choice for moderate (30-80 mL) and large (>80-100 mL) size BPH prostates respectively (2,14). TURP has been the standard surgical treatment for symptomatic BPH. TURP has high rate of success and imposes low economic burden. However, in 2.0-4.8% of cases, transfusion is required, and TURP syndrome, a dilutional hyponatremia that occurs when the irrigant is absorbed into the bloodstream, occurs in 1.1% of cases. The chance of TURP syndrome significantly increases when the prostate gland is larger than 45g, or the resection time is longer than 90 min (15). Most of all, one study showed that 14.7% of patients who underwent TURP required reoperation at the eighth year of follow-up (16). For these reasons, many alternative treatment options are been actively researched.

HoLEP is the most extensively studied laser therapy for BPH. Randomized control studies have shown that symptom improvement attained after HoLEP is comparable to that obtained following TURP, with favorable results including removed tissue weight, duration of catheterization, hospital stay, need for blood transfusions, and the absence of TURP syndrome (4,5,17-19). Similar treatment outcomes have been demonstrated when HoLEP was compared to open prostatectomy for the treatment of large prostates. Surgical time and removed tissue weight were better in open prostatectomy, but duration of catheterization, hospital stay and bleeding were better in HoLEP (9,11,20,21). The rationale behind lower amount of removed tissue weight during HoLEP comes from vaporization effect during enucleation (5). These merits, proven in long-term follow-up studies, suggest that HoLEP is a viable alternative to TURP and open prostatectomy.

However, the major disadvantage of HoLEP is a steep learning curve to attain competency. The procedure requires specialized training and equipment. Based on their own experiences, Moody et al. and Kuntz et al. argued that a minimum of 30 procedures on smaller glands (< 50 gm) should be performed before attempting enucleation of larger adenomas using HoLEP. In their retrospective study, Du et al. reported that the surgeon became more confident with the HoLEP technique after about 15 cases which involved moderate size prostates (22). Shah et al. reported in their prospective study that

Table 1 - Operative parameters related to learning curve with number of cases treated.

	Total	1-20	21-40	41-60	61-80	81-100	101-120	121-140
Prostate volume (mL)	62.0 ± 27.1	53.9 ± 17.5	50.6 ± 21.0	70.1 ± 27.3	61.3 ± 25.8	78.4 ± 36.3	57.3 ± 21.1	67.7 ± 29.8
Transitional zone volume (mL)	35.0 ± 21.2	26.5 ± 14.1	27.1 ± 21.5	42.4 ± 21.5	29.4 ± 12.7	47.8 ± 28.0	32.0 ± 18.4	39.8 ± 21.3
Enucleation time (min)	49.9 ± 23.8	74.4 ± 34.4	34.3 ± 10.8	45.7 ± 9.9	42.3 ± 13.0	60.4 ± 27.9	45.8 ± 19.6	46.2 ± 17.2
Morcellation time (min)	11.0 ± 9.7	15.1 ± 7.5	10.9 ± 8.4	12.1 ± 8.5	9.8 ± 5.9	14.2 ± 18.7	5.8 ± 3.6	9.3 ± 5.4
Enucleation weight (gm)	22.7 ± 19.1	12.2 ± 8.8	16.4 ± 13.3	25.7 ± 16.5	22.9 ± 17.7	35.9 ± 27.6	21.9 ± 20.7	24.1 ± 16.7
Enucleation ratio (gm/mL)	0.60 ± 0.28	0.45 ± 0.27	0.58 ± 0.21	0.59 ± 0.19	0.73 ± 0.37	0.65 ± 0.33	0.61 ± 0.31	0.56 ± 0.18
Enucleation efficacy (gm/min)	0.44 ± 0.27	0.20 ± 0.18	0.44 ± 0.24	0.54 ± 0.24	0.50 ± 0.24	0.53 ± 0.35	0.42 ± 0.25	0.49 ± 0.24
Enucleation ratio efficacy (gm/mL/min)	0.0133±0.0063	0.0071±0.0047	0.0178±0.0069	0.0131±0.0040	0.0176±0.0069	0.0111± 0.0052	0.0139±0.0051	0.0127± 0.0041

Data presented as mean ± standard deviation

Table 2 - Preoperative and follow-up functional outcomes.

	Preoperative (n=140)	2 wks - 1 mo (n=129)	3 mo (n=91)	6 mo (n=63)
Maximum flow rate (mL/sec)	9.6 ± 4.7	19.3 ± 9.2	20.9 ± 10.5	21.2 ± 8.2
Postvoid residual (mL)	86.5 ± 124.6	23.4 ± 29.9	27.4 ± 42.3	23.4 ± 34.1
IPSS (total)	18.8 ± 7.7	12.0 ± 7.7	6.8 ± 5.5	7.3 ± 6.5
QoL	4.2 ± 1.2	2.7 ± 1.7	2.0 ± 1.4	1.8 ± 1.5

Data presented as mean ± standard deviation. IPSS= international prostate symptom score; QoL=quality of life

Table 3 - Intra- and postoperative complications according to the number of cases treated.

	Clavien-Dindo grade	Total	1-20	21-40	41-60	61-80	81-100	101-120	121-140
Intraoperative									
Minor capsular perforation	I	5	0	1	1	3	0	0	0
Bladder mucosal injury	I	4	0	0	1	2	0	0	1
Immediate postoperative									
Re-catheterization	II	9	2	2	2	3	0	0	0
Blood transfusion	II	2	0	1	0	1	0	0	0
Transurethral coagulation	IIIa	2	0	1	1	0	0	0	0
Transient SUI	I	6	2	1	1	1	1	0	0
Urinary tract infection	II	1	0	0	0	0	0	1	0
Late postoperative									
Urethral stricture	IIIa	1	1	0	0	0	0	0	0
Bladder neck contracture	IIIb	1	1	0	0	0	0	0	0
Total sum/Total patients		31/27	6/6	6/4	6/6	10/8	1/1	1/1	1/1

SUI = stress urinary incontinence

HoLEP can be performed effectively after about 50 cases, with an outcome comparable to that of experts (12). Their conclusion was based on enucleation efficacy analysis and complication rate. Seki et al. argued that HoLEP can be taught even without a proper instructor, and in that report at least 50 cases of operative experience was needed to attain competency (13). These results were derived from a plateau curve of tissue enucleation efficacy. We found that reports based on subjective experience or confidence concluded that HoLEP competency required 20-30 cases of experience (6,11). On the other hand, reports based on enucleation efficacy concluded that at least 50 cases were needed

Figure 1. Correlation between prostate size and enucleation efficacy. (A) Total prostate volume. (B) Transitional zone volume. (Linear regression with 95% mean prediction interval).

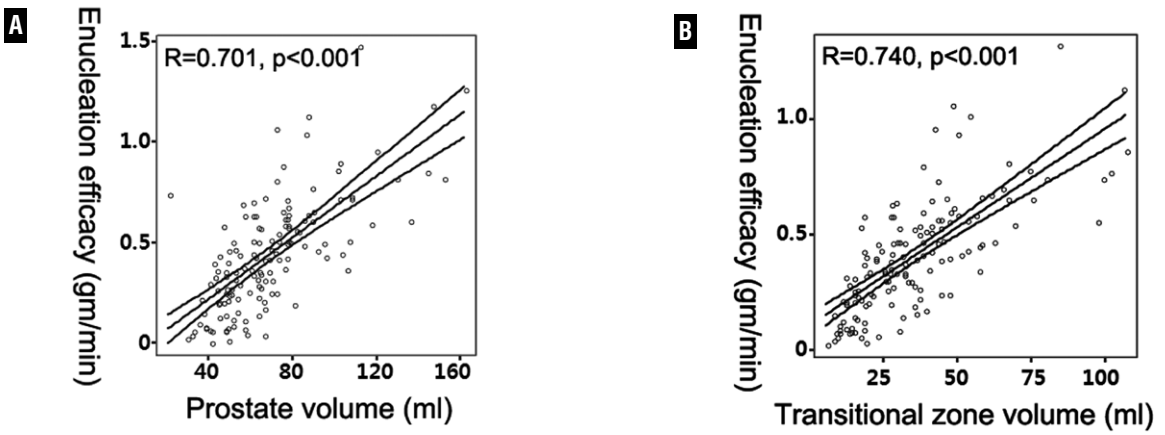
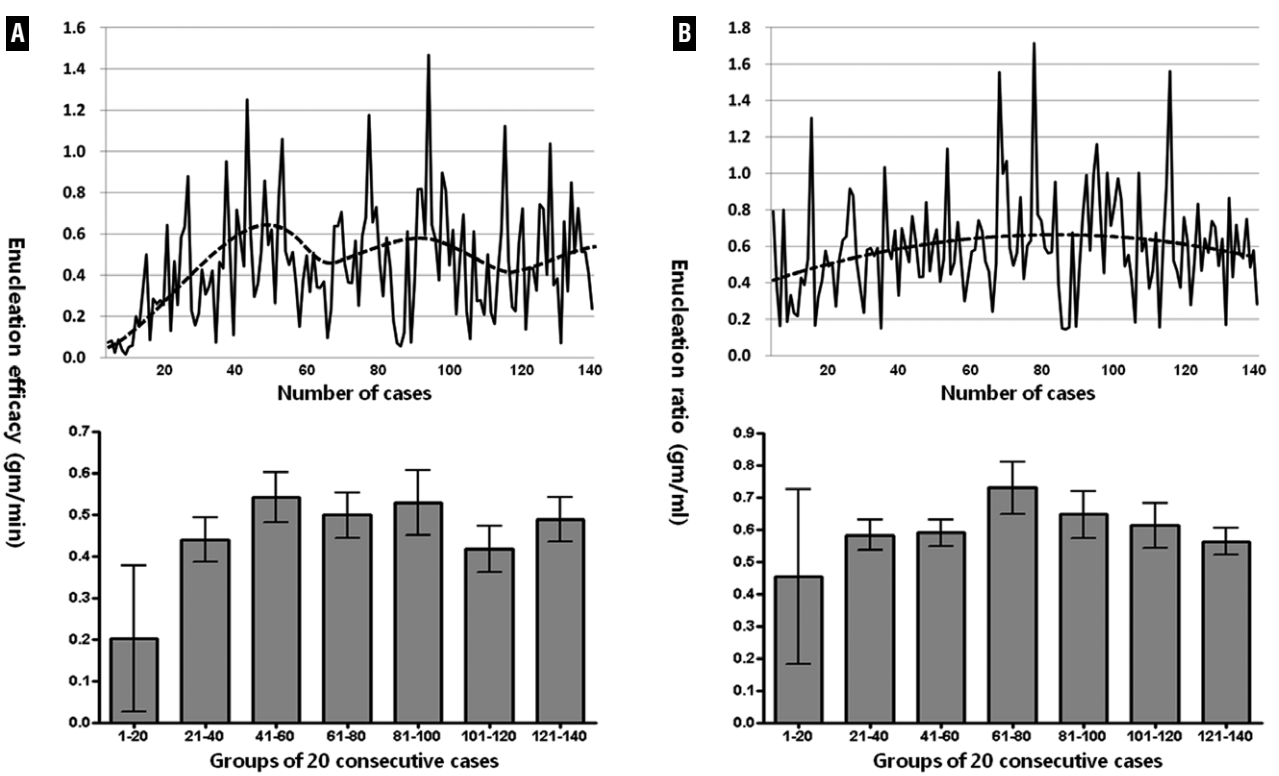
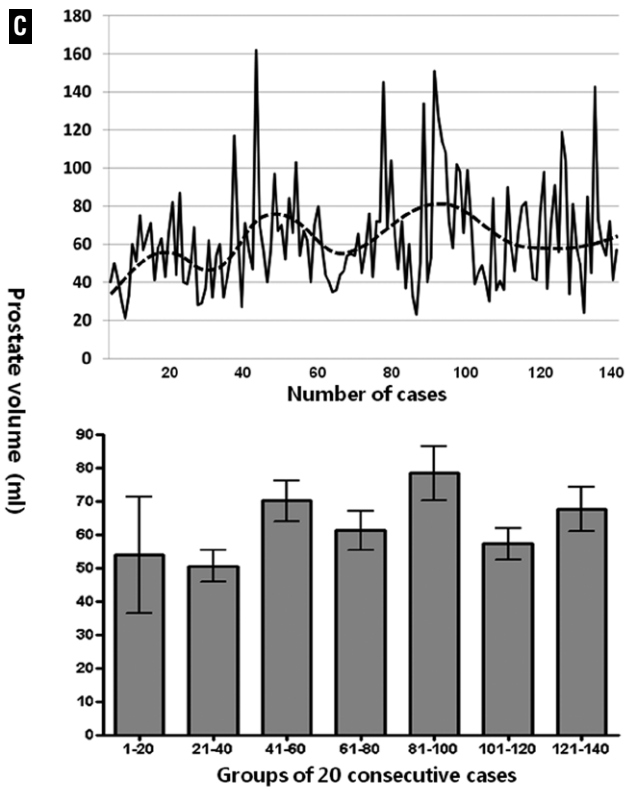
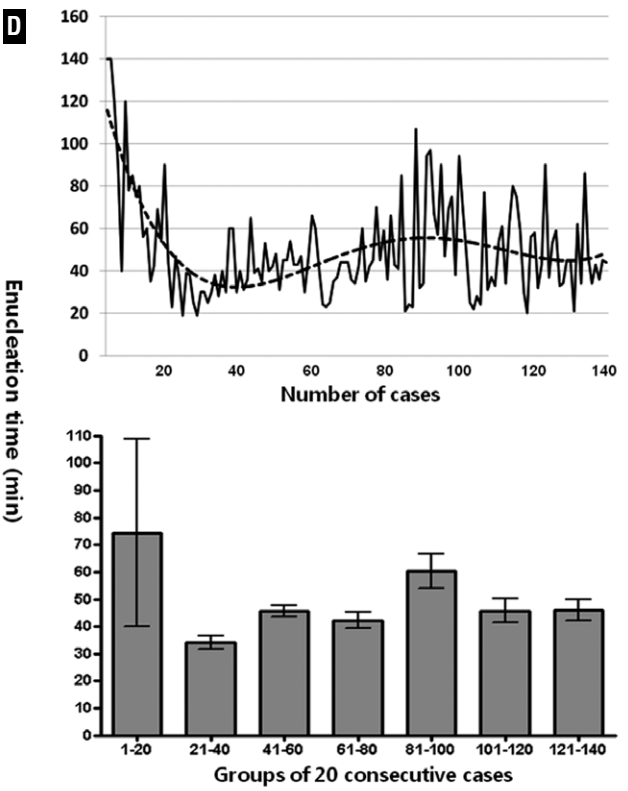
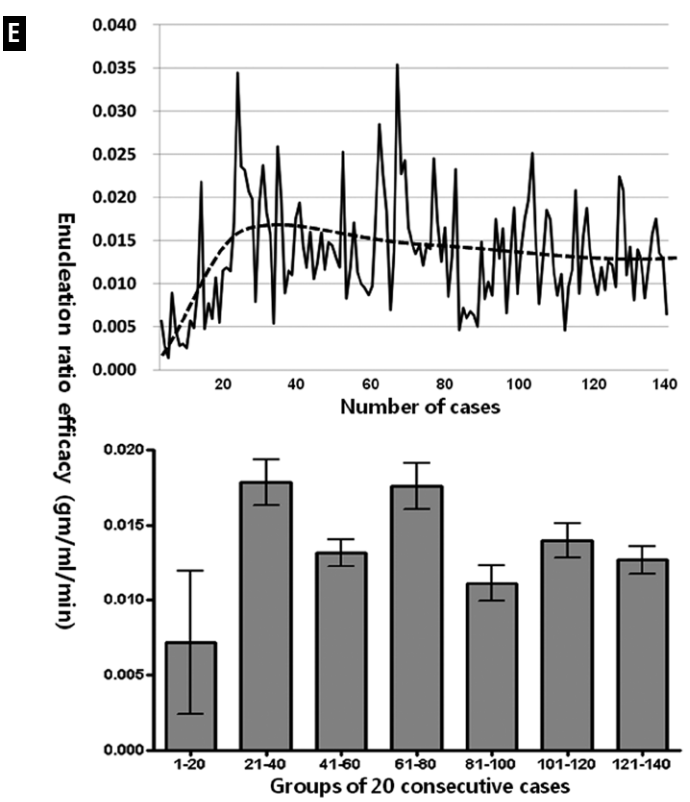


Figure 2. Changes in operative parameters related to learning curve in order of sequences. (A) Enucleation efficacy. (B) Enucleation ratio. (C) Prostate volume. (D) Enucleation time. (E) Enucleation ratio efficacy.




C
Prostate volume (ml)
Number of cases
Groups of 20 consecutive cases
1-20
21-40
41-60
61-80
81-100
101-120
121-140


D
Enucleation time (min)
Number of cases
Groups of 20 consecutive cases
1-20
21-40
41-60
61-80
81-100
101-120
121-140


E
Enucleation ratio efficacy (gm/ml/min)
Number of cases
Groups of 20 consecutive cases
1-20
21-40
41-60
61-80
81-100
101-120
121-140

to attain competency (12,13). Enucleation efficacy is considered to be a more objective parameter to estimate operative learning curve. However, there is a discrepancy between enucleation efficacy and self confidence regarding the HoLEP procedure.

In July 2008, our institution began offering HoLEP. There was no experienced urologist in the Republic of Korea at the time, and HoLEP was self-taught by reviewing expert videos. Our results, demonstrating quick symptomatic improvement and a reasonable complication rate, are consistent with that previously published (23). Moreover, while Placer et al. reported that persistent stress urinary incontinence (4.8%) occurred during initial enucleation of large-sized prostates in the self-taught situation, there was no persistent stress urinary incontinence in our series (24).

In this study, enucleation efficacy increased in the initial cases, and the plateau was reached around the fiftieth case. In contrast, the enucleation ratio did not show significant difference. However, enucleation efficacy had a strong linear correlation with prostate size. This implies that HoLEP is more effective for larger prostates, and enucleation efficacy is also confounded by prostate size. There was no difference between the first 20 cases and the next 20 cases in terms of prostate volume, but the subsequent 20 cases were of significantly larger prostates than the prior 20 cases. Thus, during the first 40 cases the enucleation efficacy has increased mainly by technical improvement, and during the later period increased efficacy was obtained due to case selection of larger prostates after the surgeon had gained confidence. This can also be observed in the enucleation time curve. This phenomenon is consistent with a previous report (24).

As a result, we propose a new parameter, 'enucleation ratio efficacy', to estimate the operative learning curve for HoLEP. It is defined as enucleation ratio divided by enucleation time and this is identical to enucleation efficacy divided by transitional zone volume. This new parameter can eliminate the confounding effect of prostate size from enucleation efficacy. In this series, the correlation coefficient between enucleation ratio efficacy and prostate size was only -0.101 (p=0.233). Furthermore, the enucleation ratio efficacy stabilized after 25 cases, and this observation was consistent with the surgeon's confidence in his operative skills. We believe this parameter reflects the surgeon's skills more accurately than enucleation efficacy by eliminating size as a confounding effect. Both enucleation efficacy and enucleation ratio efficacy reduced after reaching the plateau. We think that it occurred because the surgeon, once having gained confidence, had more time for meticulous bleeding control and completing the procedure.

The clinical outcomes were not different between the groups along cases. In all patients, objective outcomes (maximum flow rate and post void residual volume) significantly improved immediately from the first visit. In contrast, subjective outcomes, especially IPSS total score, stabilized 3 months after surgery.

While the surgeon's competency to perform HoLEP could be attained after 25 cases, complications were significantly reduced after 80 cases, although they were mild. Overall, 19.3% of patients experienced complications related to surgery. However, most of these complications were mild and transient. Clavien classification (25) III complications developed in 4 patients (2.9%), and higher-grade adverse events did not occur. Surgeons should be careful in minimizing complications after gaining confidence with their surgical skills by the lesson from this series. Seki et al. reported high incidence of urethral stricture (7%) and they laid the responsibility to the larger nephroscopes (26Fr). However, only one patient (0.7%) experienced urethral stricture in our series. We do not believe that a systematic use of a 26Fr working sheath could increase the incidence of urethral complications, if surgeon carefully performs HoLEP.

We believe that performing an extensive literature review on surgical technique and watching and analyzing videos of procedures recorded by an expert are very important. Moreover, we advocate repeated self-training with records and videos of their own cases. In doing so, the learning curve which is thought to be steep could be overcomed in a shorter period of time. This could be even more apparent if a surgeon already has considerable experience in endourological procedures. Despite the "self-taught" situation in our series, there was an overall low incidence of complications.

In the analysis, there were three patients whose prostate volumes were less than 25 mL. Their transitional zone volumes were between 8 - 9 mL and anatomical obstructions secondary to median lobe enlargement were found on urethrocystoscopy. These cases could be ideal candidates for transurethral incision of the prostate. However, we chose HoLEP rather than transurethral incision in an attempt to remove the whole adenoma. We included these cases in the study cohort, because that faithfully reflects our real practice.

Our study has several limitations. The major pitfall is the retrospective nature of the study. Notwithstanding, all consecutive patients were enrolled to avoid potential selection bias. Furthermore, all data were collected prospectively using a planned data registry form. It would be more informative if long-term follow-up results could be presented. However, we believe that surgical competency could be reasonably assessed with short-term outcomes of six months. The other limitation is that the study was conducted on patients operated by one surgeon. Our proposed parameter, enucleation ratio efficacy, should be evaluated with several surgeons in the future, to determine if it is generally acceptable.

CONCLUSIONS

Our results demonstrated that the learning curve can be overcome after about 25 cases, even in a non-mentor-aided situation. We believe that a more appropriate parameter for estimating the learning curve is enucleation ratio efficacy rather than enucleation efficacy.

ABBREVIATIONS

BPH = Benign prostatic hyperplasia;
TURP = transurethral resection of the prostate;
PVP = photoselective vaporization of the prostate;
HoLEP = holmium laser enucleation of the prostate;
ThuLEP = thulium laser enucleation of the prostate;
IPSS = International Prostate Symptom Score;
QoL = quality of life;
IRB = institutional review board;
SUI = stress urinary incontinence

CONFLICT OF INTEREST

None declared.

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EDITORIAL COMMENT

There are very consistent publication in the literature demonstrating even the superiority of Ho-Lep compared to TURP and open prostatectomy. However, the method continues to spread with difficulty, probably due to five factors. The difficulty of learning the technique, the need for morcelation, the need for investment, the existence of several other laser equipment for prostate surgery and the fact that TURP still solve the most of cases. The work has a very interesting approach, subtracting the effect on surgical time produced by large adenomas, demonstrating that the learning curve may be lower than expected and would not need a mentor. His weak point is the parameter with only one surgeon.

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EDITORIAL COMMENT

HoLEP is, so far, the most extensively studied laser technique for treatment of BPH and a "steep learning curve" is the described major drawback of this method. In the present article, the authors evaluated, in a non-mentor-aided fashion, the ideal method to assess the learning curve of HoLEP. They concluded that the best method to estimate the operative learning curve is the enucleation ratio efficacy (enucleation ratio/enucleation time), rather than enucleation efficacy. What is remarkable in their study is the low overall complication rate considering their lack of experience. This means that urologists should be encouraged to start performing HoLEP as an option for larger prostates that are not suitable for TURP, since its complications seems to be mild and not frequent.

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ORIGINAL ARTICLE

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Androgen receptor CAG polymorphism and the risk of benign prostatic hyperplasia in a Brazilian population

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ABSTRACT

Benign prostatic hyperplasia (BPH) is a very frequent age-related proliferative abnormality in men. Polymorphic CAG repeat in the androgen receptor (AR) can alter transactivation of androgen-responsive genes and potentially influence BPH risk. We investigated the association between CAG repeat length and risk of BPH in a case-control study of a Brazilian population. We evaluated 214 patients; 126 with BPH and 88 healthy controls. DNA was extracted from peripheral leucocytes and the AR gene was analyzed using fragment analysis. Hazard ratio (HR) and 95% confidence interval were estimated using logistic regression models. Mean CAG length was not different between patients with BPH and controls. The CAG repeat length was examined as a categorical variable (CAG $\leq$ 21 vs. CAG > 21 and CAG $\leq$ 22 vs. CAG > 22) and did not differ between the control vs. the BPH group. We found no evidence for an association between AR CAG repeat length in BPH risk in a population-based sample of Brazilians.

ARTICLE INFO

Key words:
Receptors, Adrenergic; Polymorphism, Genetic; Benign Prostatic Hyperplasia

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INTRODUCTION

Benign prostatic hyperplasia (BPH) is a very frequent age-related proliferative abnormality in men (1). BPH is considered a progressive disease, defined as continuous growth of the prostate, leading to intensification of symptoms and increased risk of complications, such as increased risk of acute urinary retention and BPH-related surgery (2). Studies show that the prevalence of BPH is around 40 to 50% at the age of 50 years, and approximately 80% at the age of 70 (1,3).

The pathogenesis of tumor development has been closely associated with the action of steroid hormones (4,5). The androgenic effects are mediated by testosterone and dihydrotestosterone (DHT) in the target cells and their action has been demonstrated in the morphogenesis, differentiation, cell proliferation, and secretions of the prostate gland. Androgen binding promotes the activation of the androgen receptor (AR) and recruitment of co-factors, leading to the transcription of hormone-dependent target genes (6-9).

The human AR gene is located in chromosome X, on the q11-q12 region, which contains 8 exons (10) and has an approximate size of 90 kb (11). A critical function of the AR gene product is to activate the expression of other genes. The transactivation activity lies in the N-terminal domain of the protein (encoded by exon 1). Two polymorphic microsatellites are located approximately 1.1 kb away in exon 1: a highly

polymorphic CAG repeat and a less polymorphic GGC repeat (11,12). The CAG repeat encodes a polyglutamine tract; it ranges from 8 to 31 repeats and averages approximately 20 repeats (13).

In vitro studies have shown a negative correlation between the number of CAG repeats and the transcriptional activity of the AR. The increased number of these repeats reduces transcriptional activity in the AR, whereas a reduction to zero induces increased AR (11,14,15).

Therefore, the objective of the present study was to investigate whether CAG variant can be related to the development of BPH analyzing the frequency of AR CAG polymorphism in a sample of male individuals from southern Brazil.

MATERIALS AND METHODS

Study population

This case-control study was prospectively conducted at the Universidade Federal do Rio Grande do Sul from September 2004 to January 2009. The study was approved by the local and national Ethics Committee and informed consent was obtained from every subject. Prostate hyperplasia patients were selected from the Urology outpatient clinic at the Hospital de Clínicas de Porto Alegre. Inclusion criteria were age 40-80 years old, prostate volume larger than 30g (evaluated by abdominal ultrasound to define BPH group), no past or current hormone-ablation therapy or 5α-reductase inhibitor therapy, and no concomitant neoplasia. Patients were submitted to surgery and the diagnosis of BPH was confirmed by pathological examination. Factors such as age at diagnosis, race (self-described), tumor stage and grade, total serum PSA (prostate-specific antigen) at diagnosis, and family history were recorded. Blood was collected to perform polymorphism analysis and to measure total serum testosterone. Controls were selected from a prostate cancer prevention program conducted since 2004 at the same institution. Inclusion criteria were age 40-80 years, prostate volume smaller than 30g, PSA value less than 2.0 ng/mL, normal digital rectal examination, and no concomitant neoplasia.

Genotyping

Genomic DNA for patients and controls was extracted from peripheral blood leukocytes. After erythrocyte lysis, leukocyte lysis was performed using 2 mL of specific solution (NaCl 150 mM, Tris-HCl 10 mM, pH 8.0; EDTA 10 mM, pH 8.0), 36 μL 10% SDS, and 30 μL of proteinase K (10 mg/mL), incubated at 37 °C for 18 hours. DNA was extracted and precipitated with 70% ethanol and re-suspended with specific buffer TE 10:0.1 (Tris-HCl 10 mM, pH 8.0; EDTA 0.1 mM, pH 8.0).

PCR was carried out at a final volume of 50 μL. One μL of the genomic DNA was denatured at 96 °C for 2 min in the presence of 20 mM Tris-HCl pH 8.4 plus 50 mM KCl and 1.5 mM MgCl2. After this hot start, 1.25 U of Taq DNA polymerase was added together with the same Tris-HCl buffer, 1.5 mM $MgCl_2$, 0.4 μM sense and antisense primers and 0.2 mM dNTP mix.

The primers used for polymorphism amplification were CAG primer 5'-TCCAGAATCTGTTCCAGAGCGTGC-3' (forward) and 5'-GCTGTGAAGGTTGCTGTTCCTCAT-3' (reverse). Both sense primers were labeled with FAM fluorescent dye. Amplifications were performed using an automated thermal cycler (MJ Research, Waltham, MA, USA) applying the following conditions: hot start, 2 min at 96 °C; three cycles of 40 sec at 94 °C, 30 sec at 67 °C and 20 sec at 72 °C; three cycles under the same physical conditions except for the annealing temperature, which was 64 °C; three cycles at an annealing temperature of 61 °C; three cycles at an annealing temperature of 59 °C; and 25 cycles at an annealing temperature of 55 °C. The quality of the PCR products was assessed using 1.5% agarose gel electrophoresis. Each PCR product was diluted in water (10X) for analysis, and 2 μL were mixed with deionized formamide and a fluorescent molecular weight marker [GeneMapper 500HD (ROX) Size Standard, Applied Biosystems, Foster City, CA, USA]. After denaturation for 1 min at 95 °C, each sample was submitted to capillary electrophoresis on an ABI 3100-Avant automated sequencer and the PCR products were analyzed with the GeneMapper software (Applied Biosystems, Foster City, CA, USA). The

number of CAG was calculated based on the size of the PCR products considering a series of standards obtained by direct sequencing of PCR products.

Statistical analysis

Differences between means in the continuous variables were analyzed by T test, with 95% significance. The genotype frequency between cases and controls was tested using standard χ^2 tests. Logistic regression was used to provide hazard ratio (HR), 95% confidence intervals (CI) and p-values for the risk of CAG repeat with BPH. The CAG repeat lengths were examined as categorical variables (CAG ≤ 21 vs. CAG > 21 and CAG ≤ 22 vs. CAG > 22). The categories were defined based on median analysis. Data analysis was performed using the computer software SPSS for windows (version 16.0).

RESULTS

The characteristics of the studied population are shown in Table-1. Genomic DNA from 126 BPH patients and 88 healthy male controls was examined to determine the number of CAG repeats. The participant's mean age was 62.84 ± 9.15 in the BPH group and 56.68 ± 8.08 in the control group. BPH patients were older than controls ($p < 0.001$). There was a predominance of white individuals in both groups (89.7 BPH and 82.3% controls). Total serum PSA was 0.76 (0.55-1.05) ng/mL in the control group and 1.47 (0.69-3.94) ng/mL in the BPH group. PSA was not different between the groups ($p = 0.566$). The prostatic volume was 20.00 (14.38-23.94) cm^3 in the control group and 35.68 (30.00-49.00) cm^3 in the BPH group. Prostatic volume was higher in the BPH group than in the control group ($p < 0.001$). The mean number of CAG repeats in exon 1 of the AR was 22.11 ± 2.89 in the control group and 21.62 ± 2.84 in the BPH group (Table-1). No significant difference was found between the mean CAG repeats of cases and controls. The distribution frequency of the number of CAG repeat polymorphisms is shown in Figure-1.

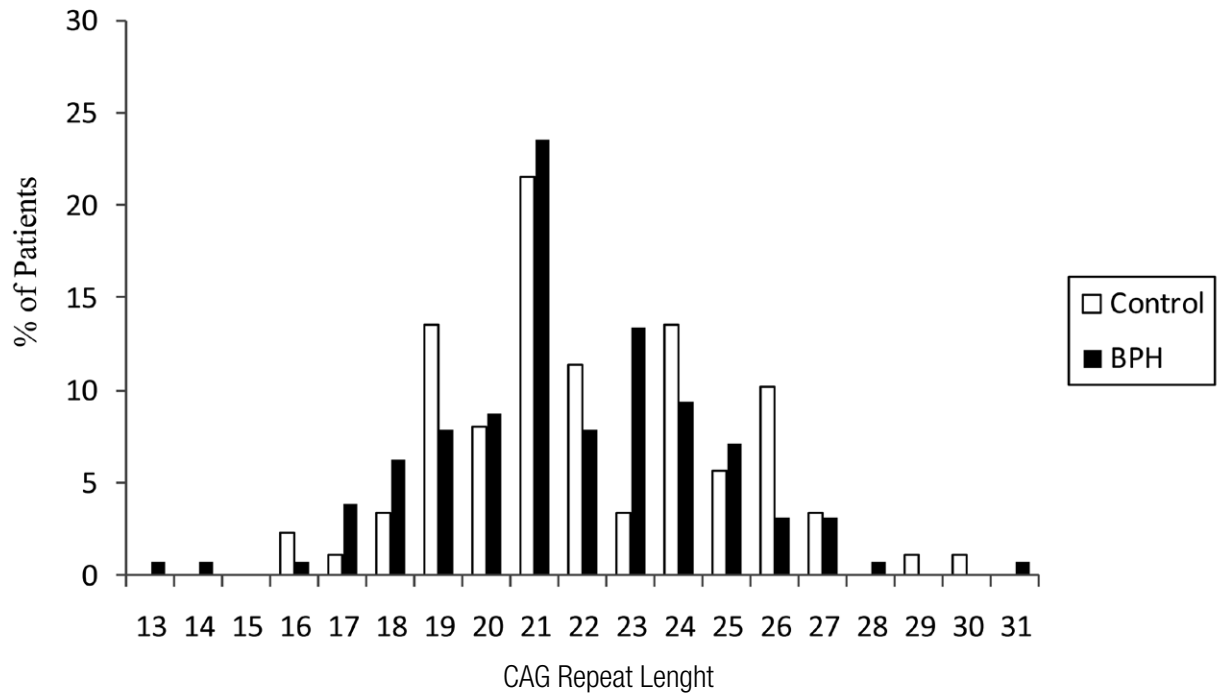
Based on the median number of CAG repeats in the control group, the population studied was dichotomously classified into different subgroups, CAG ≤ 21 vs. CAG > 21 and CAG ≤ 22 vs. CAG >

Table 1 - Characteristics of study population (n = 344).

	BPH (n=126)	Controls (n=88)
Age (years)[c]		
Mean ± SD	62.84 ± 9.15	56.68 ± 8.08
Range	41 - 82	41 - 75
Race (caucasians)[a]	113 (89.7%)	73 (82.3%)
PSA (ng/mL)[b,c]	1.47 (0.69 – 3.94)	0.76 (0.55 – 1.05)
Volume (cm^3)[b,c]	35.68 (30.00 – 49.00)	20.00 (14.38 – 23.94)
CAG repeat		
Mean ± SD	21.62 ± 2.84	22.11 ± 2.89
Median	21	21.5
Range	13 - 31	16 - 30

Values are expressed as [a]number of cases and (%), [b]median and percentile 25/75, [c]P<0.05.

Figure 1 - Frequency distribution of CAG repeats in the control and BPH groups.



22 (Table-2). According to this analysis, 66.3% of BPH group presented CAG > 21 and 56.9% presented CAG ≤ 21 (p = 0.352) and 58% of BPH group presented CAG > 22 and 58.8% presented CAG ≤ 22 (p = 0.513). The distribution frequency between the both groups was similar. There was no difference regarding any other dichotomic classification of the groups (data not show).

Considering that age is one of the major risk factors for prostate cancer and BPH development, we evaluated the risk of the genotypes described above to develop these pathologies using logistic regression analysis, considering age as a continuous variable in the regression model (Table-3). The risk of developing BPH in individuals who have CAG > 21 compared to CAG ≤ 21 was 1.013 (95%CI 0.752-1.365; p = 0.932), whereas the comparison between individuals with CAG > 22 and CAG ≤ 22 showed a risk of 0.891 (95%CI 0.488-1.627; p = 0.708). Similarly, we found no difference regarding any other dichotomic classification of the groups (data not show).

Table 2 - Frequency distribution of CAG-21 and CAG-22 genotypes between BPH patients and controls.

	n	BPH n (%)	Controls n (%)	p*
CAG ≤ 21	102	58 (56.9%)	44 (43.1%)	0.352
CAG > 21	101	67 (66.3%)	44 (33.7%)	
CAG ≤ 22	131	77 (58.8%)	54 (41.2%)	0.513
CAG > 22	81	47 (58%)	34 (42%)	

Table 3 - Risk analysis for BPH of CAG-21 and CAG-22 genotypes

	BPH HR	95%CI	p*
CAG ≤ 21	1.013	0.752-1.365	0.932
CAG > 21			
CAG ≤ 22	0.891	0.488-1.627	0.708
CAG > 22			

DISCUSSION

Our results showed no association between AR CAG allele length and risk of developing BPH.

The AR is a transactivation factor that depends on the binding of a steroid hormone. This androgen-regulated transactivation activity is a key factor in the proliferation and differentiation of prostate cells. The polymorphic variation of the AR gene regulatory region, where the polymorphisms with the highest variation are located (CAG and GGC), may alter the transcriptional activity of the receptor (11).

In the present study, we found no significant difference in the number of CAG repeats between the control and BPH groups. The few studies that investigated AR CAG repeat and the risk to develop BPH have shown conflicting results. Our findings corroborate some data from the literature that also did not demonstrate differences between CAG repeat means in BPH patients and controls (16-18). AR CAG repeats were not associated with the risk to develop BPH, but shorter AR CAG repeats and PSA non-GG genotypes were significantly associated with decreased risk in BPH patients (19). However, in a recent case-control study of 416 BPH cases and 527 controls, CAG repeat length was associated with the risk of incidence of BPH (20). In another recent study conducted in Iranian patients, the mean number of CAG repeat in BPH patients was significantly smaller than normal (19.9 vs. 21.9; $p < 0.0001$) (21).

Studies suggest that the number of CAG and GGC repeats may be related to the ethnic group. In African-American populations, the mean number of repeats was demonstrated to be lower than among Caucasians (13,22,23). The same was found in certain African subpopulations (13,23,24), while the Asian population would have a larger mean number of repeats (23,25). This might partly account for the frequency distribution of prostate cancer in the different regions of the world. In the present study, the proportion of black individuals was small and homogeneously distributed among the BPH and control groups. A separate analysis taking this factor into account did not show any differences. Moreover, in our study, information on race (or skin color) was collected by the examiner, a method that is not sufficiently adequate to define race/ethnicity. In fact, in Brazil as a whole, it is particularly difficult to assess race, due to the extensive genetic heterogeneity observed and the overlap of genetic characteristics among Europeans, Africans, and Native Americans (26). However, the patients' color distribution was the same as the one found by Parra et al., who analyzed skin color and genomic ancestry in Brazilians and found an European dominance in the south of Brazil (27). Germans and Italians are the main immigrant groups in Rio Grande do Sul, a southern Brazilian state (28). According to the last demographic census, 87.5% of the state population was classified as white, 5% as black, 7% as brown, 0.1% as Asian, and 0.4% as Amerindian; whereas in Brazil, 53.7% are white, 6.2% are black, 38.4% are brown, 0.4% are Asian, and 0.4% are Amerindians (IBGE Census 2000; http://ibge.gov.br).

It is believed that AR activity is inversely correlated with CAG repeat length based on investigations conducted using reporter-systems containing viral promoters (11,13,14,29). Furthermore, recent new results about AR activity and CAG repeats have been demonstrated. Nenonen et al. demonstrated that the CAG repeat number is not inversely associated with AR activity in vitro when analyzing CAG lengths within normal range (16,22 and 28) in a reporter-assay with the human PSA promoter as target. Using β-galactosidase as transfection control, 22CAG had the highest activity compared with 16CAG and 28CAG, whereas using renilla-luciferase the authors found that 16CAG behaved similarly to 22CAG and 28CAG, showing lower activity (30).

CONCLUSIONS

Our results suggest that specific haplotype of AR is not essential to develop BPH. In conclusion, our data suggest no evidence for an association between AR CAG repeat length and BPH risk in a population-based sample of Brazilians.

ABBREVIATIONS

AR - Androgen Receptor
BPH - Benign Prostatic Hyperplasia
CI - Confidence Intervals
DHT - Dihydrotestosterone
DNA - Deoxyribonucleic Acid
HR - Hazard Ratio
kb - Kilo-base Pair
PCR - Polymerase Chain Reaction
PSA - Prostate-Specific Antigen
SPSS - Statistical Package for the Social Sciences

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CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Surgical and functional outcomes of sigmoid vaginoplasty among patients with variants of disorders of sex development

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ABSTRACT

Purpose: To assess the use of sigmoid colon in vaginal reconstruction of some patients with disorders of sex development.
Materials and methods: The study included 31 patients with disorders of sex development of various causes. All were reared as females. Female gender was decided for all cases after complete medical assessment. All patients underwent sigmoid vaginoplasty. Assessment of surgical and functional outcomes was carried out in a follow up period of up to 6 years.
Results: The preoperative diagnoses included mullerian aplasia (16 cases), androgen insensitivity syndrome (12 cases) and previous failed vaginoplasty (3 cases). Associated surgical procedures were gonadectomy in 5 cases and gonadectomy combined with clitoroplasty and vulvoplasty in 7 cases. No intra-operative or early postoperative complications occurred. A cosmetic neovagina with adequate size was achieved in all cases. Long term follow up showed introital stenosis in 4 cases (12.9 %). Two of them responded to vaginal dilatation. The third one needed y-v plasty while the fourth one presented by acute abdomen secondary to ruptured vagina and was submitted to urgent laparotomy. Mucosal prolapse occurred in 1 case (3.2%). Reoperation rate was 9.6%. Sexual satisfaction was achieved among 9 sexually active cases. The subjective satisfaction score of the surgical outcome was 8.03.
Conclusions: For patients with disorders of sex development of various etiologies, sigmoid vaginoplasty is the preferred technique for vaginal replacement. It is a safe technique that provides the patient with a cosmetic neovagina of adequate caliber and a satisfactory functional outcome.

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Key words:
Colon; Sigmoid;
Sex Reassignment Surgery;
Genitalia; Abnormalities;
Disorders of Sex Development

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INTRODUCTION

The reconstruction of a neovagina is indicated for cases of congenital absence of vagina, genetic sexual ambiguity and vaginal loss resulting from gynecologic cancer or post traumatic injury (1,2).

The ideal surgical technique for vaginoplasty is the one that can provide the patient with a vaginal vault of sufficient size, adequate introitus and an acceptable cosmetic external appearance (3).

Several surgical techniques were described for vaginal replacement. Baldwin was

the first one to describe the use of intestinal segment for vaginoplasty in 1904 (4). Depending on this principle, Wallace was able to use the sigmoid colon successfully in 1911 (5). Later on, this procedure was discontinued due to its high morbidity rate (6). In the last few decades, the use of bowel segment for vaginoplasty was reviewed including laparoscopic approach after the reported high complication rate of other methods of vaginal reconstruction (7). In this series, we evaluate the use of sigmoid colon for vaginal replacement among patients with disorders of sex development (DSD).

MATERIALS AND METHODS

The current study was conducted from February 2003 to May 2009 at Ain Shams University Hospitals, Cairo, Egypt. It included 31 patients submitted to sigmoid vaginoplasty. Their preoperative diagnosis was mullerian aplasia (16 cases), androgen insensitivity syndrome (12 cases) (5 cases were complete and 7 cases had incomplete androgen insensitivity) and 3 cases with previous failed vaginoplasty (by skin graft) done elsewhere for treatment of vaginal aplasia.

All patients were submitted to careful history taking and physical examination. Twenty one patients seeked medical treatment due to primary amenorrhea while 7 patients (with incomplete androgen insensitivity) due to ambiguous genitalia. The remaining 3 patients with failed previous vaginoplasty had a severely stenotic vagina

All patients (except secondary cases) underwent karyotyping, pelvi-abdominal ultrasonography, pelvic CT or MRI, endocrinal and psychiatric assessment. An informed written consent was obtained from all patients or their legal guardians. The study was approved by the ethical committee of Ain Shams University Hospitals, Cairo, Egypt. All patients underwent mechanical and antibiotic bowel preparation 48 hours prior to surgery.

Surgical procedure

Under general or epidural anesthesia and in an extended lithotomy position, creation of a large and enough space between rectum and bladder at the site of perineal dimple was performed using progressively larger Hegar dilators. Next, a midline subumbilical incision was performed to select, mobilizate and isolate a segment of 12-15 cm of the distal sigmoid and its vascular bed (Figures 1A and B). The remained colon was reanastomosed. The cul-de-sac was opened over a Hegar dilator pushed through the perineal route. The isolated colonic segment was closed by 2 layers at its proximal end that was fixed to the sacral promontory. The distal end was pulled thoroughout the abdomino-perineal pouch to the perineum where it was sutured to vulvar mucosa (Figure-2A). The peritoneum was closed above the transposed bowel and the neovagina was molded with a vaginal stent (loose vaseline vaginal pack) for 48 hours (Figure-2B).

Associated procedures included gonadectomy in all cases of androgen insensitivity syndrome and clitoroplasty with vulvoplasty in the 7 cases with incomplete androgen insensitivity syndrome.

Postoperatively, all patients underwent vaginal dilatation program in the form of self dilatation and irrigation daily for 10 weeks then weekly thereafter until the patient became sexually active.

Follow-up was done from 6 months to 6 years. Physical examination to assess vaginal length and width, cosmetic appearance of the neovagina and occurrence of any complications were performed. The grade of satisfaction of the surgical outcome was estimated by a subjective satisfaction score (range from 0 = very disappointed to 10 = satisfied) (8). Personal interviews (done by another urologist of our department) were carried out for assessment of the functional outcome among the sexually active patients. Vaginogram was performed in all patients at 6 months postoperatively. Vaginoscopy was performed annually in all patients for early detection of malignancy.

RESULTS

The current study included 31 patients aged 14-28 years (mean age 18.55 $\pm$3.63 years).

Figure 1 - (A): Isolation of 12-15 cm of sigmoid colon, (B): The bowel segment has been positioned to be anastomosed to vulvar mucosa.

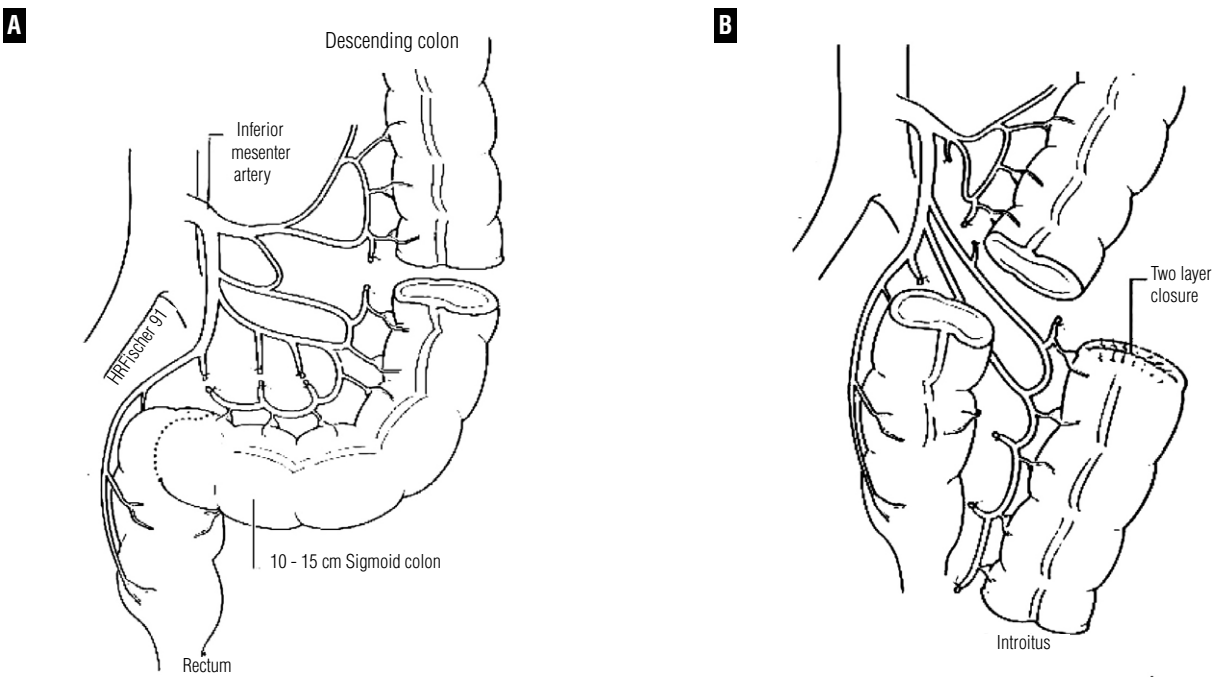
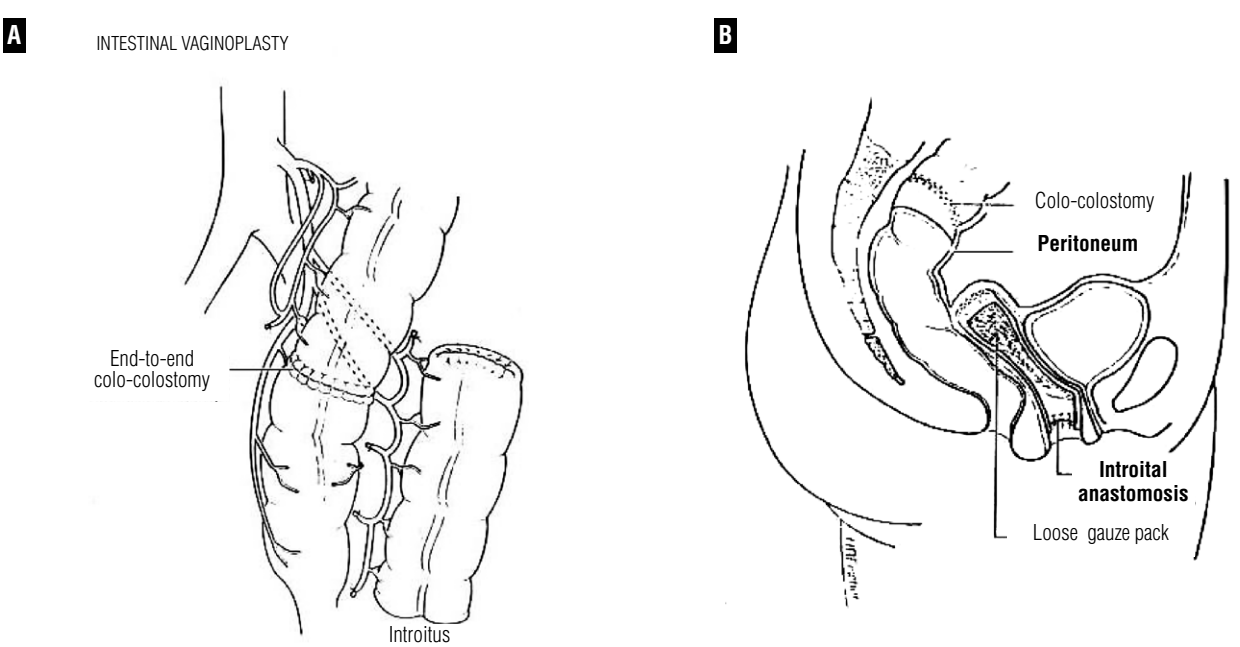


Figure 2 A and B - (A) Colocolostomy has been completed and the distal end of the colonic segment has been anastomosed to the opened rudimentary vaginal pit, (B): The peritoneum was closed above the transposed bowel and the neovagina was loosely packed with Vaseline gauze.



The preoperative evaluation revealed 16 cases with mullerian aplasia (Figure-3A). Their karyotype was 46XX. Hormonal profile regarding follicular stimulating hormone, luteinizing hormone and estradiol were normal according to age and sex matched reference ranges (9). On ultrasonography, a rudimentary or absent uterus was found. Another 12 cases were diagnosed as androgen insensitivity syndrome. All were reared as females. Their karyotype was 46XY. Inguinal gonads were felt on physical examination. Their serum testosterone level was elevated in comparison to normal age- and sex- matched reference ranges (10). Seven cases of them seeked medical attention due to ambiguous genitalia and were diagnosed as incomplete androgen insensitivity (Figure-3B). The other 5 cases who complained of primary amenorrhea were diagnosed as complete androgen insensitivity. No sexual ambiguity was found on their physical examination. The remaining 3 cases had a history of previous failed vaginoplasty. Their physical examination revealed a contractured stenotic vagina. Two of them had an ugly perineal scar (Figure-3C) secondary to previous failed vaginoplasty done by a full thickness groin skin graft.

All cases underwent sigmoid vaginoplasty. The mean operative time was 142 minutes (range 110-205 minutes). No intra-operative complications occurred and the postoperative period was uneventful. The patients were discharged from the hospital within 6 – 8 days.

During follow up, the neovagina was found to have an excellent cosmetic appearance (Figure-4). The mean vaginal length was 13.0 cm (range 10.5 – 15 cm). Vaginograms showed that all patients achieved a neovagina of adequate length and caliber (Figure-5).

Excessive vaginal discharge was found in all patients during the first 2 months postoperatively, however, it ceased markedly within the next 1 – 3 months. Three patients complained of malodor of the vaginal discharge that was managed conservatively by vaginal irrigation.

Figure 3 A to C - Some preoperative diagnoses of studied groups. (A): a case of mullerian aplasia, (B): a case of incomplete androgen insensitivity syndrome and (C): a recurrent case with stenosed vagina and scarred perineum.

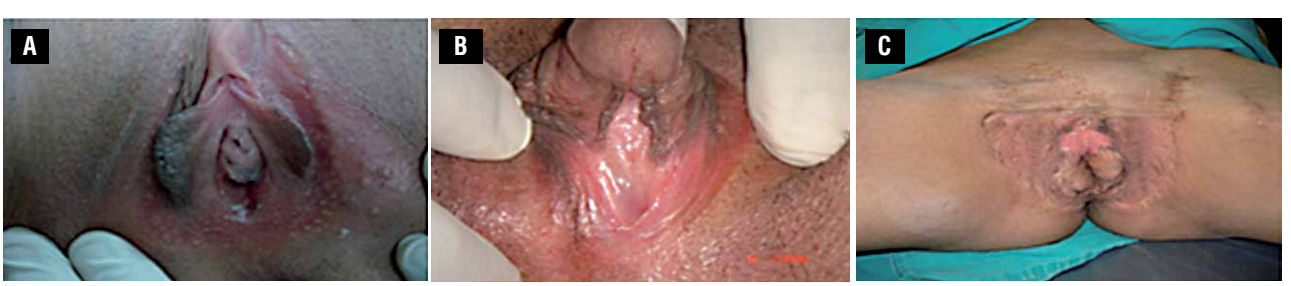


Figure 4 - A postoperative case of sigmoid vaginoplasty.

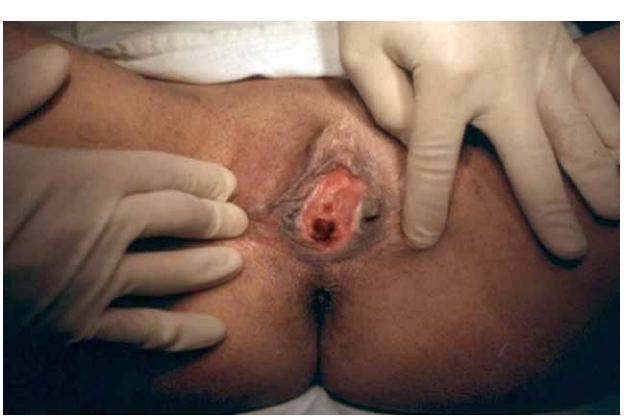
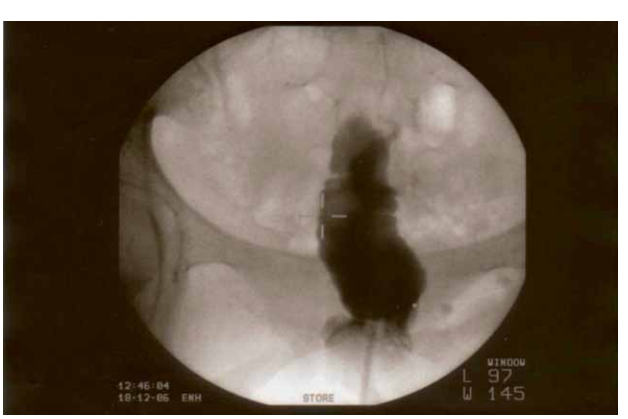


Figure 5 - Postoperative vaginogram after sigmoid vaginoplasty.



Long term follow up revealed that 4 patients (12.9%) had vaginal introital stenosis; 2 of those cases responded to dilation while one case needed y-v plasty. The remaining patient had an acute abdomen secondary to vaginal rupture that needed urgent laparotomy. Mucosal prolapse occurred in 1 patient that required surgical resection of the prolapsed tissue. The rate of surgical intervention for complications was 9.6%.

During follow up, most patients were satisfied with the surgical outcome. Mean satisfaction subjective score was 8.02 (range 6-9). Nine cases were sexually active and reported a satisfactory sexual life. Two cases with previous failed vaginoplasty regained their sexual activity 10 weeks postoperatively. Mild dyspareunia was experienced by 3 cases initially during the first few intercourses that progressively disappeared later on. None of the cases developed colitis or adenocarcinoma.

DISCUSSION

Proper diagnosis is essential for the management of patients with DSD. All patients in the current series were reared as females. After clinical, endocrinal and psychiatric assessments, female gender was confirmed and vaginal reconstruction was indicated for all of them.

Many methods of vaginal reconstruction were reported. The non operative technique which is known as Frank procedure can be used when a vaginal dimple or pouch is present and involves a progressive mechanical dilation using graduated hard dilators to create a progressive invagination of the vaginal dimple (11). Other surgical techniques reported depended on the creation of a perineal cleavage covered by a selected tissue. They include McIndoe procedure which involves insertion of a mold covered with split thickness skin graft taken from the buttocks into the created neovaginal space followed by postoperative vaginal dilation (12). Others used full thickness skin graft from the buttocks or skin flap based on labia majora (13), peritoneum from the Douglas pouch (14), amnion (15), oxidized regenerated cellulose fabric (16) and muscle flaps e.g. pudendale- thigh flap (17). The high percentage of vaginal stenosis, inadequate vaginal length, vaginal dryness and dyspareunia were reported as drawbacks of these techniques. Moreover, these modalities require long term vaginal dilatation and stenting by a vaginal mold at night which affects the patient`s psychological condition passively; these modalities must be avoided in pediatric age groups.

Intestinal segment either sigmoid, ileum or ileocecal segment can be used for vaginal replacement. Concerning the use of ileocecal segment for vaginoplasty, it is known that it can lead to metabolic disturbance and must be avoided in pediatric patients. Anatomically, the sigmoid colon is closest to the perineum and can be pulled easily with its vascular bed to the perineum and its diameter is sufficient for vaginoplasty without reconfiguration. On the other hand, the ileal mucosa was found to be more fragile and may lead to mild bleeding after intercourse. In addition, the mucus production by the ileum is more excessive and less lubricating than that of the sigmoid (18). Moreover, vaginal stenosis was found to be more frequent with ileal than sigmoid vaginoplasty (19).

In our series, no intra-operative or intestinal complications occurred. Increased morbidity with sigmoid vaginoplasty due to occurrence of intestinal leakage and intestinal obstruction was reported by another study in 3.2% of patients (18). Intestinal complications are rarely reported with sigmoid vaginoplasty and we suggest that preoperative bowel preparation, previous experience with bowel surgery and meticulous suturing are essential prerequisites to avoid such complications.

Four cases (12.9%) in this study developed stenosis at the mucocutaneous junction. Two of them were managed by progressive vaginal dilatation under anesthesia followed by regular weekly self dilatation. Another case was found to have a tight vaginal stenosis that required surgical intervention in the form of Y-V plasty. The fourth case presented in the emergency room with acute abdomen secondary to rupture vagina due to retained secretions (mucocele). Exploration revealed intraperitoneal rupture of the neovagina. Excision of the devitalized tissue, repair of the vagina, drainage of the peritoneum and dilatation of the stenosed vaginal orifice were done. Thus, regular follow up and vaginal dilatation are essential for prevention of such a hazardous complication. Variable incidence of introital

stenosis was reported with sigmoid vaginoplasty: (8.1% (18) and 19.3% (20)). The liability for anastomotic stricture can be decreased by selection of a bowel segment of adequate blood supply and length that can be mobilized and pulled easily to the perineum without tension, creation of a large enough space between bladder and rectum and generous anastomosis at the hymenal region. The need for regular vaginal dilatation after sigmoid vaginoplasty was advised by some authors (21) while others did not recommend it (22). When we analyzed our data, we found that all cases with vaginal stenosis were sexually inactive and reluctant to perform self vaginal dilatation .Therefore, we propose that regular postoperative dilatation of the anastomotic site till full sexual activity (once/week at least) is preferred to decrease the incidence of this complication.

In our series, one case of mucosal prolapse (3.2%) was observed and managed by surgical excision of redundant tissue. Another study reported mucosal prolapse in 7.6% of patients (21). Full thickness vaginal prolapse that required retroperitoneal fixation was reported by others (23). This complication rarely happens and can be avoided by selection of appropriate bowel segment length and proper fixation to the sacral promontory thus decreasing the incidence of this complication.

Excess mucosal discharge with malodour was reported by some authors in 8.3% of patients after rectosigmoid vaginoplasty (24) while others reported no mucous problem (25,26). In our series, this was not a problem as this secretion decreased markedly within 2-5 months postoperatively. Thereafter, the patients experienced just a moist vagina with no complaint. The malodor of vaginal secretions that was experienced by 3 patients was managed easily by regular vaginal irrigation 2-3 times weekly.

On vaginal examination of our patients, we found that all patients had an excellent cosmetic appearance of the neovagina with an adequate vaginal orifice. Vaginal calibration and vaginograms revealed an adequate size of the neovagina in all patients. Patients were satisfied with their surgical outcome. The estimated mean subjective satisfaction score was 8.03. Moreover, a cosmetic neovagina of adequate size was also obtained among the 3 recurrent cases with previous failed vaginoplasty using skin graft so we suggest the use of sigmoid vaginoplasty in secondary cases although the limited number of this patient`s category in our series. Other studies also reported good cosmetic and functional results of sigmoid vaginoplasty (22,27) whereas other authors reported periumbilical pain during intercourse in 5.6% of patients and considered this pain a disadvantage of this procedure (24). With assessment of the functional outcome among the 9 sexually active patients in our series, none of them complained of this pain whereas good results were obtained as all patients experienced intercourses that were satisfactory for them and for their partners. No significant dyspareunia or bleeding during intercourse was reported. None of the patients complained of vaginal dryness or need for external lubricants but instead they experienced a moist vagina due to the colonic mucosal secretion.

None of our patients developed ulcerative colitis which was reported in another study (28). Also, diversion colitis did not occur. This is a rare disorder of unknown etiology that may occur after isolation of an intestinal segment from the fecal stream (29). The risk of adenocarcinoma of the intestinal neovagina was reported and found to be rare and lower than the risk of cancer after Abbe-McIndoe procedure (30). None of our patients developed adenocarcinoma during the follow up period. Annual follow up is advisable for early detection of malignancy.

CONCLUSIONS

Our study revealed that sigmoid vaginoplasty can provide the patient with a self lubricating, esthetically pleasing neovagina of adequate size. It has a low complication rate and a low incidence of interoital stenosis with no need for daily vaginal dilatation or vaginal stenting by vaginal molds. Moreover, the reported functional outcome is excellent.

CONFLICT OF INTEREST

None declared.

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EDITORIAL COMMENT

The authors report on a retrospective study of high number of patients who underwent sigmoid vaginoplasty. They show, as do many others authors, that this surgery is effective. The cosmetic result is good and most often patients are able to have a normal sexual life. Despite there being no new information in this paper, the data presented add to the literature. However, only nine patients were sexually active and the time of follow is still not long enough to catch some complications. Outcomes that should be evaluated with this procedure include: cosmetics, vaginal discharge of mucus, adequate canal vaginal diameter, absence of dispareunia and orgasm.

Cosmetics results are usually good with this procedure, because the bowel-vaginal anastomosis is generally concealed. The authors performed a midline abdominal incision. However, abdominal scars have also to be counted in the cosmetic consideration. This procedure can be easily carried out with a low transverse abdominal incision or even by laparoscopy, regardless of the age of the patients. Vaginal mucus is commonly transitory and the vaginal diameter generally accomodates penetration without discomfort. Four patients had vaginal introital stenosis. The authors did not mention the age of the patients during surgery or whether or not they were sexually active. Also, the time of diagnosis of stenosis is not reported. When performing this procedure in sexually active patients, the vaginal penetration acts as a natural dilation and helps reduce this kind of complication. The authors reported that 9 cases were sexually active and that all had a satisfactory sexual life. The term satisfactory is vague, and information regarding number of sexual relations per month and the presence of orgasm should be stated in future trials.

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EDITORIAL COMMENT

Congenital genitourinary and intersex disorders such as Mayer-Rokitansky-Kuster-Hauser syndrome, androgen insensitivity, congenital adrenal hyperplasia, and gonadal dysgenesis result in vaginal agenesis or atresia (1). Attainment long-term functional and cosmetic outcomes with low morbidity makes reconstructive procedures a challenge for surgeons. The recommended non-surgical Frank method by the American College of Obstetrics and Gynecology is not applicable in all cases because of the requirement for long period of vaginal dilations and subsequent surgical treatment in most of treated patients (2,3).

The technique used in this research by Nowier and colleagues is based on the use of bowel graft to create a neovagina and was first described by Baldwin in 1904 (4). The authors reported their experience on the treatment of 31 patients aged 14-28 years with variants of disorders of sex development using sigmoid vaginoplasty with acceptable follow up period. The paper is worth reading as it reports functional and sexual data of this procedure. The authors address a useful technical point to fix the proximal end of the neovagina to the sacral promontory in order to prevent prolapse.

Sigmoid colon is the most common segment used when there is isolated vaginal absence. This technique is more complex than other reconstructive vaginoplasty surgeries; however,

improvements in technique and postoperative care permitted the use of sigmoid graft as first-line surgical therapy (3). The optimal timing of surgery remains a source of controversy. Many surgeons who favor the use of bowel for neovagina reconstruction do not believe that vaginoplasty should be deferred until the patient reaches adulthood (5).

One of the advantages of this procedure is the possibility of coitus shortly after surgery; in comparison to the technique of passive dilation which required an average delay of 12 months (6). Carrard et al. evaluated sexuality results of the procedure with the use of two standardized questionnaires: the Female Sexual Function Index (FSFI) and the revised Female Sexual Distress Scale (FSDS-R). They concluded that this technique provides a nearly normal sexual function for patients. Also they showed that psychologic distress related to sexuality persists in most cases and suggested a multidisciplinary support for them (3).

Herein we can appreciate an important study improving our knowledge regarding the outcome of this technique. Further studies should investigate the long term follow up with more number of cases.

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ORIGINAL ARTICLE

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Current outcome of prioritized patients for kidney transplantation

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ABSTRACT

Purpose: To analyze the outcome of deceased donor recipients given priority in allocation due to lack of access for dialysis and compare this data to the one obtained from non-prioritized deceased donor kidney transplant recipients.
Materials and Methods: we reviewed electronic charts of 31 patients submitted to kidney transplantation that were given priority in transplantation program due to lack of access for dialysis from January 2005 to December 2008. Immunological and surgical complications rates, and grafts and patients survival rates were analyzed. These data were compared to those obtained from 100 regular patients who underwent kidney transplantation without allocation priority during the same period.
Results: Overall surgical complication rate was 25.8% and 27% in the patients with priority in allocation and in the non-prioritized patients, respectively. There was no statistical significant difference for surgical complications (p = 1.0), immunological complications (p = 0.21) and graft survival (p = 0.19) rates between the groups. However, patient survival rate was statistically significant worse in prioritized patients (p = 0.05).
Conclusions: patients given priority in allocation owing to lack of access for dialysis have higher mortality rate when compared to those non-prioritized.

ARTICLE INFO

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Dialysis; health priorities; kidney; renal insufficiency; transplantation

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INTRODUCTION

Kidney transplantation is the gold standard treatment for end-stage renal disease (ESRD). However, the imbalance between organ supply and demand makes the implementation of equitable and effective organ allocation systems a major concern. In the United States, despite the increasing number of kidney transplants, more patients with ESRD are dying while waiting treatment (1). In developing countries, the situation is even worse, because only 6.2% of renal chronic patients are submitted to kidney transplantation (2).

Criteria for admission of patients to the waiting list, donor selection, tissue-typing methods, organ preservation and immunosuppressive protocols are the focus of intense debate in the literature (3-5). Efforts have been made to shorten the waiting time, to adjust for rare HLA phenotypes and homozygous and to guarantee an acceptable HLA match distribution in order to optimize the overall transplant success rate. Despite these efforts, kidney transplantation in select groups of patients may be performed with significant delay.

Priority in allocation of patients with ESRD is still a controversial subject. The best

time for kidney transplantation of highly sensitized patients or for those who lack access for dialysis is still to be determined. Lack of access for dialysis is one of the criteria for priority in allocation to be given to patients for kidney transplantation. A patient is considered to be candidate for allocation priority when all but one access for dialysis had been unsuccessfully exploited. In our service, a patient is prioritized for transplantation when there is no more arteriovenous fistula and all veins (internal jugular vein, subclavian vein, and femoral vein) are no more available for catheter placement due to thrombosis. In summary, the patient is prioritized when he is in hemodialysis and the lost of his catheter will let him without access for dialysis. The aim of this study was to analyze the outcome of the kidney transplantation in patients put in priority in allocation owing to lack of access for dialysis and to compare complication rate as well as graft and patient survival rates to non-prioritized ones.

MATERIALS AND METHODS

We reviewed electronic charts of 31 prioritized patients for kidney transplantation due to the lack of access for dialysis from January 2005 to December 2008. These patients were compared to 100 non-prioritized ones, submitted to kidney transplantation during the same period. Only patients receiving their first graft from deceased donors were included in this analysis. Highly sensitized patients and patients with incomplete data or irregular follow-up were excluded. To compare similar groups, case-matched study was conducted, where study and control groups had the same time in dialysis (mean: 6.7 years) and similar donors and recipients mismatch. Basically, immunosuppression was achieved with the triple-drug regimen, based on tacrolimus, mycophenolate mofetil, and prednisone.

All grafts were positioned retroperitoneally through an extended inguinal incision. Vascular reconstruction was performed at the external iliac vessels. The urinary tract was reconstructed using the non-standardized Gregoir technique. Drains were not routinely used. Fascial closure was performed with running 0-0 polyglycolic acid sutures. Demographic data were analyzed with T-test. Complications rates were analyzed with Chi-square test or Fisher´s Exact test. Both graft and patient survival rates were studied with the Kaplan-Meier survival curve and Logrank for survival comparison. Results were expressed in mean, standard deviation and range. Two-tailed values of $p < 0.05$ were considered statistically significant. The mean follow-up was 37.7 (range 25 – 57) months, 36.0 months (range 25 – 57) in prioritized patients and 38.2 (range 27 – 52) months in non-prioritized ones.

RESULTS

Demographic data are summarized in Table-1. There was no statistically significant difference between the groups, regarding demographic data. Overall surgical complication rate was 25.8% in the group of patients given priority in allocation. Eight surgical complications occurred in 7 (22%) patients. Early and late surgical complications in prioritized patients were: three cases of vesicoureteral reflux with symptomatic urinary tract infection that were successfully treated with bulking agent injection, two cases of retroperitoneal hematoma that were surgically removed, one incisional hernia that was surgically repaired, one lymphocele that was surgically treated and one acute urinary retention that was treated with transurethral resection of the prostate. Eight (25%) patients had humoral rejection and two (6.5%) had the acute cellular one. Overall surgical complication rate in non-prioritized patients was 27%. Twenty-seven complications occurred in 24 patients (24%). Early and late complications in the control group were: five vesicoureteral reflux with symptomatic urinary tract infection that were treated with bulking agent injection, one surgical site hematoma that was surgically drained, nine incisional hernias that were surgically repaired, three lymphoceles that were surgically treated, four urinary fistulas treated through ureteral reimplantation, one urethral stenosis that required internal urethrotomy and four renal artery stenosis that were successfully treated by angioplasty. Eighteen (18%) patients had humoral rejection and two (2%) patients had acute cellular one. The differences of surgical complication rate and graft rejection rate were not statistically significant between the groups as showed in Table-1.

Table 1 – Demographic data and post-operative complications.

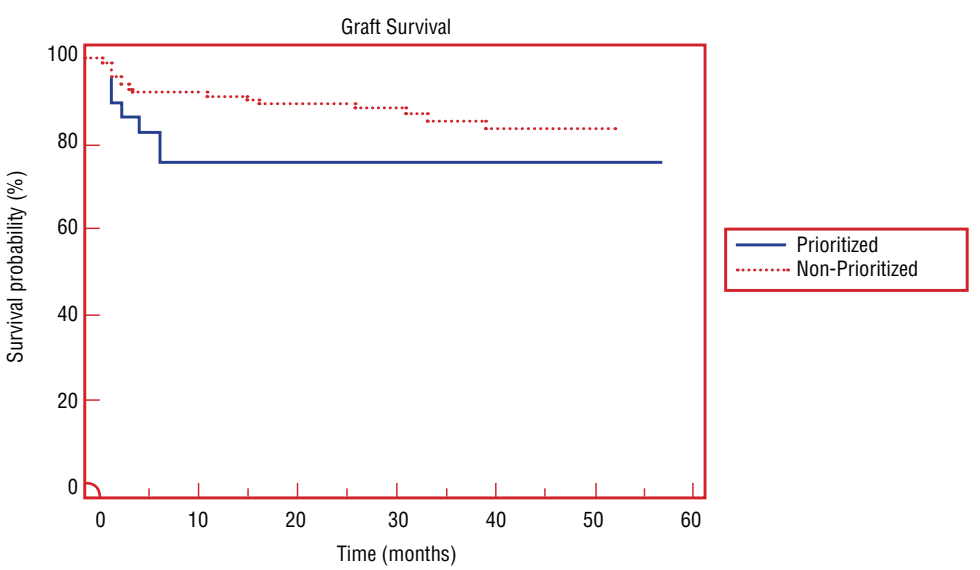
	Prioritized		Non-Prioritized		p
Number of Patients	31	(23.6%)	100	(76.4%)	-
Gender (F)	20	(64.5%)	49	(49.0%)	0.13
Age at Transplantation (years)	42.1 ± 15.7	(10 - 67)	47.0 ± 14.6	(6 - 75)	0.13
Cold Ischemia Time	18.1 ± 10.9	(10 - 44)	17. 1 ± 13.4	(10 -36)	0.18
Follow-up (months)	36.0 ± 8.0	(25 - 57)	38.2 ± 7.2	(27 - 52)	0.24
Complications (n)	18	(58.0%)	47	(47.0%)	0.28
Total Rejection	**10**		**20**		**0.21**
Humoral rejection	8	(25.8%)	18	(18.0%)	0.34
Celular rejection	2	(6.5%)	2	(2.0%)	0.23
Total Surgical	**8**	**(25.8%)**	**27**	**(27.0%)**	**1.0**
Vesicoureteral reflux	3	(9.7%)	5	(5.0%)	0.39
Hematoma	2	(6.5%)	1	(1.0%)	0.13
Incisional Hernia	1	(3.2%)	9	(9.0%)	0.45
Voiding dysfunction	1	(3.2%)	0	-	0.23
Lymphocele	1	(3.2%)	3	(3.0%)	1.0
Ureteral Leak / stenosis	0	-	4	(4.0%)	0.57
Urethral stenosis	0	-	1	(1.0%)	1.0
Renal artery stenosis	0	-	4	(4.0%)	0.57

Seven (22%) prioritized patients and fourteen (14%) non-prioritized patients had graft loss, respectively. The causes of graft loss in prioritized and non-prioritized patients are synthesized in Table-2. There was no statistical significant difference for graft survival rate between the groups (p = 0.19) – Figure-1. Regarding patients' outcome, eight (25.8%) prioritized patients and 12 (12%) non-prioritized patients died, respectively. The causes of death in prioritized patients were: six sepses, one uremia by lack of access for dialyses, and one bleeding. The causes of death in control group were: eleven sepses, and one pulmonary thromboembolism. The patient survival rate was

Table 2 – Graft loss causes.

	Prioritized	Non-Prioritized
Total	7	14
Acute rejection	3	5
Renal tumor	1	0
Renal rupture	1	1
Venous thrombosis	1	1
Arterial thrombosis	0	1
Polyomavirus infection	1	2
Would infection with abscess	0	2
Bleeding	0	1
Glomerulosclerosis recurrence	0	1

Figure 1 – Graft survival.

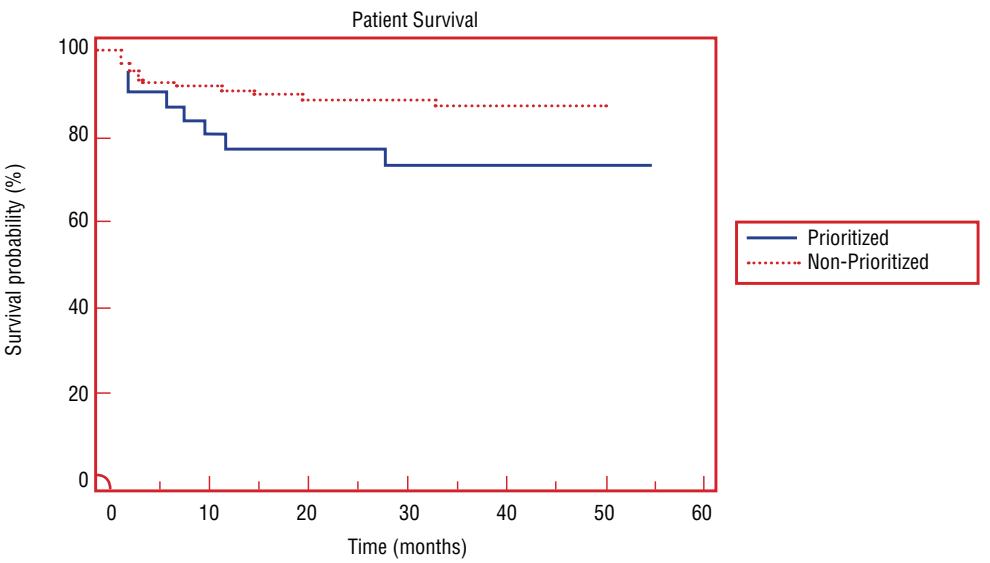


statistically significantly worse in prioritized patient (p = 0.05) – Figure-2.

DISCUSSION

In our study, prioritized patients for kidney transplantation owing to absence of access for dialysis had worse outcome when compared to non-prioritized ones. Their graft survival rate in 5-years was similar, but prioritized ones had earlier graft dysfunction. Moreover, prioritized patients had significant lower survival rate. The worst outcome of prioritized patients is probably due to several factors, including clinical and surgical conditions at the time of kidney transplantation. Our data may not guarantee that earlier

Figure 2 – Patient survival.



prioritization will put these patients in a better situation for transplantation, but it is an option. Perhaps prioritized patients may be benefited from earlier transplantation.

Great results achieved by kidney transplant programs have been motivating health care providers to search for new strategies to increase the number of deceased and living kidney donors and to find the best way to allocate these organs. In the United States, for single kidney transplants performed prior to 2008, patient survival rates at 5 years were 91% for recipients of living donors kidneys, 84% for non-expanded criteria deceased donors, and 72% for expanded criteria deceased donors (1). As a result of these good numbers, kidney transplantation centers have been increased around world; in the Latin America, kidney transplant rate increased from 3.7 per million population in 1987 to 15.4 per million population in 2006 (6).

Today, the rules to determine which candidate will receive an available organ are based on a score system that takes into account histocompatibility, blood group, age, clinical need, waiting time, negative crossmatch, and sensitization. Poli et al. (7) studied the factors impacting on deceased kidney graft survival and function, and after a multivariate analysis of a number of immunological, clinical, social, and administrative factors on transplant outcome concluded that younger donors, absence of pre-transplant transfusion, patient dialysis center and level of HLA match have a statistically significant positive association with excellent graft function at 4 years.

Dolan et al. (8) conducted an interesting study about how people wish to give priority based on certain characteristics of potential recipient of a donor kidney. Between the respondents, there was a clear consensus that one of the most important considerations is what will happen to the patient without the treatment, and so priority was given to those with poor prognosis. There was also a strong view that priority should be given to younger patients and to those with dependents. The time spent waiting for a transplant is also important, but less so. According to our results and taking into account the data published by Dolan et al., the patients with lack of access should be prioritized earlier, once the delay in kidney transplantation results in greater mortality rate. Maybe

a better communication between nephrologists, urologists, and dialysis centers could put these patients in a better situation in the waiting list.

CONCLUSION

Patients given priority in allocation due to lack of access for dialysis have higher mortality rate when compared to those non-prioritized.

CONFLICT OF INTEREST

None declared.

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EDITORIAL COMMENT

The authors, who have ample experience with renal transplant, compared the data from a period of four years of 31 patients prioritized for transplant due to lack of access for dialysis and of 100 'regular' patients.

The retrospective study concluded that mortality in the group of prioritized patients was higher. The authors were unable to identify the cause of this difference, but it could have been caused by differences in the populations; not all of which were identified or analyzed.

In any regard, the data points out the need for reviewing the criteria of patient prioritization for renal transplant, which is the merit of this study. Perhaps, as the authors pointed out, the patients could attain better outcomes if they received the transplant prior to the critical moment of the last access for dialysis. Alternatively, the prioritization criteria should be discarded as we are faced with this dire numerical picture of transplants in Brazil.

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ORIGINAL ARTICLE

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Contributing factors to complications and surgical success in mouse kidney transplantation

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ABSTRACT

Purpose: Mouse kidney transplantation is a challenging technique for novice microsurgeons. Factors that affect transplant outcomes for a clinical surgeon starting microsurgery have not yet been investigated.
Materials and Methods: 110 consecutive mouse kidney transplants were performed over a 9-month period. Data were recorded, and surgical results and complication were analyzed.
Results: Three and thirty day survival rates improved from 0 (0/6) to 92.3% (12/13) between months 1 and 9. Bleeding, arterial thrombosis, kidney failure and hydronephrosis were the most common causes of transplant failure. From month 1 to month 7, using the same surgical technique, practice significantly decreased the incidence of bleeding and increased the 3-day survival rate; however, it didn't significantly decrease the incidence of thrombosis, kidney failure, but improved the 30-day survival rate. From month 8, when surgical technique used on artery anastomosis switched from continuous suture to interrupted suture, surgical survival rate at 3 and 30 days improved significantly. Interestingly, ischemia time was not a significant factor determining the success of transplantation in this study.
Conclusions: Practice is essential for novice microsurgeons, and the choice of surgical techniques significantly affects surgical results. The use of interrupted arterial sutures can significantly improve mouse kidney transplantation outcomes compared with continuous sutures. Ischemic time was not a factor in determining successful of kidney transplantation in mice in this study.

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Key words:
Kidney transplantation; mice; urologic surgical procedures; male; survival rate

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INTRODUCTION

Mouse kidney transplantation is an ideal model for transplant research because genetically modified strains can be used to study the molecular mechanisms of renal allograft injury (1,2). However, due to the technical complexity and high mortality rates, only a handful transplant centers uses this model for research. The first mouse kidney transplantation was described by Skoskiewicz et al. in 1973 (3), later improved by Zhang and Han (4,5). To become familiar with the technique, reduce complications and increase survival rate, a relatively long learning curve is needed even for experienced microsurgeons (6).

Although there are papers describing the procedure of mouse kidney transplantation in detail, the factors that contribute to successful transplantation have not yet been evidenced-based investigated (7). In this report, a surgeon with 10 years of experience in cardiothoracic surgery started his microsurgery training on mouse. Data from the first 110 mouse kidney transplants were collected to investigate the factors contributing to successful mouse kidney transplantation. An understanding of these factors will be helpful for future researchers who plan to use this model.

MATERIALS AND METHODS

Animals

Male C57BL/6 and BALB/c mice, 6-8 weeks old and weighing between 18 and 25 g, were used for kidney transplantations. Mice were bred and maintained in the animal care facility at the William Middleton VA Hospital in Madison, Wisconsin. Procedures were performed in accordance with the Animal Care Policies at the VA Hospital and the University of Wisconsin. In total, 110 mouse kidney transplantations were performed between February and October of 2010. Surgical times, complications and results of each of the surgeries were recorded and analyzed.

Anesthesia and surgical techniques

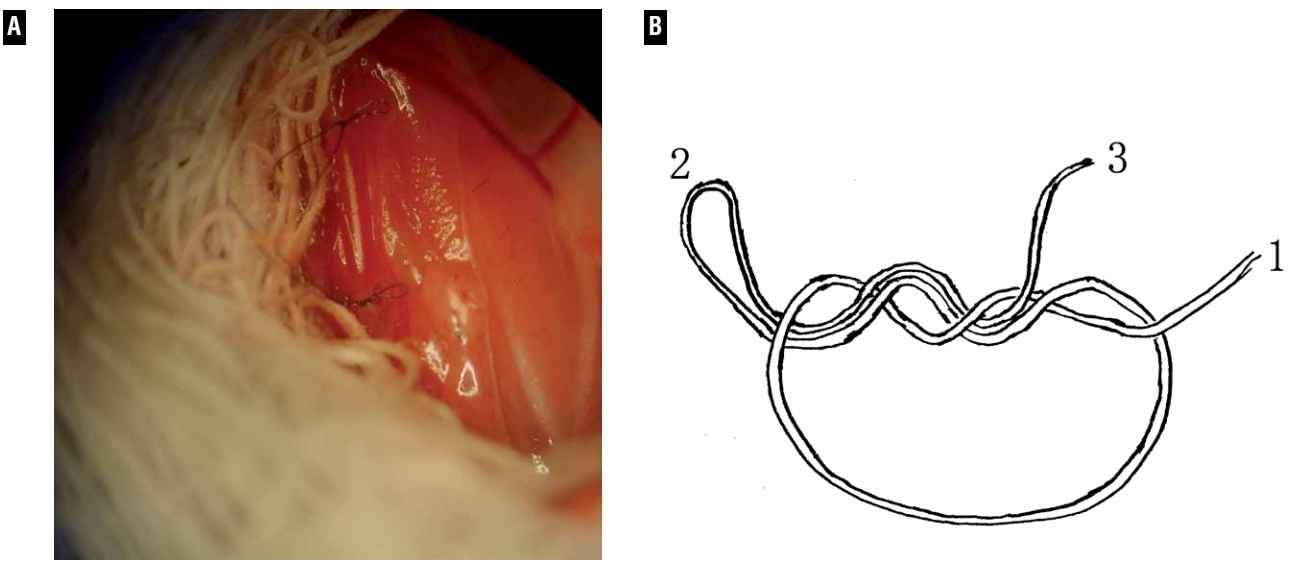
Mice were anesthetized with Isoflurane (2-3%). The transplants were performed using the technique described by Zhang and Han (4,5). All procedures were performed at 4-25X magnification using a microscope (SMZ800, Nikon, Japan) and standard microsurgical instruments.

Briefly, a midline abdomen incision was performed. The left kidney was exposed by moving the intestine, spleen and stomach to the right side. The suprarenal and infrarenal aorta and inferior vena cava (IVC) were dissected with ligation and division of the attached lumbar branches. The aorta and IVC were then dissected apart below the renal pedicle for a length of 3-4 mm. The adrenal and testicular vessels were divided and ligated with 10-0 nylon sutures (ARO Surgical, CA, USA). These ties could be used to orient the graft during the procedure of transplantation. The kidney was then separated from perinephric fat and tissues. The left ureter was dissected free down to the bladder preserving the periureteral vessels and cut with a small bladder patch. Using an 8-0 nylon suture (ARO Surgical, CA, USA), the infrarenal aorta and IVC were ligated, and the same procedure was done to the suprarenal aorta and IVC. The IVC was then transected below the renal vein. The donor kidney was then perfused via the infrarenal aorta with 1 mL cold heparinized normal saline (100 U/ mL). The aorta was cut approximately 2 mm below the renal artery. The kidney and its vascular supply was harvested with the ureter attached to the bladder patch en bloc, and then stored in 4°C normal saline until the time of transplantation.

A two-stage procedure was performed in the recipients. First, the left native kidney was removed and the donor kidney was transplanted into the recipient. Second, the contralateral native kidney was removed on post-transplantation day 10. Briefly, with a midline abdomen incision, left native kidney was removed after the vascular pedicle and ureter were ligated. The infrarenal aorta and IVC were isolated carefully and cross clamped using two 8-0 suture releasable knots (Figure-1A). The use of releasable knot can provide more room in the surgical field, and less mobilization and dissection is needed. Other benefits included less cost and very easy to tie the knot and release it (Figure-1B). Small elliptical aortotomy and venotomy were made. End-to-side vascular anastomoses were performed. A running 11-0 nylon suture (ARO Surgical, CA, USA) was used in the first continuous 86 transplantation surgeries from month 1 to 7. The last 24 surgeries at month 8 and 9 used interrupted suture for the artery anastomosis. Donor vein and recipient IVC was connected using continuous 11-0 sutures. Once the venous anastomosis was completed, the releasable knots were released and the kidney graft was perfused instantly when the anastomosis was successful.

Urinary reconstruction was established with ureteral implantation technique with some modification (5). Briefly, the recipient bladder was pierced with a fine tweezers (Dumont Twee-

Figure 1 - A, The infrarenal aorta and IVC were cross clamped using two 8-0 suture releasable knot instead of micro clamps. B, Sketch of releasable knot. Pull end 1 and 2 tighten the knot, pull end 3 release the knot.



zers #5, WPI Co. USA) and the donor ureter was pulled through the bladder. Periureteral tissue was fixed to the exterior wall of the bladder using 10-0 sutures. 2-3 mm length of the donor ureter was left and allowed to retract inside the bladder before closing the contralateral pierced wound.

Transplanted mice were monitored daily. Three and thirty-day recipient survival were counted and analyzed. According to the proposal, kidneys, urine and blood of thirty-day survival recipient were harvested for further examination. Following the harvest, mice were euthanized by exsanguination.

Statistical Analysis

Multivariable analysis was used to examine the effects of the various variables including practice (month), bleeding, thrombosis and kidney failure on surgical survival. Surgical times are presented as mean ± standard deviation, and statistical significance was assessed using unpaired t test. Fisher's exact test was used for the comparison of different anastomosis method in artery. All data analyses were conducted using SPSS v 17.0 (SPSS, Chicago IL), and $P < 0.05$ was considered statistically significant.

RESULTS

Surgical results and complications

One hundred and ten consecutive kidney transplants were performed between February and October of 2010. Three and thirty day's survival rate improved from 0 to 92.3%, and 0 to 92.3% respectively (Table-1).

Causes of death or euthanasia included bleeding, thrombosis, kidney failure, urine leakage, vena cava complication, and hydronephrosis. Bleeding, thrombosis and kidney failure accounted for over 70% of the complications in the first 3 months of training (Table-1).

Bleeding was the cause of nearly all deaths in the first two months of training. With the improvement in microsurgical technique and use of Surgicel®, the incidence of bleeding decreased rapidly after the 3rd month, accounting

Table 1 - Surgical results by month.

Month	Surgery	Bleeding (rate)	Thrombosis (rate)	Kidney failure (rate)	3-day survival (rate)	30-day survival (rate)
1	6	5 (83.3%)	1 (16.7%)	-	0	0
2	16	10 (62.5%)	3 (19.0%)	-	3 (19.0%)	0
3	15	3 (20.0%)	7 (46.7%)	-	4 (26.7%)	3 (20.0%)
4	16	2 (12.5%)	6 (37.5%)	1 (6.3%)	8 (50.0%)	4 (25.0%)
5	15	2 (13.3%)	1 (6.7%)	4 (26.7%)	12 (80.0%)	2 (13.3%)
6	8	2 (25.0%)	3 (37.5%)	0	3 (37.5%)	2 (25.0%)
7	10	0	3 (30.0%)	6 (60.0%)	7 (70.0%)	1 (10.0%)
8	11	1 (9.1%)	1 (9.1%)	1 (9.1%)	8 (72.7%)*	7 (63.6%)*
9	13	1 (7.7%)	0	0	12 (92.3%)	12 (92.3%)

From month 1 to month 7, the same surgical technique was used, there was significant effect of practice on bleeding (P = 0.017) and 3-day survival (P = 0.025), but no significant difference was found of practice on thrombosis and kidney failure (P = 0.767 and 0.080 respectively). From month 8, artery anastomosis switched from running suture to interrupted suture. 3- and 30-day survival rate increased significantly (* Compared with month 7, $P < 0.05$).

for only 10 to 20% of all subsequent deaths. The second most common complication was thrombosis, which remained a significant obstacle until month 8, when a switch to interrupted sutures on artery anastomosis improved the results. The third more prevalent complication was kidney failure. In these mice, transplantations were successful when grafts perfused very well, and the graft was pink and the renal artery patency could be observed on the second stage surgery (bilateral nephrectomy), which indicated that graft perfusion existed. However, the mice died 2 or 3 days later after the second stage surgery. In month 7, 60% mice died due to this complication. There were five cases of hydronephrosis in all surviving mice, and urine leak caused serious sickness in two mice.

Effects of practice on complications

Surgical results are always dependent on practice. In these serial surgeries, different months represented different proficiency of surgical practice. Because of the use of the same surgical procedure from month 1 to month 7, data were analyzed to determine whether practice (different month) could decrease the incidence of different complications. As indicated in Table-1, there was a significant effect of practice on bleeding (P = 0.017). However, no significant difference was found of practice on thrombosis or kidney failure (P = 0.767 and 0.080 respectively).

Association between practice (month) and surgical survival

Since survival is the most important endpoint in microsurgery, the data was investigated further to determine the association between the practice (month) and the survival rate. From month 1 to month 7, the same surgical technique was used (continuous artery anastomosis). The effect of practice on surgical survival rates was analyzed. Findings showed that practice improved the 3-day survival rate (P = 0.025). How-

ever, there was no better change in the elevation of 30-day survival rate (P = 0.221), which indicated that other factors may be involved in the improvement of the long-term survival rate.

Difference between using continuous and interrupted suture on artery anastomosis

From month 8, interrupted artery suture method was used. Obvious difference was found at 30-day survival rate. Data of month 7 and month 8 which used continuous and interrupted suture in artery anastomosis was analyzed separately. The use of interrupted suture method resulted in a higher 30-day survival rate than using the continuous suture method; this difference was probably due to a lower incidence of kidney failure in the interrupted suture method group (Table-2). Significant difference was also found in 3-day survival rate between these two methods (P = 0.012). There was no significant difference in the warm ischemia time between these two methods (Table-2).

Surgical Time

With practice, surgical time on donor and recipient reduced to 50 minutes and 100 minutes respectively (Figure-2A). The total ischemia time (TIT) in the transplantation consisted of two parts: cold saline preservation time (Cold ischemia time, CIT) and warm ischemia time (WIT). CIT was the time duration of donor kidney preserved in cold saline. WIT was the time duration to complete the vessel anastomosis between donor kidney and recipient, out from the cold saline. Practice could decrease the ischemia times gradually (Figure-2B), and there was significantly difference (P < 0.05).

Impact of ischemia time on the results

One of the most important factors impacting transplantation results is ischemia time. When the continuous artery anastomosis technique was used (from month 1 to month 7), the ischemia time differed among the groups of 30-day survival, thrombosis and kidney failure. It seemed as if there was a relationship among the groups, but there were no significant differences. Ischemia time is important, but it was not associated with surgical survival in this procedure (Table-3).

Table 2 - Surgical results of different method of artery anastomosis.

Method	Number	Bleeding (rate)	Thrombosis (rate)	Kidney failure (rate)	WIT	3-day survival (rate)	30-day survival (rate)
Continuous suture	10	0	3 (30.0%)	6 (60.0%)	29.58 ± 5.12	7 (70.0%)	1 (10.0%)
Interrupted suture	11	1 (9.1%)	1 (9.1%)*	1(9.1%) *	31.67 ± 6.12	8 (72.7%)*	7 (63.6%)*

Kidney transplantation data using interrupted suture in Month 8 was compared with the use of continuous suture in Month 7. Interrupted suture method significantly decreased the incidence of thrombosis and kidney failure, and significantly improved surgical survival rate. There was no significant difference on warm ischemia time (p = 0.398). *: P < 0.05, Compared with continuous suture group. WIT: Warm ischemia time. Time duration on vessel anastomosis (out of cold saline preservation).

DISCUSSION

The mouse kidney transplantation model is challenging for microsurgeons and has a steep learning curve. In addition to technical requirements, the beginner should also understand variable factors including ischemia time, complications and suturing methods which are related to surgical outcomes. Here we described our experience in 110 consecutive mouse kidney transplants.

Figure 2 A and B - (A) Surgical time in mouse kidney transplantation, STR: Surgical time on recipient, STD: Surgical time on donor, TIT: Total ischemia time. (B) Ischemia time in mouse kidney transplantation. TIT: Total ischemia time, CIT: Cold ischemia time, WIT: Warm ischemia time.

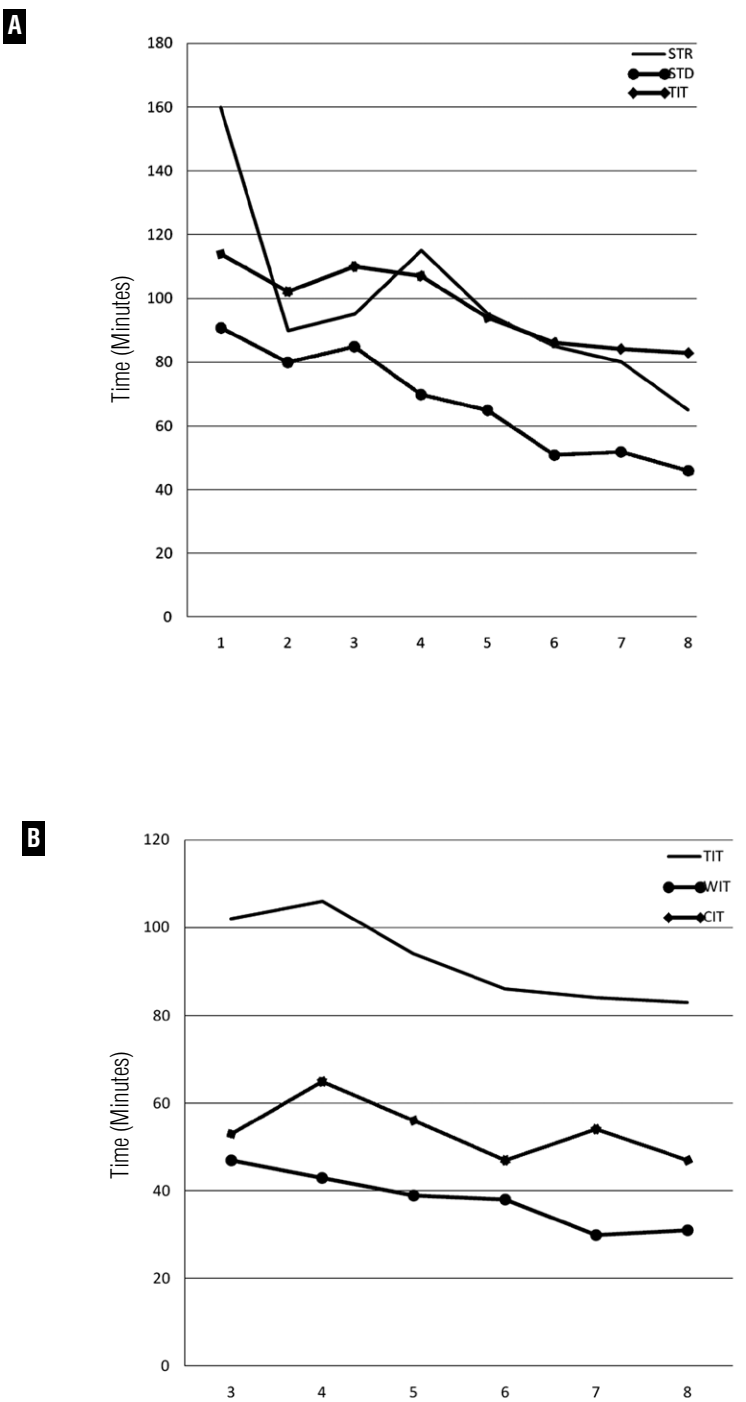


Table 3 - Ischemia times in different groups.

	TIT	p	WIT	P	CIT	p
Thrombosis	97.50 ± 17.77	-	37.50 ± 10.34	-	60.42 ± 19.12	-
Kidney failure	85.77 ± 17.67	# 0.057	34.62 ± 7.217	# 0.397	51.54 ± 9.12	# 0.116
30-day survival	82.50 ± 8.80	* 0.052 ** 0.657	34.17±5.85	* 0.433 ** 0.914	46.67 ± 6.06	* 0.054 ** 0.476

No significant differences were found among mice of thrombosis, kidney failure and 30-day survival on various ischemia times.
#: Compared with group of thrombosis. *: Compared with group of thrombosis.
**: Compared with group of kidney failure.
TIT (Total ischemia time): Time duration without perfusion. WIT (Warm ischemia time): Time duration on vessel anastomosis (out of cold saline preserve). CIT (Cold ischemia time): Time duration preserved in cold saline.

As a 10-year experienced cardiothoracic surgeon, microsurgery on mouse was still a challenge work which amalgamating manual precision and technical experience in equal measure. For the author, it took nearly 2 months and 30 mice surgery to get the first long-term survival kidney transplantation mouse. Practice is one of the basic factors for surgical results in surgical training. In this study, practice could significantly decrease the incidence of bleeding and increase surgical short-term survival rate. The condition was similar in clinical surgeon training. However, no statistical difference was found of practice on long-term survival rate and complications including thrombosis and kidney failure. This may due to surgical techniques used in the suture of artery. When the suture method was switched from continuous to interrupted sutures on artery anastomosis, the incidence of thrombosis and kidney failure decreased significantly, and the long-term survival rate improved significantly. This indicated that in microsurgery procedure, besides practice, selection of the right surgical technique was equally important for the surgical results.

Successful mouse kidney transplantation is a surgical masterpiece. A variety of complications can lead to death or surgical failure. In our operation, surgical complications included bleeding, thrombosis, kidney failure, hydronephrosis, urine leakage, vena cava complication (stenosis and distortion) and breakdown of the skin suture. Bleeding is the most frequent cause of early postoperative mortality early in the learning curve. Mice have low tolerance to blood loss because of their relatively small blood volume. For a 20g mouse, 0.14 mL blood loss (10% total blood volume) can cause early postoperative mortality. In addition to improvement of microsurgical technique, Surgicel® is an effective material that prevents bleeding. Thrombosis of the arterial anastomosis is another significant problem in mice kidney transplantation, and it was the most common technical complication after bleeding in this project. Similar to the experience of other researchers (6), we also found that mismatch of the recipient aortotomy and donor aorta was a factor leading to arterial thrombosis. Researchers should consider the factors that lead to complications when developing standard operating procedures.

Ureter implantation technique was used to reconstruct the ureter-bladder connection. This technique could significantly reduce ureter attachment time and the incidence of urine leakage (5). A common complication in this study was hydronephrosis, with a total of five cases in survived mice. Among the five cases, four cases appeared in Month 5 which was part of the reason resulting in low 30-day survival rate with high 3-day

survival rate. The obstruction was always located in the junction of the ureter-bladder connection. This complication occurred when continuous suture was used. We expected that this suture method would prevent urine leakage. However, there was no urine leak in mice using three interrupted stitches around the junction of ureter-bladder connection, and also there were no hydronephrosis cases when this sewing method was used.

Ischemia time is one of the important factors impacting transplantation results (8). Ischemia itself can cause severe damage because of the potential for a build-up of metabolic wastes. Reperfusion after a period of ischemia can actually be more damage than the ischemia itself. Ischemia-reperfusion results in vascular edema and leukocytes building up in small capillaries and obstructing them which accelerated the process of thrombosis and renal failure after transplantation. In this study, the average total ischemia time ranged from 65 to 135 minutes, and warm ischemia time from 20 to 55 minutes, but there were no significant difference among 30-day survival, thrombosis and kidney failure mice. This shows that within a certain range, ischemia time was not the key factor for successful kidney transplantation. The same result was found by Tian (9), who found that the kidney graft can be stored in a cold solution for more than 2.5 hours, and there was no significant difference in success rate compared with a kidney graft stored for less than 1 hour.

Kidney failure is one of the most common complications in our procedure. The transplanted kidney looked pink with the renal artery pulse observed before closing the abdomen incision, and even on day 10 of post-transplant. However, the mice died 2 or 3 days after the other native kidney was took out. In this study, no relationship was found between kidney failure and ischemia time. When interrupted artery anastomosis was used, complication of kidney failure decreased significantly. Making the artery anastomosis with continuous suture would be more time-efficient and could decrease bleeding. But it may form a stenosis ring around the anastomosis site and limit the relaxation of the vessel. This is a significant problem for microvessels. The caliber of the mouse aorta is extremely small (< 0.4 mm), so a 0.1 mm diameter change in a 0.4 mm diameter vessel can result in a nearly 60% cross-sectional area decrease. But in surgery, the 0.1 mm is a very small change. After bilateral nephrectomy, blood flow can't increase proportionately because of the constriction of the continuous suture. In addition, the constriction ring could lead to formation of micro-thrombosis. As hypothesized, the incidence of kidney failure decreased significantly after adoption of interrupted suture. For the anastomosis of artery in small animal like mouse, the experience of this study indicated that interrupted suture method would be a better choice compared with continuous suture.

In summary, successful mouse kidney transplantation is a challenging microsurgical procedure. In our experience, practice is the base of microsurgery success, and surgical techniques choice can change the surgical results significantly. There are some factors such as practice and the use of interrupted arterial sutures that are essential to prevent bleeding, thrombosis and kidney failure, and to increase the long-term survival rate. In this study, however, ischemic time was not a factor in determining the success of kidney transplantation in mice.

CONFLICT OF INTEREST

None declared.

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EDITORIAL COMMENT

The basic research is so important as clinical trials, but few studies have been published about some topics.

Kidney transplant in mouse model is quite useful and valuable for studying transplant immunology and surgery techniques. However, the technical difficulty and high mortality are a huge obstacle in its widespread use.

Animal models are commonly used to study the pathogenesis of acute and chronic diseases. The use of mouse as models are much valuable in researches because the development of gene knockout and transgenic animals can lead to better control of the studies.

In this study the authors evidenced which factors affected kidney transplants in a mouse model. Although in recent review of the literature (1), Ge and Gong, found that the warm and cold ischemia time should be less than 35 five minutes and 2.5 hours, this study evidenced that ischemic time was not a factor in determining the successful of kidney transplantation.

The information obtained by the authors in this study have contributed to improve what Zheng et al. have had describe (2), no matter the mouse kidney transplants successful have still a challenge.

The renal failure treatment by transplants have had excellent outcomes nowadays, although these outcomes, physicians who have been working in this Field keep on their researches up to date as the way to improve the outcomes of transplants, so the data showed in this article will help them to improve their basic researches.

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EDITORIAL COMMENT

Clinical kidney transplantation has become the preferred method of renal replacement therapy worldwide. Testing new pharmacological and biological agents, and identifying new methods to diagnose and treat rejection depends on preclinical investigation. The use of large animal models such as the monkey, dog, cat, pig, etc. in transplant research has become problematic due to high cost, the need for specialized animal care, longer gestation and life spans, and cultural sensitivities regarding certain species.

The rat is of sufficient size (300-400 grams) to permit solid organ transplant experiments using microsurgical techniques, but has been less well characterized than the mouse in molecular biology. However, the adult mouse (30-50 grams) is 5-10 times smaller than the rat, more difficult to perform microsurgery, and ultimately more prone to technical failures (1).

The mouse model has been used for research since there are more than 1300 mutant mice with which to study the biology of diseases, particularly the complexity of the immune system (2). Indeed, the mouse has many inherent advantages: low cost, no specialized animal care, short gestation, short life spans, and no culture sensitivities. The mouse model of a kidney transplant was first reported in 1973 by Skoskiewicz and associates (3). However, the demanding techniques and mortality rates have impeded widespread use of this model.

The learning curve in kidney transplantation in mice is substantially longer than in rats and the mortality rate is as high as 50%, even in the hands of experienced microsurgeons and long-term survival rates are sparse. Most animals died due to surgical related complications.

The end-to-side procedure for vascular reconstruction is widely used for establishing a mouse model of kidney transplant. Continuous sutures are preferred for vascular anastomosis with high success (4-8).

The authors describe a change in the surgical technique carrying out the arterial anastomosis with an interrupted suture which factor was responsible for the highest survival index at the thirtieth day, probably due to a lesser incidence of kidney failure without however altering the time of warm isquemia. This technique must be reproduced in future studies aiming to diminish technical failures consequently high levels of animal mortality, with the objective to stimulate a wider use of such kidney transplants as models for a long term follow-up.

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ORIGINAL ARTICLE

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Comparison of the efficacy and safety of topical diltiazem and nitroglycerine for pain relief during transrectal ultrasound guided biopsy of the prostate

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ABSTRACT

Introduction and Objective: Transrectal ultrasound biopsy of prostate is a painful procedure. The introduction of the rectal probe is one of the major contributors to the pain associated with this procedure. Drugs that relax the anal sphincter should theoretically decrease this pain. This study was done to compare the efficacy and safety of two topical medications that relax the anal sphincter, diltiazem and nitroglycerine, in decreasing the pain associated with transrectal ultrasound guided prostate biopsy.

Materials and Methods: 66 patients who were to undergo their first prostate biopsy were randomized to receive either 2 mL of 2% topical diltiazem or 2 mL of 0.2% topical nitroglycerine or placebo 20 minutes before prostate biopsy. All patients also received 15 mL of intrarectal lignocaine. A 10-point visual analogue score was used to record the pain immediately after the insertion of the probe, during biopsy and at the end of the procedure.

Results: The pain scores due to probe insertion, during biopsy and at the end of the procedure in patients who received topical diltiazem or nitroglycerine were significantly lower compared to the placebo group ($p < 0.001$). There were no significant differences in the pain scores between the patients receiving diltiazem compared to those receiving nitroglycerine. Higher incidence of headache and fall in blood pressure was noted in patients who received nitroglycerine compared to those receiving diltiazem.

Conclusion: Topical diltiazem and nitroglycerine are equally effective in reducing the pain associated with transrectal prostatic biopsy. Diltiazem is safer compared to nitroglycerine.

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Prostate; biopsy; analgesia; lignocaine; diltiazem; nitroglycerin

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INTRODUCTION

Transrectal ultrasound guided biopsy (TRUS-Bx) of the prostate is an established method for the diagnosis of prostatic cancer. The procedure is most commonly performed on an out-patient basis. It is an invasive procedure and is associated with pain thus requiring some form of analgesia (1,2). The pain associated with the procedure has two main causes, first the insertion of the transducer of the ultrasound into the rectum and second the pain due to insertion of the biopsy needle into the prostate (3,4). Numerous methods and agents have been tried in the past to decrease this pain. These can range from topical agents, oral agents, inhalational agents, injections, etc. Topical, intrarectal application of lignocaine is a commonly used modality for this procedure as it anaesthetises the

rectal mucosa and the prostatic nerves. Periprostatic nerve block (PPNB) with lignocaine infiltration is now being promoted as the standard form of anaesthesia for this procedure. However, periprostatic injection of lignocaine which is given bilaterally to cause nerve block may itself be associated with significant pain and can make the whole procedure uncomfortable for the patient (5). PPNB is ineffective in decreasing the pain associated with the insertion of the transducer of the ultrasound into the rectum. It has been found in various randomized trials that the pain of insertion of probe is actually the most painful part of this procedure (6,7). Relaxing the anal sphincter may help reduce this pain. Theoretically, combining a drug which provides analgesia (e.g. topical lignocaine) with a drug that relaxes the anal sphincter can provide optimum pain control during TRUS-Bx of the prostate. Diltiazem and nitroglycerine have been used topically to decrease sphincter tone in patients with anal fissures (8,9). Thus, these drugs may help decrease the pain of TRUS-Bx of the prostate. We, in this study, evaluated and compared the efficacy and safety of the topical application of diltiazem with nitroglycerine in controlling the pain associated with different stages of the prostatic biopsy.

MATERIALS AND METHODS

The study was conducted from December 2010 to August 2011. Ethical clearance was obtained from the institutional ethical committee. In all, 66 patients, who were to undergo their first prostate biopsy, were enrolled in the study. These patients either had an elevated prostate specific antigen (PSA) level or/and abnormal digital rectal examination. Each of the participants provided informed and written consent for the study. Exclusion criteria included history of previous transrectal prostate biopsy, chronic prostatitis, prostatodynia, chronic pelvic pain, bleeding diathesis, anticoagulation therapy, anorectal conditions like haemorrhoids, anal fissure, anal stenosis, allergy to lignocaine, diltiazem or nitroglycerine, neurological conditions, recent use of phosphodiasterase-5 inhibitors, recent use of narcotics, analgesics or antiplatelet drugs, systolic blood pressure of less than 100 mm Hg and severe hepatic, cardiac or renal disease. Antibiotic, in the form of oral ciprofloxacin 500 mg twice daily, was started from the day before the procedure and was continued until the fifth post-procedural day. Cleansing enema was given in the morning of the biopsy.

The patients were randomized by the help of a computer program that generated random numbers in three groups. The patients' serial number decided the type of analgesia received i.e. intrarectal administration of either 2 mL of placebo (group 1) or 2 mL of 2% diltiazem gel (group 2) or 2 mL of 0.2% nitroglycerine gel (group 3). All patients received 15 mL of topical lignocaine gel intrarectally. The gels were applied by another operator (S.N.M.). The gels were applied 20 minutes before the biopsy. First, placebo or diltiazem or nitroglycerine was administered following which 15 mL of lignocaine gel was administered. The patients did not know about the medication they had received.

All biopsies were performed by a single operator (T.J.) who was blinded regarding the type of medication received by the patients. The biopsies were taken in the left lateral decubitus position using an 18-G, 26 cm long, tru-cut biopsy needle driven by a biopsy gun. A 7 MHz ultrasound probe was used for guiding the prostate biopsies (Shimatzu, SDU-450 XL). In all cases, 12 core biopsy samples were taken in a schematic manner. The pain score using the standard ten-point visual analogue scale was done, scored 0 meaning no pain, 1-3 meaning mild, 4-6 moderate, 7-9 severe and 10 meaning unbearable pain. The score was done during the insertion of the probe, during the biopsy per se and at the end of the procedure (to determine the overall acceptability of the procedure). This simultaneous assessment of the pain score was done to avoid recall bias. The vitals of the patients were recorded and they were observed for any complications during and after the study (for 72 hours).

The statistical analysis was done using SPSS Statistical data editor (version 17). Due to the non-parametric nature of the data, median values were calculated. The Mann Whitney test and ANOVA were used to calculate the statistical

significance in the study groups. A p value of less than 0.05 was considered to be significant.

RESULTS

The median age, volume of the prostate, PSA levels and time taken during biopsy were similar in all the groups (Table-1).

During the insertion of the probe, the patients who received either diltiazem or nitroglycerine with lignocaine reported significantly less pain scores compared to those who received placebo with lignocaine ($p < 0.001$). The pain scores at the time of biopsy and at the end of procedure too were significantly lower in the patients who received diltiazem or nitroglycerine with lignocaine compared to those receiving placebo and lignocaine ($p < 0.001$). There were no significant differences between the pain scores at the time of insertion of probe, during the biopsy or at the end of the procedure, between the patients receiving diltiazem with lignocaine when compared to those receiving nitroglycerine with lignocaine (Table-2).

Two of the twenty two patients (9%) who received nitroglycerine reported headache within the first hour following biopsy. The blood pressure of these patients reduced to a systolic pressure of less than 90 mm of Hg. The patients were managed by bed rest, limb elevation and fluid infusion. There were no such complications in patients who received diltiazem or placebo. Complications like hematuria, hematospermia, mild fever and dysuria were noted in 9 patients out of the total study population (13%).

Table 1 - Characteristics of the patients included in the study.

Variable	Placebo and lignocaine (group 1)	Diltiazem and lignocaine (group 2)	Nitroglycerine and lignocaine (group 3)	p value (ANOVA)
Number of patients	22	22	22	-
Median Age in years (range)	61 (55 - 78)	64 (54 - 74)	62 (50 - 74)	0.58
Median volume of the prostate in mL (range)	69.5 (48 - 96)	67 (47 - 93)	64 (50 - 96)	0.82
Median PSA (ng/mL) level (range)	11 (4.2 - 21)	12.6 (4 - 22)	9.8 (4 - 24)	0.76
Time (seconds) taken for biopsy (range)	223 (208 - 256)	228 (209 - 239)	224 (210 - 242)	0.9

Table 2 - Pain scores in the three groups during transrectal biopsy of the prostate.

Variable	Placebo and lignocaine (group 1)	Diltiazem and lignocaine(group 2)	Nitroglycerine and lignocaine (group 3)	p1	p2	p3
Median VAS score during probe insertion (range)	5 (3 - 7)	2 (1 - 4)	2 (1 - 4)	< 0.001	< 0.001	0.35
Median VAS score during biopsy per se (range)	5 (4 - 7)	3 (1 - 4)	3 (1 - 4)	< 0.001	< 0.001	0.13
Median VAS score at the end of the procedure (range)	5 (4 - 8)	3 (1 - 4)	2 (1 - 4)	< 0.001	< 0.001	0.29

p calculated by Mann Whitney test. p1: (group 1 v/s group 2); p2 (group 1 v/s group 3); p3 (group 2 v/s group 3).

DISCUSSION

Prostate cancer is the most common malignancy in aging men in the Western world. Prostate biopsy is the gold standard exam to identify the patients harbouring the malignancy. With the increasing use of screening for prostatic cancer with the help of digital rectal examination and serum PSA levels, more and more patients are being subjected to prostate biopsy which is most commonly performed under ultrasound guidance using a rectal transducer. TRUS guided prostatic biopsy is an invasive procedure associated with pain and thus requires analgesia/anaesthesia in some form (1,2). The analgesia can be provided by topical, oral, inhalational or injectable agents. Topically applied local analgesics are often used to alleviate this pain as they can be easily administered. Lignocaine, following topic intrarectal application, diffuses into the periprostatic tissue through the rectal wall and blocks the periprostatic nerves which carry the pain sensation. It has been reported in most studies to reduce the pain associated with the TRUS biopsy by more than 50% (10). Prilocaine and lignocaine mixture has also been used and has been shown to have good results (4). Controversy exists regarding the efficacy of non-steroid anti- inflammatory agents in reducing the pain of biopsy. Oral agents like rofecoxib have not been found to be effective while on the other hand, diclofenac suppositories, one hour before the biopsy have been shown to reduce the pain of biopsy (11,12). Sedation with agents like entonox, midazolam, meperidine, propofol etc. have also been tried but they need strict monitoring as they may risk the patient to cardiopulmonary depression (13-15). Soloway et al., in the year 2000, introduced another method of blockage of the periprostatic nerves with the help of lignocaine infiltration known as PPNB (16). Numerous trials have been conducted which have found PPNB to significantly reduce the pain of prostatic biopsy but few trials have shown that lignocaine gel is as effective as PPNB while a couple of other trials have shown that PPNB is not effective in pain control (17-19). This is probably related to the technique of the operator and the pain threshold of the study population (6). PPNB has also been reported to increase the pain of prostatic biopsy as it requires intrarectal injections. Pudendal nerve block has also been tried, albeit occasionally, with good success, either alone or in combination with PPNB.

Pain during prostate biopsy has two components, one due to the insertion of probe (due to the stretching of the anal sphincter) and another due to the biopsy per se. Luscombe et al. found that the pain of insertion of the probe was equally bad or even worse than the pain of biopsy in 27% of the patients (7). Raber et al. too confirmed this finding and showed that 15% of men had pain scores of 6 at the time of probe insertion which is significantly high (20). This component of pain is not taken care of by either topical application or infiltration of lignocaine (6,21). Thus, combining pharmaceutical agents that can cause periprostatic nerve block e.g. lignocaine and those which can relax the anal sphincter can reduce the pain associated with prostatic biopsy.

Diltiazem and nitroglycerine have been topically used in the treatment of anal fissures. They relax the anal sphincter, decrease the pain and promote healing of the fissure (8,9). They are commonly used drugs for this indication and are easily available. The notion that this action of these drugs can be of use during the prostate biopsy led to their evaluation in the present study. We used lignocaine gel which can block the periprostatic nerves and thus decrease the pain of needle insertion and nitroglycerine/diltiazem which relax the anal sphincter thus decreasing the pain due to insertion of the probe. Though there are a few studies available which have used nitroglycerine, ours is the first study that evaluates the efficacy and safety of diltiazem and also compares it with nitroglycerine (6,22). Our study also addresses the issue of recall bias which has been often quoted as an issue in the previous studies as pain scores were assessed at each step of the procedure.

Our study found that the pain at the time of insertion of the rectal probe is significantly reduced by application of either diltiazem or nitroglycerine gel along with lignocaine. Median pain scores reduced from 5 (moderate, according to the VAS grading) in the placebo arm to 2 (mild, according to the VAS grading) in the diltiazem or nitroglycerine arm ($p < 0.001$). The pain at the time of biopsy per se was

also reduced in our study in patients who had an application of diltiazem or nitroglycerine gel compared to the placebo arm ($p < 0.001$). The reason for this is that at the time of biopsy, the operator has to position the probe either at apex, middle or base of the prostate. This probe manoeuvring places the sphincter on stretch and causes pain. Diltiazem and nitroglycerine decrease this component and thus decrease the pain associated with the biopsy. The patients who had diltiazem or nitroglycerine application reported significantly less pain scores at the end of the procedure.

Our study found that two patients who received nitroglycerine reported headache and their systolic blood pressure fell to values less than 90 mm of Hg. On the other hand, patients who received diltiazem did not have any such side effects. Thus, in our study, diltiazem appears to be safer compared to nitroglycerine, though no conclusive statistical inference can be drawn on this aspect.

CONCLUSIONS

Our study proves that topical diltiazem and nitroglycerine are better than placebo in reducing the pain associated with the prostate biopsy. Both these drugs are equally effective in reducing the pain. Diltiazem appears to be safer as compared to nitroglycerine due to absence of significant side effects and may be preferred.

CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Clinical and laboratorial study of HPV infection in men infected with HIV

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ABSTRACT

Objectives: To determine the prevalence of precursor lesions of penile cancer, to establish the concordance of diagnostic techniques (PCR, Hybrid Capture (HC) and peniscopy with acetic acid 5%) in the diagnosis of Human Papilloma Virus (HPV) of the penis of men infected with HIV and to evaluate the influence of the immune status.
Patients, Methods and Results: 276 men were studied, with a median age of 34.6 years. Prevalence of High Risk HPV, Low Risk HPV and infection with both, according to HC, was 43%, 32% and 22%, respectively. PCR showed 50% of positivity for HPV DNA. Peniscopy was positive in 27% of individuals. Peniscopy showed good specificity and low sensitivity for the detection of penile HPV, and low concordance with PCR. Men with white lesions had a 3.6 higher relative risk of positivity for HPV. The most common clinical lesion observed was vegetation, identified in 29% of patients. PCR and HC techniques showed high sensitivity for HPV DNA and there was an excellent correlation between them. Immunosuppressed individuals with CD4< 200 cells/mm3 had the highest prevalence of pre-malignant lesions that were observed in 10% of the studied individuals.
Conclusions: Peniscopy was important for identification and treatment of subclinical lesions. PCR and HC techniques were sensitive methods for the detection of HPV DNA with high concordance. Severely immunosuppressed individuals showed a higher prevalence of pre-malignant lesions of the penis.

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Key words:
DNA Probes; HPV; Men; HIV

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INTRODUCTION

In the literature, several studies point out that 10% to 20% of sexually active adults have HPV infection, although only 1% presents classic condyloma and 2% visible lesions after acetic acid application (1). According to world literature, it is rational to expect the existence of 3 to 6 million males infected with HPV (2).

The relationship between HPV infection and cervical cancer is well established and there are strong evidences that it may also be implicated in the etiology of anal and genital cancer (3).

The studies related to the determination of prevalence of HPV infection in males are very important, since they can present a subclinical and asymptomatic infection and become potential source of infection of HPV to their male or female sexual partners (4).

In view of all these facts, our study was designed to determine the prevalence of precursor lesions of penile cancer, to establish the concordance among different diagnostic techniques (PCR, Hybrid Capture (HC) and Peniscopy with acetic acid 5%) in the diagnosis of infection with the Human Papilloma Virus (HPV) of the penis of

HIV positive males and to evaluate the influence of the immunologic status on the occurrence of the lesions.

MATERIAIS AND METHODS

This is a cross-sectional descriptive study of men affected with HIV attended at the Fundação de Medicina Tropical Dr. Heitor Vieira Dourado (FMT-HVD). Data including demographic, epidemiologic and clinical characteristics of patients were collected. Physical examination (urological inspection and peniscopy with acetic acid 5%), molecular biological tests (PCR in house and Hybrid Capture II, Digene & Co®) and conventional histopathology study were also performed. Criteria for inclusion were: HIV positive males, with ≥ 18 years old, who provided written informed consent to join the study. Criteria for exclusion were: HIV negative males, Indians, psychiatric patients and those that didn't complete all steps of the study. Data were collected at Epi Info® version 6.04 platform and the statistical analysis was made through Statistical Package for Social Sciences® (SPSS) version 16.0 for Windows.

Patients lied in supine position in order to be submitted to the urological and scrotal inspection. A surgical brush with saline was rubbed against the foreskin, balanopreputial sulcus, glans and navicular fossa of the penis. The brush was immersed in an Eppendorf vial containing 1 mL of T1 buffer (commercial kit for nuclear extraction Spin Tissue-Macherey-Nagel®). The vial was tightly closed and sent to the laboratory, where was maintained at -70ºC until PCR analysis.

Another brush was used to Hybrid Capture (HC) for High and Low Risk HPV, and it was stored in an appropriate kit.

After the cytological collection, we proceeded with peniscopy and penile and scrotal inspection. A gauze soaked with acetic acid 5% was placed around the penis for 10 minutes. Positive lesions (white lesions) were biopsied, except in patients with previous histopathological or laboratorial diagnosis or that didn't allow the procedure. The samples were fixed in buffered formalin 10% and were sent to histopathological studies.

For statistical analysis, it was used Pearson's Chi-Square test with Yates correction whenever necessary; Fisher's exact test was used to categorical variables for values under 5 and significance analysis including Odds Ratio (OR) and 95% confidence intervals was performed. Significance was established for $p < 0.05$ (5%).

For concordance analysis, it was used the kappa (k) associative test.

RESULTS

Two hundred and seventy six HIV-positive male patients older than 18 years old were included. Median age was 34.6 years. Table-1 depicts socio-demographics characteristics of patients. Table-2 shows the variables related to sexual behavior, use of condoms and previous STDs and Table-3 those related to HIV virus.

Peniscopy was positive for white lesions in 27% of patients. The most frequent lesion was vegetation (29%) (Table-4). Biopsy was obtained in 22% of participants; some of them had more than one lesion and a total of 75 fragments of skin were collected for conventional histopathological study (Table-5). Pre-malignant lesions were observed in 10% of patients (Table-6), and most of them (59%) had CD4 < 200 cells/mm3.

According to HC, the prevalence for High Risk, Low Risk and both High and Low Risk HPV infection were 43%, 32% and 22%, respectively. PCR had 50% of positivity for HPV DNA.

The concordance between peniscopy and PCR was observed in 62% of samples, revealing a "weak concordance" according to kappa associative test (k = 0.2317). Patients with white lesions observed at peniscopy had a 3.6 higher risk of HPV infection.

Peniscopy was considered a diagnostic test with high specificity (86%) and low sensitivity (37%). Positive and negative predictive values were 73% and 58%, respectively. Exam accuracy was 62%.

When PCR and HC for High Risk and Low Risk HPV results were compared, 88% of samples showed similar results. There was an "excellent concordance" between the different techniques according to kappa associative test (k = 0.7522).

Table 1 - Socio-demographic variables of 276 men with HIV+/AIDS.

Demographic indices	N	%
Age		
18-29 years	101	36
30-49 years	151	55
≥ 50 years	24	9
Race/Colour		
Brown	207	75
White	47	17
Black	18	7
Yellow	4	1
Marital status		
Single	158	57
Married	53	19
Fixed partner	56	20
Divorced	7	3
Widow	2	2
Education		
Illiterate	2	1
Primary	97	35
Secondary	125	45
Terciary	52	19
Use of unlawfully drugs		
Yes	66	24
No	210	76
Smoking		
Yes	75	27
No	201	73

Table 2 - Distribution of sexual behavior variables, use of condoms and previous STDs of 276 HIV+/AIDS males.

Variables	N	%
Sexual Orientation		
Homosexual	101	36
Heterosexual	107	39
Bisexual	68	25
Beginning of sexual activity		
≤ 15 years	105	55
15-19 years	151	38
≥ 20 years	20	7
Nº of sexual partners during last year		
0	24	9
1	97	35
2-9	93	34
≥ 10	62	22
Use of condom prior to HIV+		
Occasionally	230	85
Always	7	3
Never	39	14
Use of condom after HIV+		
No sexual relations	35	13
Occasionally	28	10
Always	206	75
Never	7	2
Prior STDs*		
Yes	177	64
No	99	36

*sexually transmitted diseases.

Table 3 - Variables associated to the presence of HIV virus.

Variables	N	%
Time to HIV diagnosis		
≤ 3 years	198	72
4-6 years	38	14
7-9 years	15	5
≥ 10 years	25	9
Opportunistic Diseases Associated to HIV		
Yes	134	48
No	142	52
Use of ART*		
Yes	152	55
No	124	45
ART* time of use		
< 1 year	79	52
1-2 years	16	10
2-3 years	12	8
> 3 years	45	30
Lymphocyte count TCD4		
< 200	103	37
200-349	68	25
350-500	41	15
> 500	40	15
No exam	24	9
Viral load		
Undetectable	68	25
< 30.000	108	39
> 30.000	71	26
Superior limit (> 500.000)	4	1
No exam	25	9
HIV infection phase		
AIDS	179	65
Carrier	97	35

*antiretroviral therapy

Table 4 - Distribution of dermatological lesions observed in 75 positive peniscopies. After: Rook's 2010 (5).

Peniscopy Lesion	N	(%)
Vegetation	25	29
White lesion	13	15
Ulcer	10	12
Papule normochromic	13	15
Crust	1	1
Papule Hypochromic	4	5
Papule Hyperchromic	4	5
Macula hypochromic	9	10
Macula hyperchromic	3	4
Eritema	1	1
Hyperchromic Plate	2	2
Total	85	100

DISCUSSION

The prevalence of HPV in our study was higher than of the study of Goldstone et al. They evaluated 602 HIV negative males who were engaged in sex with other males and observed a prevalence of 18.2% of HPV infection of the penis using also PCR (6).

Peniscopy showed high specificity and low sensitivity. However, most studies showed a weak specificity of the exam and also a good sensitivity (7-9). We believe that our results were biased due to the high prevalence of HPV in the studied population (around 50%), explaining the good specificity, and that most of the patients had subclinical or latent infection with HPV, that impaired the identification through peniscopy, only with biomolecular techniques, explaining the low sensitivity.

Some risk characteristics for HPV infection of penis of HIV positive males were identified in our study, including heterosexual behavior (higher rate of penile HPV infection compared to bisexuals and homosexuals, probably due to the high rate of female infection in our population, demonstrated in several studies done in Manaus (10-12).

PCR and HC techniques had high concordance and sensitivity for the detection of HPV. Rodrigues et al. (13) demonstrated that HC and PCR techniques for the detection of HPV in clinical samples had a fair concordance, including conventional and real time techniques (k = 0.338). When they compared conventional PCR with real time PCR they observed an almost perfect concordance (k = 0.818).

There are very few studies related to intraepithelial neoplasms or penile cancer of HIV positive men. Kreuter et al. studied 263 HIV-positive homosexual men and found penile intraepithelial neoplasms of penis in 11 (4.2%) and of anus in 156 (59.3%) (14).

The limitations of our study included the small size of sample that prevented a strong association among the techniques. The study was conducted in an AIDS ambulatory. However, most

Table 5 - Distribution of histopathological findings of patients with clinical lesions detected.

Histopathological Findings	N	(%)
Angioceratoma	1	1
Chronic inespecifc balanitis	2	2
Inespecific ulcerated balanitis	1	1
Inespecific chronic balanopostitis	2	2
Condyloma Acuminata	19	27
Flat Condyloma	1	1
Epidermodysplasia	2	2
Nonspecific chronic eczema	1	1
Hypermelanose	1	1
High grade intra-epitelial lesion (HSIL)	4	6
Fungal infection	1	1
Scleroathrofic lichen	2	2
Lichen planus	1	1
Low grade Intra-epitelial lesion (LSIL)	18	25
Molluscum Contagiosum	6	9
Melanocytic nevi	1	1
Bowenoid papulosis	5	7
Chronic inespecific postitis	1	1
No significative alterations	6	9
Total	75	100

Table 6 – Distribution of histopathological findings of 27 pre-malignant lesions.

Pre-malignant lesions	
Epidermodysplasia Verruciformis Like	02
High grade intra-epitelial lesion (HSIL)	04
Low grade Intra-epitelial lesion (LSIL)	17
Bowenoid papulosis	03
Bowenoid papulosis+ LSIL+Bowenoid papulosis	01
Total	27

HIV+/AIDS patients from Amazonas are attended at FMT-HVD, which we believed allowed the study of a significant sample of patients.

We believe that the present results can be used to delineate preventive programs for early detection of penile cancer, in individuals with higher risk, including immunosuppressed patients. Diagnosis and treatment of male partners infected with HPV would also allow a reduction of sexually transmitted diseases.

CONCLUSIONS

Prevalence of DNA HPV was approximately 50%.

Peniscopy proved to be a high specific and low sensitive exam.

Concordance of peniscopy and PCR for the detection of HPV was low.

Concordance of PCR and HC for HPV detection was excellent.

We observed a prevalence of 10% of patients with pre-malignant lesions determined by histopathological studies and that most of them were severely immunosuppressed (TCD4 < 200 cells/mm^3).

CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Antioxidant supplementation decreases the cell death rate in the prostatic stromal tissue of long-term castrated rats

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ABSTRACT

Objective: The purpose of this study was to compare the effects of castration on cell death rate of the adult rat prostates and to evaluate the benefic action of alpha tocopherol supplementation to avoid apoptosis post-orchiectomy.
Material and Methods: Thirty male Wistar rats weighing 250-300g were divided into three groups: group I – they were subjected to bilateral orchiectomy and sacrificed eight weeks after the procedure; group II – subjected to bilateral orchiectomy and alpha-tocopherol supplementation for four weeks preceding the procedure; and group III – subjected to bilateral orchiectomy and alpha-tocopherol supplementation for four weeks preceding the procedure and for eight weeks afterwards. At the end of the experiment, the prostatectomy was performed in all rats. The presence of oxidative stress was determined by assaying the blood level of 8-isoprostane and the occurrence of apoptosis was evaluated by identification of active caspase-3 through immunohistochemical analysis.
Results: The statistic analysis of active caspase-3 showed that in the long-term castrated group the detection was higher than in groups were the alpha-tocopherol was supplemented (p=0.007). Analysis of 8-isoprostane levels showed higher concentrations of reactive oxygen species in group I compared to other groups (p<0.05). Groups II and III presented active caspase-3 lower than in group I (p<0.05).
Conclusion: Our exploratory analyses demonstrate a method to study the aging process and its influence on oxidative stress of prostatic tissue and cells death rate. Based on our results we can suggest that alpha tocopherol supplementation can decrease the apoptotic process as well as the oxidative stress levels induced by androgen deprivation of the prostate gland.

ARTICLE INFO

Key words:
Prostate; Testosterone; Oxidative Stress; alpha-Tocopherol; Apoptosis

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INTRODUCTION

During the male aging process, prostate growth occurs in the form of benign prostatic hyperplasia and prostatic carcinoma. The androgens have a central role in regulating the growth of the prostate with the ability to stimulate proliferation and inhibit cell death rate. The androgen ablation by castration leads to rapid cell death, mainly in the ventral prostate lobe of rats, through apoptosis. Defects on the mechanism of programmed cell death have great participa-

tion in the pathogenesis of several proliferative changes and attempts to activate apoptosis represent a possibility of therapeutic approach (1).

Androgens may be involved in the epithelial stroma interaction. In mature prostate, androgens are known to cause several changes in prostatic epithelium through androgen receptors located in the stroma. Immunocytochemical studies have shown that prostatic smooth muscle cells are uniformly androgen receptor-positive. This fact indicates that smooth muscle located in prostatic stroma may be an important target for androgen action and able to regulate the expression of prostate growth factors (2).

Apoptosis is an important physiological process associated with aging (3,4). It is defined by a set of morphological and biochemical changes in different cellular levels (5), and the result is the elimination of unwanted cells, leaving the surrounding tissue intact. In relation to aging, apoptosis has a primary negative effect, by destruction of essential and often irreplaceable cells. But it also eliminates non-functioning cells and protects the organs against cancer or hypertrophy (6).

Reactive oxygen species (ROS) act probably on nucleic acids, causing cellular damage by reaction with membrane lipids and proteins, and modulate the expression of some genes related to cellular differentiation and proliferation. Due to these facts, they may trigger the signaling routes that lead to cell apoptosis (7). It has been suggested an imbalance between apoptosis and cell proliferation that results in the development of the HPB (benign prostatic hyperplasia). Zang et al. (8) demonstrated close relationship between a high rate of proliferation of stromal region and a high rate of apoptosis in glandular epithelium in BPH. Therefore, the development of BPH may be associated with stromal growth, through the proliferation of stromal cells mesenchyma active, and the epithelial growth, due to reduction in cellular apoptosis.

Recent experimental study showed the beneficial action of vitamin E in reducing the activation of the intrinsic pathway of apoptosis and cell death rate induced by moderate oxidative stress (9). It can react directly with a variety of oxi-radicals such as superoxide, the hydroxyl and also with the singlet oxygen (10). Bell (11) showed the protection obtained by vitamin E against oxidative damage of unsaturated fatty acids tissue.

Considering the exposed above, we aimed to study the occurrence of apoptosis in prostatic stroma in the presence of low levels of testosterone, yet assessing the beneficial action of alpha-tocopherol to prevent the state of oxidative stress induced by orchiectomy.

MATERIALS AND METHODS

Animals and Diet

Thirty male Wistar rats, 3 months old, were used in these experiments. Rats were housed at the university´s animal facility on a 12-hour light/ 12-hour dark cycle and allowed access to food and water ad libitum.

Grouping of animals

The animals were divided into 3 groups of 10 animals each: Group I: the rats were subjected to bilateral orchiectomy. Group II: subjected to bilateral orchiectomy and received supplementation with alpha-tocopherol for four weeks preceding the castration procedure. Group III: subjected to bilateral orchiectomy, with supplementation of alpha-tocopherol for four weeks preceding the procedure and for eight weeks afterwards. After 8 weeks of the beginning of the experimental protocol, the animals were sacrified and venous blood was collected from the dorsal vein of the tail, in order to assay the 8-isoprostane and testosterone levels. The groups II and III received alpha-tocopherol dissolved in 0.1 mL of sesame oil (Galênica, SP – Brazil) at a dosage of 1000 UI/kg/week intramuscularly. The prostate was quickly removed and formalin-fixed and paraffin–embedded and submitted to histological and immunofluorescence studies.

Alpha-tocopherol assay

Alpha-tocopherol was quantified by high-performance chromatography, using fluorescence with an excitation wavelength of 292 nm and an emission wavelength of 340 nm. The values were expressed in ng/mg.

Measurement of testosterone

Blood was collected from the dorsal vein of the tail, in order to assay total serum testosterone by competitive radioimmunoassay (testosterone direct radioimmunoassay kit, Immunotech, Brazil, cat # 1119).

Oxidative Products

Enzimatic immune assay of oxidatively modified product isoprostane 8-epi-PGF2α was performed, as previously reported (14). Briefly, the concentration of 8-isoprostane in the samples was measured using a commercial enzymatic immunoassay kit (Cayman Chemical). The assay was based on competition between the 8-isoprostane present in the sample and 8-isoprostane conjugated with acetylcholinesterase by a limited concentration of anti-8-isoprostane antibody. The new complex formed between 8-isoprostane conjugated with antibody combined with another antibody was detected by means of a reaction with 5.5 -dithiobis (2-nitrobenzoic acid)-DTNB- (absorbed at 405 nm), which was hydrolyzed by the acetylcholinesterase of the conjugate. The concentration of 8-isoprostane was determined by interpolation on a standard curve. The detection limits were from 6 to 500 pg/mL. The samples were diluted by a factor of four and all values obtained were within the detection limits. Microtiter plates were scanned by means of a computer software that allowed wavelength adjustment and plate reading (EL x 800 instruments). The results were expressed in pg/mL.

Determination of apoptosis

To prepare tissue sections for immunostaining it was used paraffin clearing of the slides placing them in fume hood, overnight, at 60ºC. After that, the paraffin was cleared with xylene for 30 minutes and the slides were moved to a fresh dish of xylene for an additional 30 minutes. The slides were rinsed twice for 10 minutes in 80% alcohol (18:1:1 100% ethanol: 100% methanol: 100% isopropanol). The slides were rinsed five times with fresh deionized water.

The Antigen Retrieval Method was made placing slides face-up in incubation tray and covering each section with 1% SDS in PBS (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na2HPO4, and 1.47 mM KH2PO4). After that, they were incubated for five minutes at room temperature, followed by three five minute washes with phosphate buffered saline (PBS). Then, the slides were immersed in blocking buffer 1 (3% Molico®, diluted 1:10 in PBS) - 3g non-fatted cow milk (Molico®): 100 mL PBS. After that, they were incubated overnight at 4º C.

To prepare the incubation with primary antibodies (rabbit anti-caspase 3) the sections were covered with blocking buffer 2 (3% Molico®, diluted 1:10 in PBS, plus 200µL Tween). Tissue sections were covered with primary antibody (rabbit anti-caspase-3 - C8487) diluted in 1:500 blocking buffer 2 and incubated at 370C for one hour. The excess liquid was blotted from slides and rinsed three times in PBS for five minutes each wash. To prepare the incubation with secondary antibodies (chicken anti-rabbit IgG) the tissue sections were covered with secondary antibody (chicken anti-rabbit IgG - F0382) diluted 1:40 in blocking buffer 2 and incubated at 370C for one hour. The excess liquid was blotted from slides and rinsed three times in PBS for five minutes each wash.

To prepare the counterstaining and visualization (nuclear staining) the slides were immersed for 15 minutes in 100µL solution of propidium iodide diluted 1:10 in PBS. The slides were rinsed in PBS for five minutes. The coverslip was placed for visualization by fluorescence microscopy. The volumetric density of the distribution of active caspase-3 was analyzed by overlaying the M-42 grid system on the computed morphological image of the slide.

Statistical Analysis

In all data the criterion for significance was $p<0.05$. Futhermore, for 8-epi-PGF2α and testosterone statistical analysis, the nonparametric Kruskal-Wallis test was used, with the Bonferroni correction test. The results were considered statistically significant when $p<0.05$. The data obtained from caspase-3 were analyzed using the SPSS 12.0 statistics software (SPSS Inc., USA) and were expressed as mean ± standard deviation. The nonparametric Newman-Keuls test for multiple

comparisons was used to assess the differences between the independent samples.

RESULTS

Alpha-tocopherol

The concentration of alpha-tocopherol was 0.69 ng/mg in group II; 1.73 ng/mg in group II and 1.83 ng/mg in group III. The comparative analysis showed that the values in groups II and III were statistically greater than those of groups I and II (p = 0.003).

Testosterone measurements

The serum testosterone concentrations immediately before sacrifice of the animals of the orchiectomized groups with or without alpha-tocopherol replacement were lower than 20 pg/mL, the lower limit of sensitivity of the method applied. This demonstrated the presence of a significant hormone deficit at the time of the sacrifice of these animals.

Marker of Oxidative Stress

Analysis of the 8-isoprostane levels demonstrated statistically higher values (p<0.0003) in group I (5.1 pg/mL), i.e. among the orchiectomized rats without alpha-tocopherol replacement, in relation to groups II (2.4 pg/mL) and III (2.6 pg/mL).

Caspase-3

The statistical analyses showed that the volumetric density of active caspase-3 was greater in the orchiectomized group without alpha-tocopherol supplementation (32.4%) than in the other groups (group II, 18.8%; group III, 18.6%) (p = 0.007). In addition, there was no statistically significant difference between the group that received alpha-tocopherol supplementation before orchiectomy and the group that received it both before and after orchiectomy (p=0.55) (Table-1).

The Figures 1-3 show the immunohistochemical method used for the detection of active caspase 3 on the prostatic tissue in the three different groups. The positive reactions are seen in green and the nucleus in red.

Table 1 – Quantitative analysis of active Caspase-3 in tissue.

	% active caspase-3 in tissue
GROUP I	32,4*
GROUP II	18,8
GROUP III	18,6

* p <0,05

Figure 1 - Group I. Microscopic fluorescence immunohistochemistry for the detection of active caspase-3. Increase 10x.

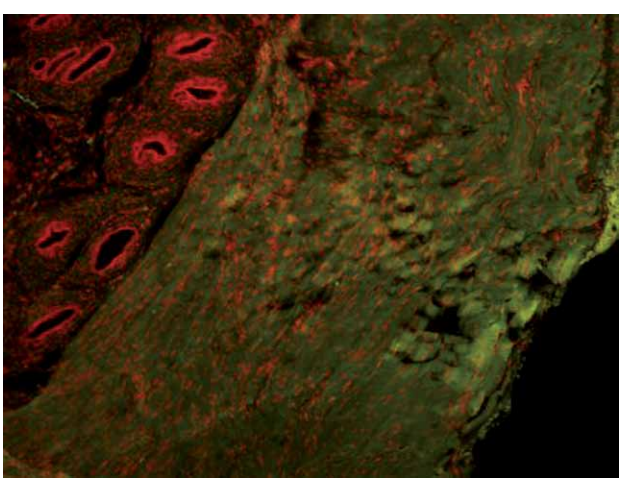


Figure 2 - Group II. Microscopic fluorescence immunohistochemistry for the detection of active caspase-3. Increase 10x.

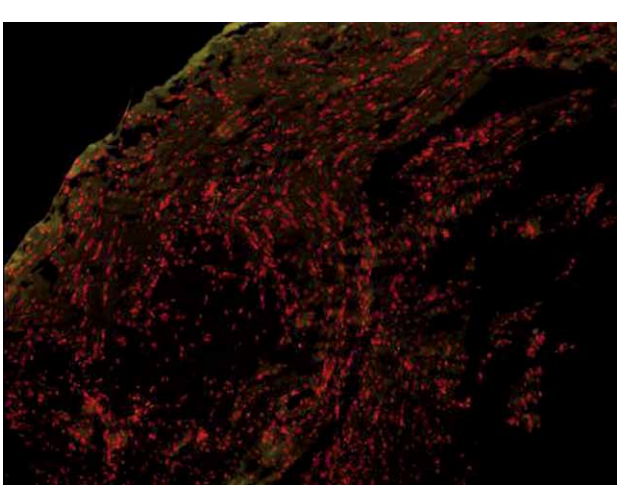
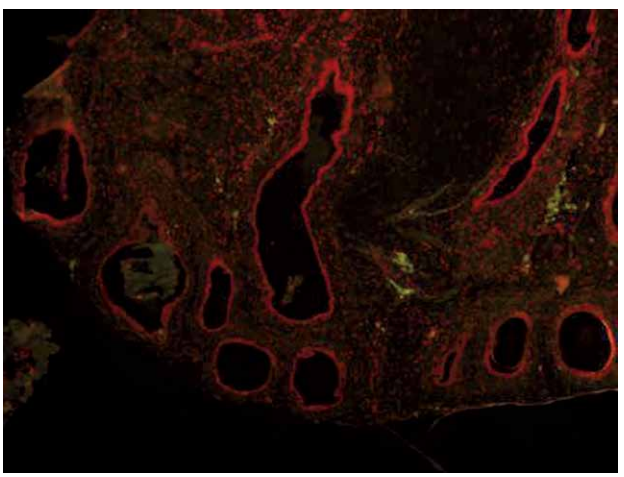


Figure 3 - Group III. Microscopic fluorescence immunohistochemistry for the detection of active caspase-3. Increase 10x.



DISCUSSION

In the mid-1950s, the theory of free radicals to explain the aging process was proposed, speculating that endogenous oxygen radicals were produced in cells and resulted in a pattern of cumulative damage. Only a decade later, with the identification of the enzyme superoxide dismutase (SOD), responsible for the conversion of superoxide radical to hydrogen peroxide, there was scientific support for that hypothesis (12).

Oxidative stress, or oxidative damage, is present when there is an imbalance between the natural antioxidants and reactive oxygen species. With aging, there is a decrease of antioxidant mechanisms, resulting in prevalent cellular damage caused by free radicals (13).

However, there is increasing evidence of the role of oxidizing agents specific in signaling molecules in both physiological and pathophysiological conditions (12).

The generation of reactive oxygen species is related to the maintenance of homeostasis, as an example, in combating infectious processes through the generation of ROS by phagocytic cells and regulation of the proliferative response. Regardless of how or where they are produced, increasing intracellular levels of ROS has two important effects: damage of various cellular components and induction of activation of specific signaling chains; both effects can influence several processes related to the process of aging and development of associated diseases (12).

There is evidence showing that the aging process contributes to the accumulation of oxidative damage in different cells and molecules (14).

In this study, a dosage of 8-isoprostane was used as a marker of oxidative stress to check the presence of oxidative stress after eight weeks of bilateral orchiectomy. This marker has been used in animals and humans. In general, the isoprostanes are present in blood in nanomolar concentrations and are increased in many pathological conditions, such as renal and myocardial ischemia, pulmonary hypertension, hypercholesterolemia and neurogenic detrusor hyperactivity (15,16).

Corroborating the literature, we demonstrated high levels of 8-isoprostane serum in rats undergoing orchiectomy, without replacement of alpha-tocopherol (group I), reflecting increased oxidative stress associated with hypoandrogenism. A statistically significant reduction was observed in the estimation of 8-isoprostane after supplementation of alpha-tocopherol in both groups that received supplementation, demonstrating the protective effect of alpha-tocopherol to reduce the serum levels of oxidative stress, and its action, especially by preventing the generation of radicals.

Antioxidants are substances that when present in small concentrations, compared with those oxidizable substrate, significantly delay or inhibit the oxidation of the substrate and may act at different levels of the oxidative sequence.

The use of alpha-tocopherol was based on several studies that demonstrated its beneficial action in various urological diseases (17,18); moreover, it is one of the most powerful and effective exogenous antioxidants, preventing the peroxidation and avoiding the effects of oxidative stress (19,20). We chose the use of alpha-tocopherol because of its well established pharmacokinetics, which assisted the determination of the dose to be employed oral or parenterally (20).

There are reports in the literature of the protection against damage related to aging by using vitamin E in Sprague-Dawley rats with removal of 8-isoprostane and reduction of oxidative damage (21).

Apoptosis is an important physiological process that is related to aging (3,4). It is defined as a set of biochemical and morphological changes in different cellular levels, resulting in the elimination of unwanted cells (5). However, with aging, apoptosis has a negative effect since it destroys vital and often irreplaceable cells (6).

Among the various ways of identifying the process of apoptosis, we chose the detection of active caspase-3, since the caspase enzymes are involved in various stages of apoptosis, from the initial signal to the final lysis of cellular components. The active caspase-3 is the final effector of cell lysis, so we decided to use it as a marker of irreversible apoptosis.

The prostatic overgrowth in the form of BPH or CaP observed during aging is credited to an imbalance between proliferation and cell death rates. Recent studies have indicated not only the rate of cell proliferation, but also the rate of cell death associated to tumor size. Previous studies have demonstrated the induction of apoptosis in prostate of rats through the male hormone decline induced by aging or by orchiectomy (1,22,23). The castration causes loss of weight of the gland and reduction of cell size in all lobes, but the apoptosis in response to androgen withdrawal occurs predominantly in the ventral lobe. The castration has little effect on DNA content or rate of apoptosis in dorsal lobes, lateral and anterior. Although the apoptotic index was lower in dorsal, lateral and anterior lobes compared with the ventral lobe, the percentage of apoptotic cells observed in the 4 prostate lobes were significantly reduced with age (1).

This study demonstrated a statistically significant difference between the levels of active caspase-3 in animals of the groups that received alpha-tocopherol supplementation (groups II and III) in relation to the group without supplementation, that was higher in those without supplementation. It was observed that in groups with supplementation, the dosage of 8-isoprostane was significantly lower in the castrated group without the use of antioxidant, suggesting that the serum oxidative stress, detected by 8-isoprostane, has a role in apoptosis of castrated rats. These findings demonstrate that the probable pathway of apoptosis induced by androgen ablation is the oxidative stress, since the use of vitamin E reduced both the dosage of 8-isoprostane and quantification of active caspase-3.

The findings demonstrated in this study show that oxidative stress may have an important role in the process of apoptosis in prostate stroma of rats, due to low levels of testosterone (hypoandrogenism). It was found that supplementation of alpha-tocopherol after bilateral orchiectomy decreased the apoptotic index, but it is necessary to emphasize that this work was designed to evaluate animals submitted to orchiectomy as a cause of aging. This study will follow the line of research that intents to clarify the factors involved in the development of benign prostatic hyperplasia and a possible better and less invasive therapeutic approach.

CONFLICT OF INTEREST

None declared.

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Missed iatrogenic partial dysruption of the male urethra, caused by catheterization

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This 37-year-old male presented at the emergency room with shortness of breath (SOB), a distended abdomen and a history of anuria for 2 days. Clinical examination revealed a left pleural effusion, cardiomegaly and ascites. An abdominal ultrasound confirmed the presence of massive ascites. The bladder appeared to be distended on ultrasound. The abdomen was not tender to palpation. The admission temperature was 37.6 C, BP 134/58, PR 86, and RR 20. Blood-chemistries showed the RBC 4.2 mil, WBC 6800, BUN 32, creatinine 2.6, glucose 106, K 4.2.

In view of the anuric status and apparently distended bladder, retrograde placement of a Foley catheter was attempted by the nurse. When she failed, a junior resident made a second attempt, but failed likewise. He did not introduce a parallel micro-catheter, which could have been used to inject contrast-laced jell to further outline the path of the urethra. A scout film of the abdomen revealed an ominous deviation of the tip of the catheter to the left (Figure-1 arrow), which was not appreciated. Urologic consult was requested. After a 6 hour delay the urologist noted the penile shaft to be swollen, the scrotum to be distended with fluid. The patient's temperature was now 38.4°C, and he complained of vague prepubic pain.

A computed tomogram of the pelvis with sagittal reconstruction was performed, demonstrating a distended bladder and significant fluid accumulation with interspersed gas in the pre- and retrourethal space (Figure-2 arrow). The tip of the catheter (Figure-2 dotted arrow) appeared to be outside the urethra and angled posteriorly. A suprapubic catheter was then inserted, decompressing the bladder. Prior to removal of the Foley catheter a small amount of dilute non-ionic contrast medium was injected to delineate the urethra. The urethra was noted (Figure-3 arrow) to be displaced downward and posteriorly by massive urine extravasation in the anterior peri-urethral space (Figure-3 arrow-heads). Furthermore, note the subcutaneous air delineating the corpora cavernosa in the penile shaft (Figure-3 curved arrow). Finally an 8 French catheter was passed with an antegrade approach, via the suprapubic access site

Figure 1 – Ominous deviation of the tip of the catheter to the left (arrow).

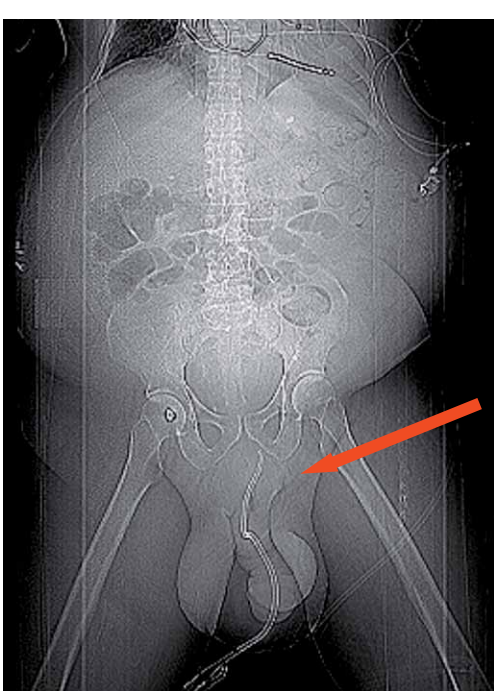


Figure 2 – Distended bladder and significant fluid accumulation with interspersed gas in the pre- and retrourethal space (arrows). Tip of the catheter (dotted arrow) appeared to be outside the urethra and angled posteriorly.

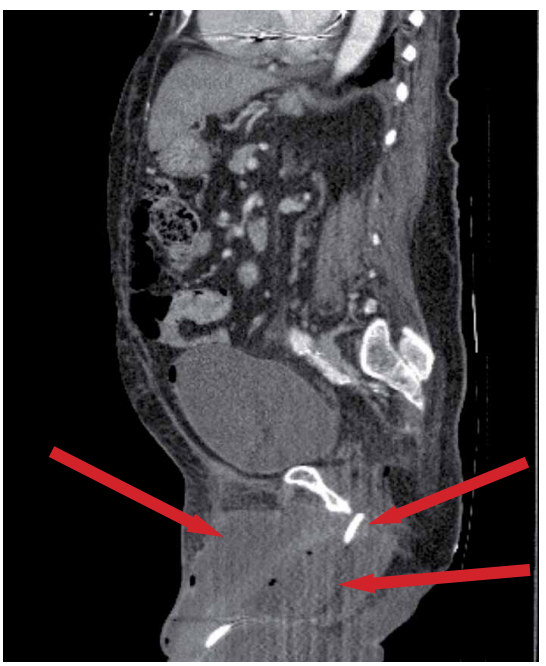
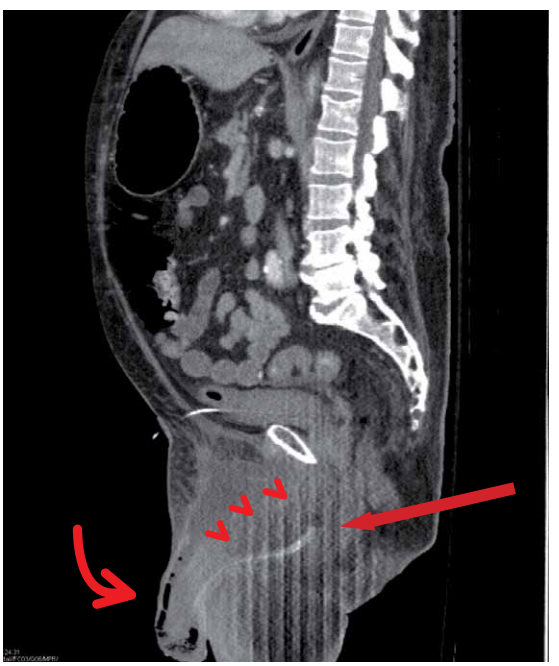


Figure 3 - Urethra was noted (arrow) to be displaced downward and posteriorly by massive urine extravasation in the anterior peri-urethral space (arrow-heads). Furthermore, note the subcutaneous air delineating the corpora cavernosa in the penile shaft (curved arrow).



under fluoroscopic guidance across the only partly dehiscent urethra. It was left in position for 8 weeks as was the suprapubic catheter. Satisfactory healing, without a stricture at this point in time, resulted.

Seating of a Foley catheter in the male patient has a high rate of complication when performed by marginally or inexperienced operators (1). In male patients introduction of a parallel microcatheter to the point of perceived obstruction and delineation of the further path of the urethra by a mixture of non-ionic contrast medium and gel, may allow fluoroscopic guided placement of a guide-wire, which can then be safely followed by the Foley catheter. CT with sagittal and coronal reconstructions is the procedure of choice to identify leakage from partial or complete urethral dehiscence (2). Compared to the CT cysto-urethrogram, the retrograde cysto-urethogram may identify only 17.8% of partial dehiscence with minimal leakage (2). Moreover, clinical symptoms are not reliable to predict urethral injury. TRUS however is a reliable technique for assessing injury to the female urethra (3).

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Evaluation of supernumerary kidney with fusion using magnetic resonance image

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Urogenital anomalies are frequent and correspond to 33% of all congenital anomalies (1). Horseshoe kidney and renal crossed ectopy with fusion are the most frequent renal anomalies (2). Supernumerary kidney is the rarest renal anomaly and it can be encapsulated or totally apart from the original kidney or connected to it by a sheath of connective tissue. Usually the supernumerary kidney is located caudally to the kidney of the same side and is associated with bifid ureter or more infrequently a double ureter (2,3).

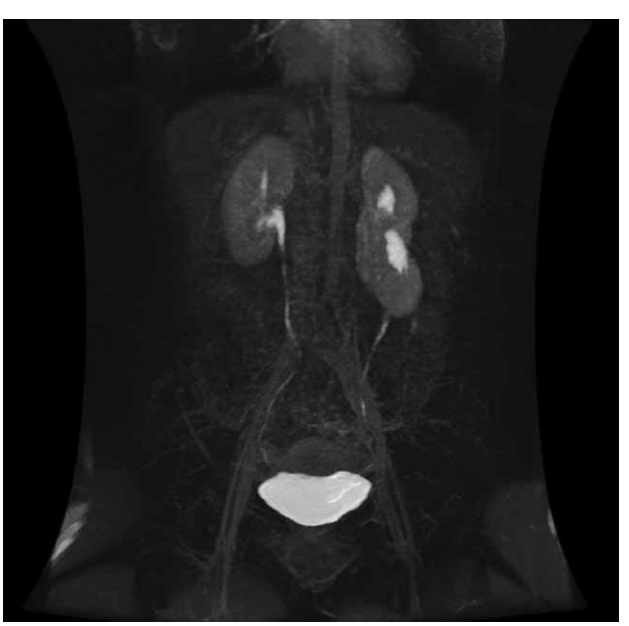
There are several reports of supernumerary kidney with fusion (2) but this is the first one described in the literature evaluated with magnetic resonance image.

A female patient of 27 years old presented with left flank pain 3 days duration, dysuria and fever (100oF). Physical exam of abdomen was inconclusive and ultrasound suggested left duplication of the renal pelvis. Urine exam suggested urinary infection. After the administration of sedatives and antibiotics the patient was submitted to magnetic resonance image (MRI) of abdomen and pelvis (1.5T system - GE Signa Excite). T1 and T2 weighted pre- and post-gadolinium enhancement were obtained.

MRI showed a left moety fused to the upper pole of the supernumerary kidney (Figure-1). The supernumerary kidney showed a defect of rotation and had no dilation of the collecting system. The MRI also showed the presence of individual ureters (Figure-2). After the clinical treatment, the patient got better and was discharged.

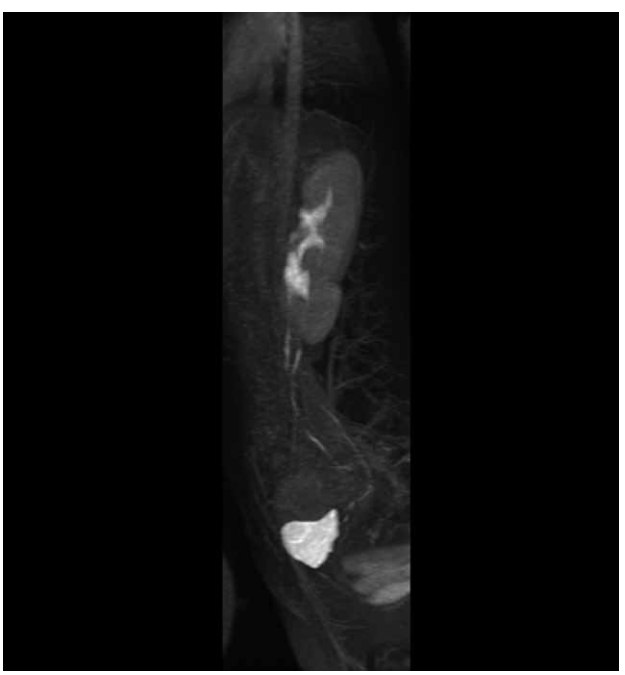
There are several theories to explain the occurrence of a supernumerary kidney: a) bifurcation of the ureteral bud with independent penetration of the buds in the metanephrogenic blastema that develop and divide in two kidneys; b) two independent ureteral buds that penetrate the metanephrogenic blastema that divides in two and c) fragmentation of the metanephrogenic blastema due to linear infarcts (3). The supernumerary

Figure 1 - Magnetic ressonance image of abdomen and pelvis of a coronal reconstruction frame in T2. It is possible to observe the fusion of the lower pole of left kidney to the superior pole of the supernumerary kidney.



kidney, independently of its origin, does not present characteristic symptoms and in most cases is separated from the same side kidney.

Figure 2 - Magnetic ressonance image of abdomen and pelvis of a sagital reconstruction frame in T2. It is possible to observe the two independent collector systems without dilation with two ureters running to the bladder.



The occurrence of supernumerary kidney with fusion and independent ureter is an extremely rare anomaly, and there are no previous reports of the evaluation of this anomaly with MRI. In the present case, the supernumerary kidney is connected to the same side kidney apparently by a portion of parenchyma and not by connective tissue and has an independent ureter that runs to the bladder, making this case even more rare and special.

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VIDEO SECTION

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Laparoscopic radical prostatectomy for high risk localized and locally advanced disease

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ABSTRACT

Purpose: The indication for surgery in locally advanced prostate cancer is growing considering and long-term follow-up shows that 60-80% of patients can be free of clinical recurrence. The aim of this video is demonstrate the modifications in traditional laparoscopic surgery that permit to observe the oncological principles reproducing open surgery.
Materials and Methods: A 55 years-old male presented with an initial PSA = 25ng/dL, the digital rectal examination found a prostate with hardened nodules bilaterally (clinical stage T2c). Prostate biopsy showed an adenocarcinoma Gleason 7, the patient´s disease was classified as a localized high-risk prostate cancer. Surgery was offered as initial therapeutic option and the critical technical points were: transperitoneal approach to evaluate if separation of rectum from prostate and seminal vesicles was possible, extended pelvic lymphadenectomy, opening of endopelvic fascia lateral to the prostate, bladder neck section without preservation, pedicle control without neurovascular bundle preservation, meticulous dissection of apical region, reconstruction of posterior bladder neck before the anastomosis.
Results: The operative time was 240 minutes without conversion to open surgery and an estimated blood loss around 520 mL. Neither intraoperative nor postoperative complications occurred and the hospital stay was about 36 hours. Pathological report confirmed a prostate adenocarcinoma Gleason 4+4, negative margins and stage pT3a pN0 pMx.
Conclusions: Laparoscopic surgery adopting oncological principles can be utilized with efficacy to selected patients with high risk localized and locally advanced prostate cancer maintaining the advantages of minimally invasive surgical approach.

ARTICLE INFO

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EDITORIAL COMMENT

The authors describe and then demonstrate the technique of pure laparoscopic radical prostatectomy and extended pelvic lymph node dissection in a patient with high risk prostate cancer. They appropriately comment that surgery is able to achieve reasonable cancer control in this challenging group of patients with aggressive cancers. They clearly address the two main surgical principles that allow optimizing the procedure: Wide excision of the neurovascular bundles and bladder neck and the completion of an extended lymph node dissection. The video demonstrates the gross appearance of an adequately performed lymph node dissection on the left side with clear anatomical definition of the distal common iliac vessels, full mobilization of the external iliac artery and vein and a clean obturator fossa. More importantly, they demonstrate that the procedure may be performed safely and efficiently through a pure laparoscopic approach in centers with surgeons experienced in advanced laparoscopy and without the need of the robot. This is of relevance as many centers in Latin America currently do not have access to robotic technology.

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VIDEO SECTION

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Single Port Transvesical Prostatectomy

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ABSTRACT

Purpose: To describe a case of a transvesical prostatectomy performed by a single port technique.
Patient and Methods: JLS, 64y, diabetic and hypertense, under treatment of LUTS for 8 years with 4mg doxazosin and 5mg finasteride. The IPSS score was 26. The digital rectal exam showed a more than 60g benign prostate. The Body Mass Index was 28.9. The total PSA was 5.4ng/mL and the free/total PSA was 22%. A 12-fragments prostate biopsy showed BPH. The sonography revealed a 106g prostate and the maximum urinary flow was 12 ml/s. The patient was under general anesthesia and was positioned in dorsal decubitus with Trendelemburg. The bladder was filled until that a bexigoma was visible. A 2 cm longitudinal infra-umbelical incision was done. The Gel Point Single Port System (Applied, Ca, USA) was placed inside the bladder and the pneumovesicum was done until 10mmHg. A peri-bladder neck incision was done and the adenoma dissection was performed until its remotion. The hemostasia was done under vision. A 3-way 24-Fr Foley catheter and an 8-Fr plastic catheter were placed inside the bladder. The adenoma was removed and the bladder and the abdominal wall were closed.
Results: The procedure took 55 minutes and the blood loss was 180 ml. The patient evolved uneventfully, the bladder irrigation stayed for 24 h, the hemoglobin drop was 2.4g/dL and the patient was discharge after 36 hours. The urethral catheters stayed for 5 days. The postoperative IPSS was 6 and the maximum flow was 26 ml/s.
Conclusion: The surgery was safe and effective, showing that the single port transvesical prostatectomy can be an option in the surgical treatment of large prostates.

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EDITORIAL COMMENT

In this video by Vicentini et al., the authors employ a novel single port transvesical approach for the surgical management of benign prostatic hyperplasia (BPH). I applaud the authors on employing such a well suited minimally invasive surgical approach to BPH refractory to current medical therapies. As a host of alternate surgical therapies arise for BPH including Holmium-YAG laser enucleation/vaporization and Greenlight laser vaporization, the ultimate question is which one provides the best outcomes in terms of long-term improved urinary stream, minimal irritative post-treatment voiding symptoms, optimal surgical outcomes (i.e. quickest convalescence, less perioperative pain, decreased blood loss/necessity for blood transfusion, and lowest risk of complications), as well as the ever increasing concern of treatment specific healthcare cost. Clearly, this is a complex question which can't be answered through a single video presentation or case series but will require multi-arm prospective studies to be conducted. I encourage the urological society at large to consider doing such studies because it is only though such efforts that we can ultimately answer the question of what is the best option. It is likely that certain patients may do better with one specific surgical option versus another based on the size of the gland, specific surgical anatomy e.g. prominent median lobe, and baseline irritative voiding symptoms.

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Original Article: Original articles should contain a Cover Page, Abstract, Introduction, Materials and Methods, Results, Discussion, Conclusions, References, Tables and Legends, each section beginning in a separate page and numbered consecutively. Original articles should cover contemporary aspects of Urology or experimental studies on Basic Sciences applied to urology. The manuscript text should contain no more than 2500 words, excluding the Abstract. The number of authors is limited to five. References should contain no more than 30 citations, including the most important articles on the subject. Articles not related to the subject must be excluded.

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Abstract (maximum 200 words) and should contain

- **Main findings:** Report case(s) relevant aspects
- **Case(s) hypothesis:** Proposed premise substantiating case(s) description
- **Promising future implications:** Briefly delineates what might it add? Lines of research that could be addressed

Full text (maximum 2000 words):

- **Scenario:** Description of case(s) relevant preceding and existing aspects;
- **Case(s) hypothesis and rational:** precepts, clinical and basic reasoning supporting the case(s) hypothesis and the raised scenario. Why is it important and is being reported?
- **Discussion and future perspectives:** what might it add and how does it relate to the current literature. 'Take-home message' - lessons learnt;
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Books:
▪ Sabiston DC: Textbook of Surgery. Philadelphia, WB Saunders. 1986; vol. 1, p. 25.

Chapters in Books:
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Introduction must exclude unnecessary information. It should briefly describe the reasons and objective of the paper.

Materials and Methods should describe how the work has been done. It must contain sufficient information to make the study reproducible. The statistical methods have to be specified.

The **Results** should be presented using Tables and Figures whenever possible. Excessive Tables and Figures must be avoided. The tables should not be repeated on the text.

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Conclusions must be strictly based on the study findings.

References should contain no more than 30 citations, including the most important articles on the subject. Articles not related to the subject must be excluded.

The Abstract must contain up to 250 words and must conform to the following style: Purpose, Materials and Methods, Results and Conclusions. Each section of the manuscript must be synthesized in short sentences, focusing on the most important aspects of the manuscript. **The authors must remember that the public firstly read only the Abstract, reading the article only when they find it interesting.**

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