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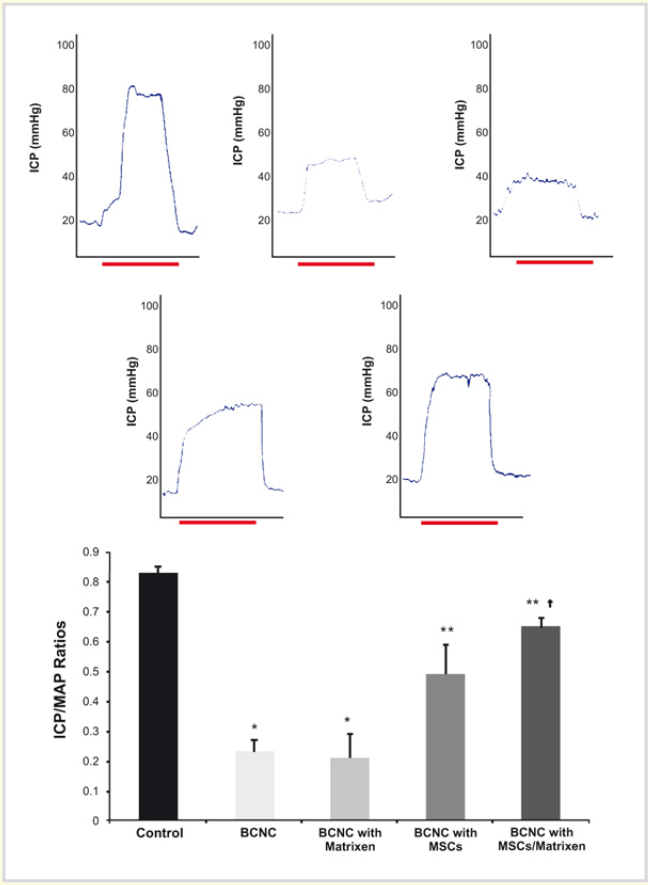

Erectile functional assessment under cavernous nerve stimulation (CNS) at week 4 in control (A); bilateral cavernous nerve (BCNC) crushing (B); BCNC with Matrixen (C); BCNC with mesenchymal stem cells (MSCs) (D); BCNC with MSCs mixed with Matrixen group (E). The voltage dependent erectile response to CNS represented by the ratios of intracavernous pressure (ICP) / mean arterial pressure (MAP) (F). *P < 0.05 compared with the control group, **P < 0.05 compared with BCNC group, †P < 0.05 compared with BCNC injected with MSCs group. (Page 837)

SOCIEDADE BRASILEIRA DE UROLOGIA

EDITOR'S COMMENT

The November-December 2012 issue and the TOP 5 in 2012

The November-December 2012 issue of the International Braz J Urol presents original contributions from many different countries such as Brazil, France, Italy, Taiwan, Egypt, Puerto Rico, Republic of Korea, China, Turkey and USA, and as usual the editor´s comment highlights some papers.

Doctor Favorito and collegues from Urogenital Research Unit – State Universtiy from Rio de Janeiro, Brazil, performed on page 802 a interesting basic research about morphologic alterations in the foreskin of patients with phimosis, submitted to topic treatment with betamethasone in association with hyaluronidase. The success rate of the phimosis topic treatment with coerticosteroids is significant, with satisfactory results (67%–95%). In this study the topical treatment of phimosis with betamethasone 0.2% + hyaluronidase was effective with a success rate of 85%. Patients in whon topical steroid treatment failed had fewer elastic fibers, which is a characteristic of the healing processes, as well as an amplification of the collagen type III, a recently found collagen that is associated with muscular retraction. The betamethasone + hyaluronidase cream leads to significant histological alterations in the prepuce.

Dr. Gonzaga Silva and collegues from Ceara Cancer Hospital of Ceara Federal University, Brazil, performed on page 750 a elegant study about the testicular-sparing surgery X classic emasculation in patients with penile cancer. The authors studied172 patients with Penile cancer and 16 (9%) underwent emasculation. The authors conclude that the finding of minimal testicle infiltration in only one of 32 testicles, even in the presence of clinically apparent scrotal invasion, suggests that emasculation without bilateral orchiectomy is a safe treatment option for patients with locally advanced PC.

Doctor Esmat and collegues from Ain Shams University, Egypt, performed on page 779 a study about the Yang-Monti principle. The authors studied Sixteen patients underwent ileal ureter replacement using the Yang-Monti principle to overcome long ureteric defects. They concluded that the reconfigured ileal segment for ureteric substitution is a safe technique with an excellent outcome. It uses short ileal segments for reconstruction of an ileal tube of adequate length and optimal caliber that permits easy antireflux implantation into the bladder so it not associated with excess mucus production or metabolic abnormalities and offers a durable preservation of renal function without urinary obstruction.

Doctor Kim and collegues from the Pohang University of Science and Technology (POSTECH), Pohang, Republic of Korea performed on page 833 an interesting basic research about the Effect of Mesenchymal Stem Cells (MSCs) Associated to Matrixen on the erectile function after bilateral cavernous nerve injury. They studied 35 rats and induced bilateral cavernous nerve injury. The authors concluded that the erectile function was more preserved in MSCs/Matrixen group compared with the administration of MSCs alone in the rats with bilateral cavernous nerve crushing injury. Therefore, they consider that the use of transplant cell carrier such as Matrixen may help the implantation of MSCs and improve the therapeutic effect of MSCs.

Doctor Ornellas and collegues from the Brazilian National Cancer Institute from Brazil in collaboration with the Mário Kröeff Hospital from Brazil and with the University of Milano-Bicocca,

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Milan, Italy performed on page 739 an interesting basic research about the cancer markers in penile cancer. They analyzed the plasma of 36 healthy subjects and 25 patients with penile carcinoma who underwent surgical treatment. The plasma was collected and analyzed by the ClinProt/MALDI/ToF technique. Then the peptides were identified from the C8 MB eluted fraction of patients' and control subjects' plasma by LIFT MS/MS. They authors showed that as the disease progresses, the fragments C3 and C4 A/B are less expressed in comparison with healthy subjects. These results may be useful as prognostic tools.

Dr. Sidney Glina

Editor-In-Chief
Internacional Braz J Urol

Editorial TOP 5 in 2012

In 2012 the International Brazilian Journal of Urology editorial board would like to highlights some papers of great interest.

Oncology

Doctor Botrel and collegues (1), performed a interesting meta-analysis about the Immunotherapy with Sipuleucel-T (APC8015) in patients with metastatic castration-refractory prostate cancer. The authors studied several databases including MEDLINE, EMBASE, LILACS and CENTRAL. The endpoints were overall survival (OS), time to progression (TTP) and side effects. The final analysis included 3 trials comprising 737 patients. The authors concluded that Sipuleucel-T prolongs overall survival in patients with asymptomatic or minimally symptomatic metastatic castration-refractory prostate cancer.

Doctor Lang and Collegues (2) performed a interesting study about the small renal masses in CTs. They performed a retrospective search for incidentally discovered small renal masses, less then 1 cm in size, was carried out in the files of 6 major US medical centers. 4822 such lesions had been reported over a 12 year period. The authors concluded that is questionable whether the small number of malignant neoplasms (0.4%), inflammatory lesions (5%) and renal medullary necrosis (6%) justify routine follow-up CTs and exposure to radiation. The delay in intervention in neoplastic lesions probably didn't influence tumor-free survival potential and clinical symptoms would soon have revealed inflammatory conditions. With exception of ambivalent lesions, clinical surveillance appears adequate.

Gender reassignment surgery

Doctor Rossi-Neto and collegues (3) performed a report on surgical outcomes from 332 patients who underwent male to female gender reassignment surgery (GRS). The results showed a great number of adverse events, although functionality preserved. Comparision of the outcomes with recent

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publications additionally showed that treatment options provide satisfying results. Moreover, outcomes reaffirm penile inversion vaginoplasty in combination with glans-derived sensate clitoroplasty as a safe technique. Nevertheless, discussing and improving surgical techniques in order to reduce complications and their influence on patient's quality of life is still strongly necessary and theme of our future reports.

Male Health

Doctors Da Ros and Averbeck (4), a prospective study about the stimulating effect of clomiphene citrate (CC) in the endogenous testosterone production pathway and to address the applicability of this medication as a therapeutic option for symptomatic hypogonadism. They studied 125 patients with hypogonadism and low libido and concluded that the CC was effective in stimulating the endogenous production of testosterone. A lower level of total cholesterol was verified after three months of treatment. This medication should be considered as a therapeutic option for some patients with symptomatic male testosterone deficiency.

Endourology/Laparoscopy

Doctor Tsivian and Collegues (5) performed a interesting study about the Simplified hemostatic technique during laparoscopic partial nephrectomy. They studied 34 patients with Tumor size ranged from 17-85 mm. Median warm ischemia time was 23 min (range 12-45) and estimated blood loss 55 mL (30-1000). There were no intraoperative complications or conversions to open surgery. No urine leaks or postoperative bleedings were observed. They concluded that this simplified technique appears reliable and quick, and therefore may be attractive for many urologic surgeons. Furthermore, the avoidance of routine use of additional hemostatic maneuvers may provide an economical advantage to this approach with no compromise of the surgical outcome.

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Dr. Luciano A. Favorito

Associated Editor
Internacional Braz J Urol

REVIEW ARTICLE

Vol. 38 (6): 717-727, November - December, 2012

Immunotherapy with Sipuleucel-T (APC8015) in patients with metastatic castration-refractory prostate cancer (mCRPC): a systematic review and meta-analysis

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ABSTRACT

Objective: To perform a systematic review and meta-analysis of all randomized controlled trials comparing the efficacy of Sipuleucel-T versus placebo for asymptomatic or minimally symptomatic metastatic castration-refractory prostate cancer (mCRPC).
Materials ans Methods: Several databases were searched, including MEDLINE, EMBASE, LILACS, and CENTRAL. The endpoints were overall survival (OS), time to progression (TTP) and side effects. We performed a meta-analysis (MA) of the published data. The results are expressed as Hazard Ratio (HR) or Risk Ratio (RR), with their corresponding 95% confidence intervals (CI 95%).
Results: The final analysis included 3 trials comprising 737 patients. The TTP was similar in patients who received Sipuleucel-T or placebo (fixed effect: HR = 0.89; CI 95% = 0.75 to 1.05; p = 0.16), with no heterogeneity detected on this analysis (Chi2 = 2.14, df = 2 (P = 0.34); I2 = 6%). The results showed a higher overall survival in patients treated with Sipuleucel-T (fixed effect: HR = 0.74; CI 95% = 0.61 to 0.89; p = 0.001; NNT = 3). We found no heterogeneity on this analysis either (Chi2 = 1.46, df = 2 (P = 0.48); I2 = 0%). The incidence of adverse events (grade > 3) was the same in both groups.
Conclusion: Sipuleucel-T prolongs overall survival in patients with asymptomatic or minimally symptomatic mCRPC.

ARTICLE INFO

Key words:
Immunotherapy; Sipuleucel-T; Review Literature as Topic; Meta-analysis

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INTRODUCTION

Prostate cancer is the most common non-cutaneous malignancy and the second leading cause of cancer mortality amongst men in the Western world (1). Up to 40% of men diagnosed with prostate cancer will eventually develop metastatic disease (1). Although androgen deprivation is the standard of care for advanced prostate cancer, patients with metastatic disease eventually progress to a castration-resistant state (2,3).

The average survival for patients with metastatic castration-refractory prostate cancer (mCRPC) is 2 to 3 years (1). Other denominations have also been used in the definition of CRPC, including: hormone-resistant prostate cancer, hormone-refractory prostate cancer, hormone-independent prostate cancer and androgen-independent prostate cancer. CPRC is the most widely accepted term, because even those patients considered resistant to castration, may still show some response to secondary hormonal manipu-

lations as documented in studies with new drugs such as Abiraterone (4) or MDV3100 (5).

Until recently, we have had few effective therapeutic options for the management of CRPC. Mitoxantrone was the first chemotherapeutic agent that demonstrated clinical activity in CRPC; it was approved in 1996 based on a reportedly improved quality of life (6). New treatment strategies have since been developed. Docetaxel was shown to modestly improve overall survival (OS) in two phase III trials (7,8). Based on these results, docetaxel chemotherapy was approved in 2004 by the US Food and Drug Administration (FDA) as the first-line standard treatment of mCRPC (9).

In 2010, the autologous cellular immunotherapeutic product Sipuleucel-T also received FDA approval for use in patients with asymptomatic or minimally symptomatic mCRPC. This approval indicates immunotherapy as a feasible treatment for CRPC patients (10,11). Currently, most studies are investigating the real efficacy of immunotherapy in patients with metastatic CRPC (11).

Sipuleucel-T, also referred to as APC8015, is an autologous active cellular immunotherapy product designed to stimulate an immune response against prostate cancer. Sipuleucel-T consists of autologous peripheral blood mononuclear cells, including antigen-presenting cells, that have been activated in vitro with a recombinant fusion protein. The recombinant fusion protein PA2024 is composed of prostatic acid phosphatase, an antigen expressed in the majority of prostate adenocarcinomas (12-14).

The first randomized study, published in 2006, showed an increase of 4.5 months in median overall survival, in favor of Sipuleucel-T in patients with mCRPC (15).

However, 3 years later, the final results of the study D9902A were presented and no differences were observed in overall survival between the groups evaluated (14).

In 2010, a larger phase III study, the IMPACT trial, was subsequently published and showed an increase of 4.1 months in median overall survival in favor of the Sipuleucel-T group (16).

To confirm these survival findings, our objective was to analyze all published randomized controlled trials (RCTs) that compared the efficacy of Sipuleucel-T against placebo for asymptomatic or minimally symptomatic mCRPC.

MATERIALD AND METHODS

Study Selection Criteria

Types of Studies

We included RCTs with parallel design that compared the use of Sipuleucel-T (autologous active cellular immunotherapy) and placebo.

Types of participants

The selected studies included patients with radiologic evidence of metastases, asymptomatic or minimally symptomatic castration-refractory prostate cancer (progressive disease with serum testosterone level of less than 50 ng/dL or 17 nmoL/ liter) and without visceral metastases.

Search strategy for identification of studies

A wide search on the main computerized databases was conducted, including EMBASE, LILACS, MEDLINE, SCI, CENTRAL, The National Cancer Institute Clinical Trials service, and The Clinical Trials Register of Trials Central. In addition, the abstracts published in the proceedings of the American Society of Clinical Oncology (ASCO), the European Society for Medical Oncology (ESMO), Society of Urologic Oncology (SUO) were also searched.

For MEDLINE, we used the search strategy methodology for randomized controlled trials (17) recommended by the Cochrane Collaboration (18). For EMBASE, we used adaptations of this same strategy (17), and for LILACS, we used the search strategy methodology reported by Castro et al. (19). We performed an additional search on the SCI database looking for studies that were cited on the included studies. We added the specific terms pertinent to this review to the overall search strategy methodology for each database.

The overall search strategy was as follows: #1 ("Immunotherapy"(Mesh) AND "Prostatic Neoplasms"(Mesh); #2 Random*

Searches of electronic databases combined the terms: #1 AND #2 for these study designs:

Humans, Clinical Trial, Meta-Analysis, Practice Guideline, Randomized Controlled Trial, and Review.

Critical Evaluation of the Selected Studies

All the references retrieved by the search strategies had their title and abstract evaluated by two of the researchers. Every reference with the least indication of fulfilling the inclusion criteria was listed as pre-selected. We retrieved the complete article of all pre-selected references. They were analyzed by two different researchers and included or excluded according to the previously reported criteria. The excluded trials and the reason of their exclusion are listed in this article. Data was extracted from all the included trials.

Details regarding the main methodology characteristics empirically linked to bias (20) were extracted with the methodological validity of each selected trial assessed by two reviewers (T.E.A.B and O.C). Particular attention was given to some items such as: the generation and concealment of the sequence of randomization, blinding, application of intention-to-treat analysis, sample size predefinition, loss of follow-up description, adverse events reports, if the trial was multi-centric and the sponsorship.

Data Extraction

Two independent reviewers extracted data. The name of the first author and year of publication were used to identify the study. All data were extracted directly from the text or calculated from available information when necessary. The data of all trials were based on the intention-to-treat principle, so they compared all patients allocated in one treatment with all those allocated in the other.

The primary endpoints were overall survival (OS) and time to progression (TTP). TTP was defined as the time from randomization to the time of disease progression. Disease progression included any of the following: 1) progressive disease on serial radiographic imaging tests; 2) new cancer-related pain associated with a radiographic anatomical correlation; or 3) other clinical events consistent with progression such as spinal cord compression, nerve root compression, or pathologic fracture.

Other clinical outcomes evaluated included the number of patients that presented adverse events (AEs) (grade ≥ 3): chills, fatigue, fever (pyrexia), back pain, headache, arthralgia, asthenia, nausea, anemia, vomiting and prostate-specific antigen (PSA) reduction of ≥ 50%.

Analysis and Presentation of Results

Data were analyzed using the Review Manager 5.0.24 statistical package (Cochrane Collaboration Software) (21).

Dichotomous clinical outcomes are reported as Risk Ratio (RR) and survival data as Hazard Ratio (HR) (22). The corresponding 95% confidence interval (CI 95%) was calculated, considering P values less than 5% ($p < 0.05$). A statistic for measuring heterogeneity was calculated through I2 method (25% was considered low-level heterogeneity, 25-50% moderate-level heterogeneity and > 50% high-level heterogeneity) (23,24).

To estimate the absolute gains in TTP and OS, we calculated the meta-analytic survival curves as suggested by Parmar et al. (22). A pooled estimate of the HR was computed by a fixed-effect model according to the inverse-variance method (25). Thus, for effectiveness an HR or RR greater than one favors the standard arm (Placebo) whereas an HR or RR less than 1 favors the Sipuleucel-T treatment.

If statistical heterogeneity was found in the meta-analysis, we performed an additional analysis using the random-effects model described by DerSimonian and Laird (26), that provides a more conservative analysis.

To assess the possibility of publication bias, we performed the funnel plot test described by Egger et al. (27). When the pooled results were significant, the number of patients needed to treat (NNT) to cause or to prevent one event was calculated by pooling absolute risk differences in trials included in meta-analyses (28-30). For all analyses, a forest plot was generated to display results.

RESULTS

The diagram represents the flow of identification and inclusion of trials, as recommended by the PRISMA statement (Preferred Reporting

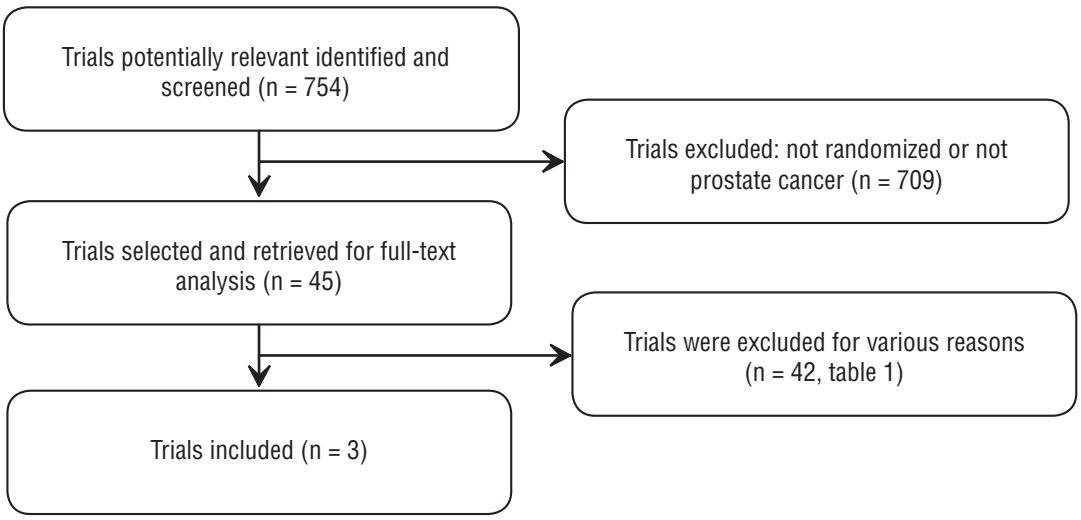
Items for Systematic Reviews and Meta-Analyses) (31) (Figure-1).

Overall, 754 references were identified and screened.

Forty-five studies were selected and retrieved for full-text analysis. Of these studies, 42 were excluded for various reasons, described on Table-1 (additional material).

The final analysis included 3 trials comprising 737 patients. All trials were randomized (Sipuleucel-T or control), double-blinded, placebo-controlled and multi-centric. The primary endpoint was TTP in two studies (14,15) and OS in one study (16) (Table-2).

Figure 1 - Trial selection flow.



Patients were randomly assigned in a 2:1 ratio to receive either Sipuleucel-T or placebo every 2 weeks, for a total of three infusions. In all studies, concurrent bisphosphonates therapy and previous chemotherapy were allowed, but patients with visceral metastases were excluded (14-16).

Patients without prior bilateral orchiectomy continued on gonadal suppression with a luteinizing hormone-releasing hormone agonist throughout the trials. In all studies, pretreatment was performed with acetaminophen and an antihistamine such as diphenhydramine (14-16).

Reductions in PSA levels ≥ 50% were equally detected in both groups (fixed effect: RR = 0.98; CI 95% = 0.96 to 1.00; p = 0.07). We found no heterogeneity on this analysis (Chi^2 = 0.68, df = 1 (P = 0.41); I^2 = 0%) (Figure-2).

The TTP was similar in patients who received Sipuleucel-T or placebo (fixed effect: HR = 0.89; CI 95% = 0.75 to 1.05; p = 0.16) without indications of heterogeneity on this analysis (Chi^2 = 2.14, df = 2 (P = 0.34); I^2 = 6%) (Figure-3).

The results showed a higher overall survival in patients treated with Sipuleucel-T (fixed effect: HR = 0.74; CI 95% = 0.61 to 0.89; p = 0.001; NNT = 3), again without heterogeneity (Chi^2 = 1.46, df = 2 (P = 0.48); I^2 = 0%) (Figure-4).

The incidence of grade ≥ 3 adverse events was the same in both groups: chills (RR = 6.13; CI 95% = 0.81 to 46.73), fatigue (RR = 0.94; CI 95% = 0.26 to 3.39), fever (pyrexia) (RR = 0.66; CI 95% = 0.16 to 2.61), back pain (RR = 0.89; CI95% = 0.40 to 1.98), headache (RR = 2.04; CI 95% = 0.23 to 18.12), arthralgia (RR = 0.96; CI 95% = 0.35

Table 1 - Characteristics of Excluded Studies.

Study	Reason for Exclusion
Beer 2011 (39)	Androgen-dependent prostate cancer
Sanda 1999 (40)	Not a randomized trial
Madan 2009 (41)	Not a randomized trial
Antonarakis 2010 (42)	Not a randomized trial
Cha 2011 (43)	Not a randomized trial
Joniau 2011 (44)	Not a randomized trial
Madan 2010 (45)	Not a randomized trial
May 2011 (46)	Not a randomized trial
Morse 2010 (47)	Not a randomized trial
McLeod 2011 (48)	A subgroup analysis
Fizazi 2011 (49)	Androgen-dependent prostate cancer
Beer 2011 (50)	Androgen-dependent prostate cancer
Penson 2006 (51)	Integrated analysis of studies analyzed (Small and Higano)
Sonpavde 2011 (52)	Not a randomized trial
Beltran 2011 (1)	Not a randomized trial
Drake 2010 (53)	Not a randomized trial
Cha 2010 (2)	Not a randomized trial
Harzstark 2009 (54)	Not a randomized trial
Mohebtash 2008 (55)	Not a randomized trial
Vaishampayan 2008 (56)	Not a randomized trial
Harzstark 2008 (57)	Not a randomized trial
Barqawi 2007 (58)	Not immunotherapy
Arlen 2007 (59)	Not a randomized trial
Di Lorenzo 2007 (60)	Not a randomized trial

Table 2 - Characteristics of Included Studies.

Study	N	Patients	Desing	Interventions	Primary endpoint
Small 2006 (15)	127	Asymptomatic metastatic CRPC	Randomized, double-blind, placebo-controlled, multicenter	Sipuleucel-T Placebo	Time to disease progression
Higano 2009 (14)	98	Asymptomatic metastatic CRPC	Randomized, double-blind, placebo-controlled, multicenter	Sipuleucel-T Placebo	Time to disease progression
Kantoff 2010 (16)	512	Asymptomatic or minimally sympto-matic CRPC	Randomized, double-blind, placebo-controlled, multicenter	Sipuleucel-T Placebo	Overall survival

Figure 2 - Comparative effect in reduction of PSA > 50% of Sipuleucel-T versus Placebo.

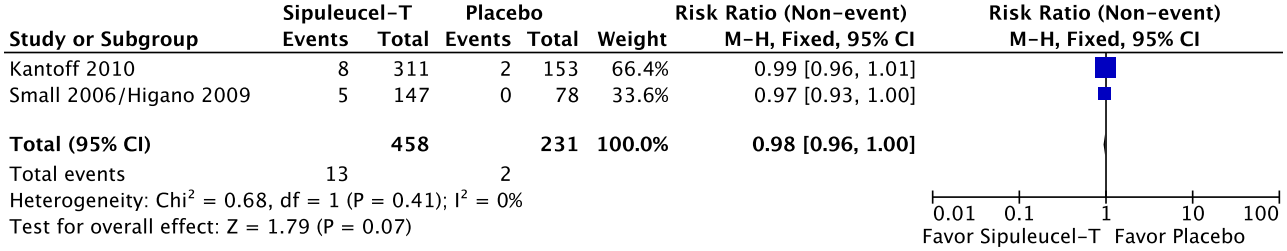


Figure 3 - Comparative effect in time to progression of Sipuleucel-T versus Placebo.

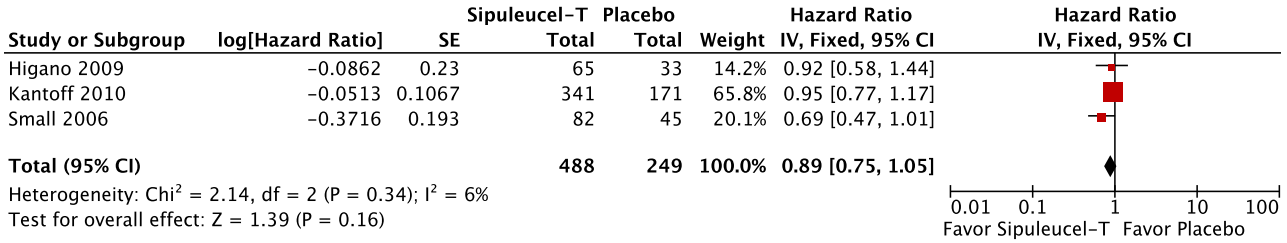
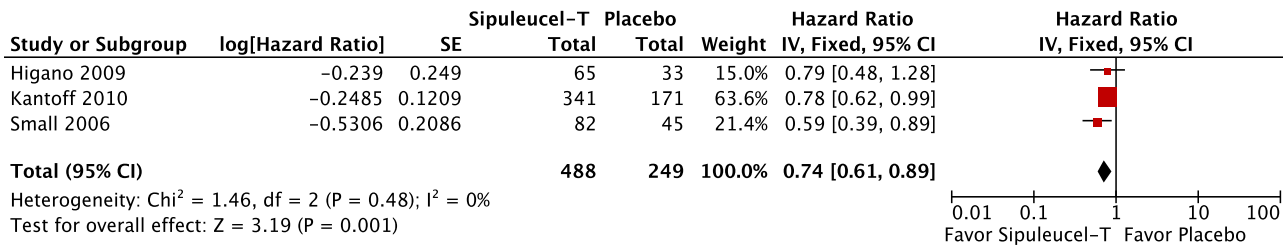


Figure 4 - Comparative effect in overall survival of Sipuleucel-T versus Placebo.



to 2.66), asthenia (RR = 1.49; CI 95% = 0.30 to 7.31), nausea (RR = 2.03; CI 95% = 0.23 to 18.10), anemia (RR = 0.78; CI 95% = 0.31 to 1.92) and vomiting (RR = 1.56; CI 95% = 0.06 to 37.86) (Figure-5).

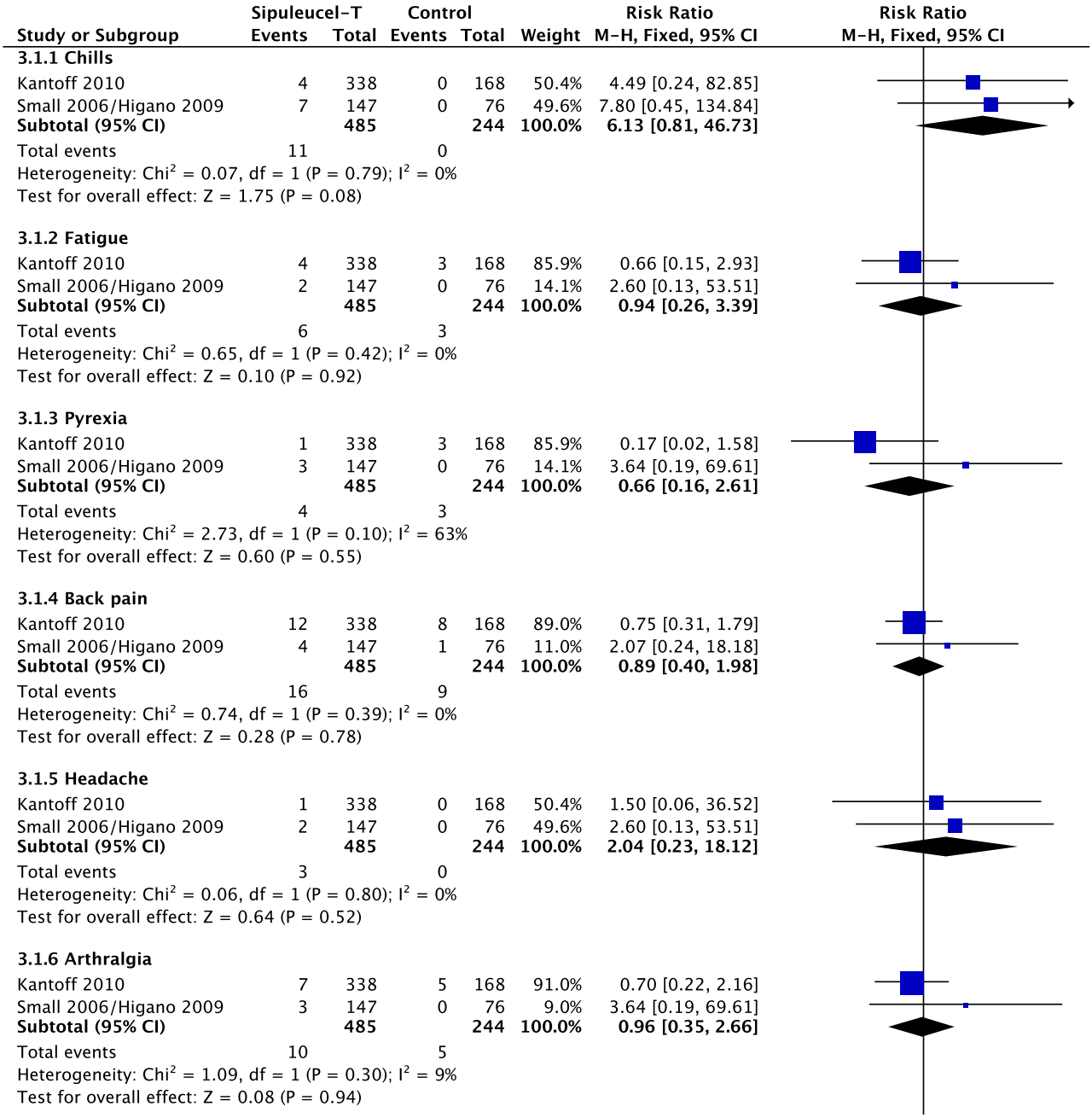
According to the funnel plot analysis (27) the possibility of publication bias was low for all of the endpoints.

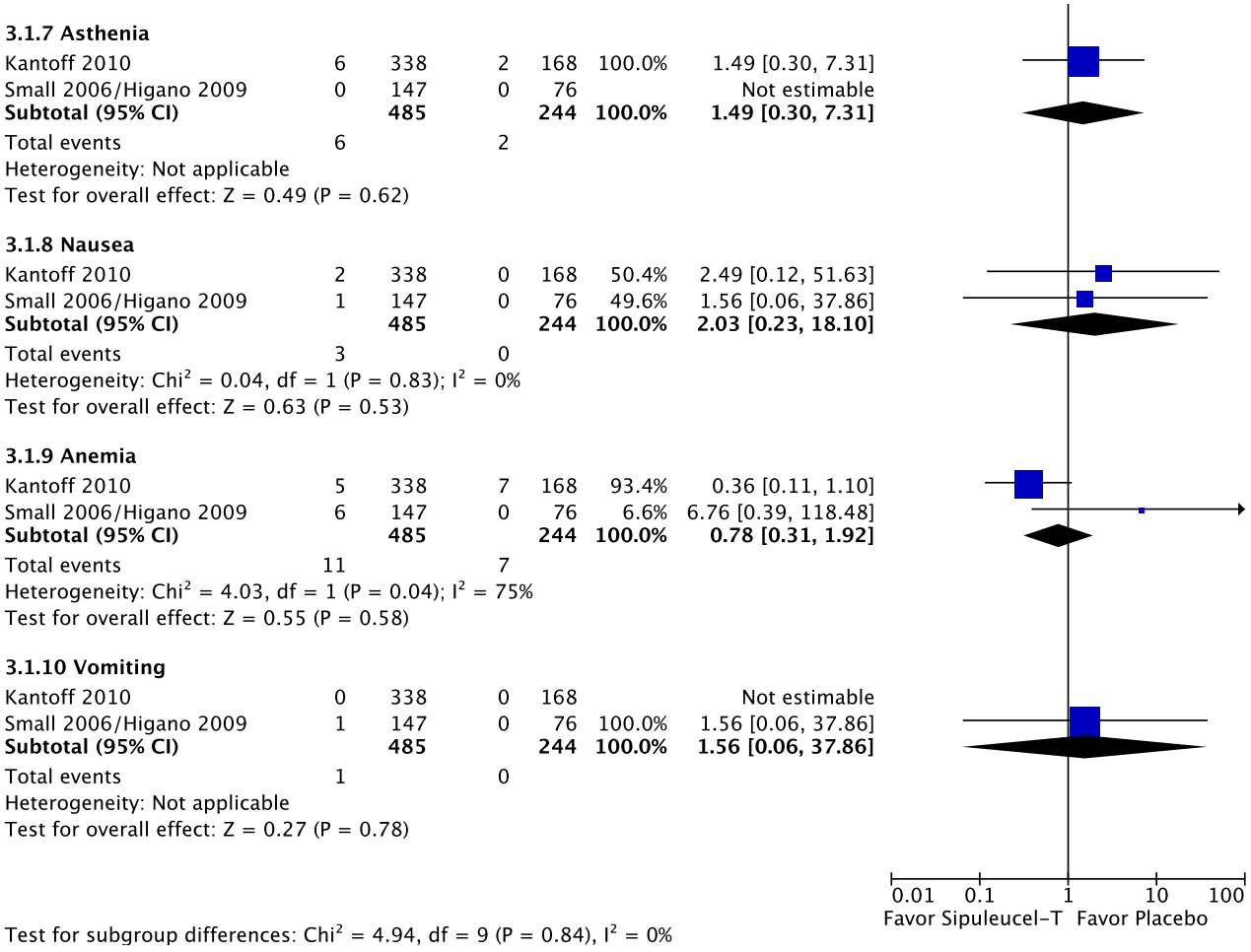
DISCUSSION

Vaccine-based therapies seek to directly stimulate a specific immune reaction against a single or multi-tumor antigens (11).

The four main types of vaccines that have been investigated for CRPC can be classified as

Figure 5 - Adverse events (grade > 3) of patients treated with Sipuleucel-T versus Placebo.





viral vector based, cell based, DNA vaccines, and autologous (derived from a patient's own tumor cells) (11).

Sipuleucel-T in this meta-analysis reduced the risk of death by 26%. In contrast to overall survival, the time to progression for the respective diseases and reduction in PSA levels did not differ significantly between Sipuleucel-T and placebo. This may be due to the delayed onset of antitumor responses after active immunotherapy, relative to objective disease progression (16). Other randomized clinical trials have also demonstrated that benefits in overall survival were not linked to effects in time to progression (32) or vice versa (16,33).

Similarly, PSA level declines have not consistently been a surrogate endpoint for survival, particularly in CRPC trials. The TAX327 trial is one example (34). In that trial, the docetaxel given every 3 weeks arm and the weekly docetaxel arm had essentially similar PSA > 50% decline rates (45% and 48%, respectively) yet only the every 3 week regimen demonstrated a survival advantage compared to mitoxantrone (35).

In the study by Kantoff (16) most patients had a PSA response rate measured at least twice during treatment. In the integrated analysis of two other studies included in this analysis (14) only 26% of patients had ≥ two PSA values measured at least 4 weeks apart. Therefore the PSA response rate may be underestimated (14).

The most common AEs associated with Sipuleucel-T treatment were chills, fatigue, fever (pyrexia), back pain, and headache. These AEs are generally consistent with cytokine release, as observed after the administration of other immunotherapies (36). Most AEs developed within one day

of infusion were grade 1/2 in severity and resolved in 2 days or less (37).

Docetaxel and Cabazitaxel, other FDA approved therapies that confer a survival advantage in mCRPC cases, are associated with grade 3/4 hematological toxicities and infections in 5% to 82% of patients (7,37,38). Abiraterone acetate, a potent inhibitor of CYP17 and androgen synthesis, also demonstrated improved survival in men with metastatic CRPC with progression after docetaxel chemotherapy. The most common adverse events included fluid retention, hypokalemia and hypertension (4). None of these have been compared in head to head trials or against Sipuleucel-T so we still do not know which is the better option.

Despite the encouraging results, there are many unresolved questions regarding immunotherapy, including the best clinical setting for immunotherapy (the rational combination and proper sequencing of Sipuleucel-T with other newly approved agents) and the definition of relevant clinical and immunological endpoints (2).

CONCLUSIONS

Sipuleucel-T prolongs the survival of patients with asymptomatic or minimally symptomatic mCRPC. The time to progression was the same of both groups. No grade 3/4 AEs were reported in 5% or more of patients.

CONFLICT OF INTEREST

None declared.

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Penile Cancer Disparities in Puerto Rican Men as compared to the United States Population

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ABSTRACT

Purpose: This study compares incidence and mortality of penile cancer in Puerto Rico (PR) with other racial/ethnic groups in the United States (US) and evaluates the extent in which socioeconomic position index (SEP) or its components influence incidence and mortality in PR.

Materials and Methods: Age-standardized rates were calculated for incidence and mortality based on data from the PR Cancer Registry and the US National Cancer Institute's Surveillance, Epidemiology and End Results program, using the direct method.

Results: PR men had approximately 3-fold higher incidence of penile cancer as compared to non-Hispanic white (Standardized rate ratio [SRR]: 3.33; 95%CI=2.80-3.95). A higher incidence of penile cancer was also reported in PR men as compared to non-Hispanic blacks and Hispanics men. Mortality from penile cancer was also higher for PR men as compared to all other ethnic/racial groups. PR men in the lowest SEP index had 70% higher incidence of penile cancer as compared with those PR men in the highest SEP index. However, the association was marginally significant (SRR: 1.70; 95%CI=0.97, 2.87). Only low educational attainment was statistically associated with higher penile cancer incidence (SRR: 2.18; 95%CI=1.42-3.29).

Conclusions: Although penile cancer is relatively uncommon, our results support significant disparities in the incidence and mortality rates among men in PR. Low educational attainment might influence the high incidence of penile cancer among PR men. Further studies are strongly recommended to explore these disparities.

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Key words:
Urogenital Neoplasms; Penile Neoplasms; Epidemiology; Minority Health; Healthcare Disparities

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INTRODUCTION

Increased insight has been gained into the pathogenesis of penile cancer, since reports indicated that several risk factors such as phimosis with chronic inflammation, lack of circumcision, human papillomavirus (HPV) infection, poor hygiene and smoking history are associated with pe-

nile cancer (1). Some of these risk factors, including HPV infection, circumcision, smoking and hygiene, have been previously identified as modifiable risk factors that might lead to a reduction of penile cancer incidence and mortality (2).

The incidence of penile cancer varies enormously among different populations, being highest in developing countries (3). An evaluation of cancer registries around the world shows that although penile cancer is a rare disease with lowest rates in Israel (4), Western Europe and the United States (US) (<1 per 100,000) (5), incidence rates of 4.0 and 3.7 per 100,000 have been reported in Uganda and Brazil, respectively (6). In the US, although penile cancer represents less than 1% of new cancers in men, rates among Hispanics (USH) are 72% higher compared to Non-Hispanics (7). These higher estimates of penile cancer among Hispanic men correlate with the high incidence of cervical cancer among USH women in the US compared with non-Hispanic whites (NHW) (8). Penile incidence rates from a limited time period (1995-1999) also shows a high incidence rate in PR men (2.6 per 100,000) (9).

Despite the low burden of penile cancer incidence and mortality in the US, it is important to assess the recent distribution of this malignancy within specific racial/ethnic groups, particularly those of Hispanic origin. This will be important in order to identify vulnerable groups and evaluate the impact that HPV-related cancers in men might have on cervical cancer. Within the Hispanic/Latino population, in Puerto Rico (PR), particularly, where rates of cervical and oropharyngeal cancer are higher than Non-Hispanic Whites (NHW) (8,10), no studies have been performed recently to evaluate penile cancer incidence and mortality rates beyond descriptive epidemiology (11). Therefore, in this study we estimated trends in penile cancer occurrence for each racial/ethnic group from 1992 to 2004; then, we compared penile cancer incidence and mortality in men in PR with USH, NHW and Non-Hispanic Blacks (NHB), during the period 2000-2004. Finally, given the potential impact of socioeconomic factors on the burden of penile cancers in men, we evaluated the effect of socioeconomic indicators of health on penile cancer incidence and mortality among PR men.

MATERIALS AND METHODS

Data sources

Incidence statistics from PR were obtained from the Puerto Rico Central Cancer Registry (PRCCR). The PRCCR is the fourth oldest population-based cancer registry in the world collecting information on cancer in PR since 1951. The PRCCR uses the Surveillance, Epidemiology, and End Results (SEER) program and the North American Association of Central Cancer Registries (NAACCR) standards for coding data; thus, the registry is fully comparable with both SEER and NAACCR data. All penile cancer cases diagnosed since 2001 are reported using the third edition of the International Classification of Disease for Oncology (ICD-O-3) (C60.0-C60.9). Cases from 1992 to 2000 which were originally reported using previous editions of ICD-O were converted to ICD-O-3 codes. Mortality information for PR from 1998-2004 was obtained from the PRCCR as reported by death certificates prepared by the PR Department of Health.

Penile cancer incidence statistics for USH, NHW and NHB were obtained from those released by the SEER program. The SEER program identifies Hispanic ethnicity by a combination of medical record review and matching surnames against a list of Hispanic surnames. The term Hispanic used throughout our report does not account for racial differences within the USH population. Penile cancer mortality information for USH, NHW and NHB was obtained from the SEER program as reported by the National Center for Health Statistics (NCHS). US mortality cases were obtained for all states except Connecticut, Maine, Maryland, Minnesota, New Hampshire, New York, North Dakota, Oklahoma and Vermont because of the large number of individuals with unknown origin or ethnicity (≥10.0% missing) for several years. Thus, the "Hispanic Index" as developed by the National Cancer Institute (NCI) was used to exclude states where mortality statistics for Hispanics were deemed unreliable (12).

Statistical analysis

Age-standardized rates: For each racial/ethnic group, we applied the direct method to esti-

mate the penile cancer age-standardized incidence and mortality (per 100,000 persons) for three time periods (1992-1996, 1997-2000, and 2001-2004) using the World Standard Population as reference. These rates were identified by ASR (World), either for incidence or mortality. The change in the ASR from the earliest and the latest studied period (1992-1996 and 2001-2004) was calculated as a percentage as follows:

$$\%change = \frac{Rate_{2001-2004} - Rate_{1992-1996}}{Rate_{1992-1996}} * 100$$

The significant percentage of change was determined by the construction of the 95% confidence intervals (CI) using the formulas from the U.S. Census Bureau (http:// www.census.gov/acs/www/Downloads/data_documentation/Accuracy/PercChg.pdf). If zero was not included in this interval, significant changes were declared with p--value less than 5%.

Group differences: To assess racial/ethnic group differences, the ASRs (World) were grouped during the study period, as follows:

$$ASR(World)_i = \sum_{j=1}^{3} w_j \frac{\sum_{k=2001}^{2004} d_{ij}^k}{\sum_{k=2001}^{2000} n_{ij}^k}$$

Then, the ratio of two standardized rates

$$ASR(World)group_i \Big/ ASR(World)group_j$$

between any two groups was estimated with 95% CIs, to assess differences in penile cancer incidence and mortality between PR group and USH, NHB and NHW groups. This ratio if referred to as the standardized rate ratio (SRR). In addition, age-specific incidence (per 100,000 persons) and mortality rates for different age groups (<60, 60-70, >70) was computed for the period 2001-2004. On the basis of these rates, the relative risks (RR) were estimated with 95% CIs to determine relative differences among study groups using the Poisson regression model. The reference groups in the age--specific RR estimation were NHW, NHB, and USH.

SEP: The socioeconomic assessment was performed only for PR because the geographical level of analysis available to define the socioeconomic characteristics in PR was different from the geographical level in the US. PR penile cases diagnosed from 2001-2004 and cancer deaths from 2001-2004 were linked to the 2000 PR Census data. Briefly, principal component analysis (PCA) was used to obtain the SEP index at municipality level. The PCA transform a set of correlated variables to a new set of uncorrelated variables. Therefore, we initially considered 14 socioeconomic indicators available in the US Census 2000 for PR. Then, the most correlated socioeconomic indicators, based on the Pearson correlation index and PR data, were used for PCA. As a consequence the following eight-census based socioeconomic indicators were used for the PCA: unemployment rate, median family income, %population living below the poverty level, %population aged > 25 years with less than 12 years of education, %employed civilian population aged > 16 years in management, professional, and related occupations (used to define white-collar occupations), %occupied housing units without telephone, %population fluent in both English and Spanish, and %occupied housing units without car (13). The first principal component was used to define the SEP due to the fact, that the rest of the principal components had a variance lower that one (criterion of Kaiser). Once the SEP was computed for every municipality, the index was categorized in five groups using quintiles as the cut-off points; such as the municipalities with the lowest socioeconomic position (highest socioeconomic deprivation) were identified by SEP1 and the municipalities with highest socioeconomic position (lowest socioeconomic deprivation) were identified by SEP5. To assess the socioeconomic effect, the two extreme SEP categories were used (SEP1 vs. SEP5) to compute the relative ratio (RR) of the ASR's of penile cancer incidence and mortality with 95% confidence intervals. The ratio of the ASR's in these SEP categories was used to determine the socioeconomic disparity by the SEP index and the

eight-census based socioeconomic indicators of penile cancer incidence and mortality among men in PR for the period 2000-2004.

The statistical comparisons were performed using the STATA System release 11.0 (STATA Corp, College Station, TX, USA).

RESULTS

Penile Cancer Trends

In PR, a total of 587 cases of penile cancer were diagnosed between 1992-2004. Comparing the period of 2001-04 vs. 1992-1996, penile cancer incidence decreased for all racial/ethnic groups. Increasing percent change in mortality of penile cancer was only observed among NHB and PR men. A slightly increase percent change was observed among NWH (Table-1).

Penile Cancer Rates (2000-2004)

The age-standardized incidence (per 100,000) of penile cancer ranged from 0.84 in NHW to 2.8 in PR. Puerto Rican men had 3 times higher incidence, than NHW (SIR: 3.33; 95%CI=2.80, 3.95) and NHB (SIR: 3.04; 95%CI=2.21, 4.36). PR men had more than 2 times higher incidence than USH (SIR: 2.59; 95%CI=1.99, 3.43) (Table-2).

The age-specific incidence increased with age among all ethnic/racial groups (Table-2). However, Puerto Rican men had a significant higher incidence ($p<0.05$) of penile cancer in all age group categories studied (Table-2). Particularly higher incidence of penile cancer was observed in Puerto Rican men of younger ages (<60 years) as compared to their USH, NHB and NHW counterparts ($p< 0.05$). Puerto Rican men younger than 60 years old had approximately 5 times higher incidence

Table 1 - Incidence and mortality for penile cancer among men in PR and racial/ethnic groups in the US: 1992-1996, 1997-2000, and 2001-2004.

Incidence (x 100,000)					
Racial/Ethnic Group	1992-1996	1997-2000	2001-2004	% change*	CP 95% CI
PR	3.46	3.19	2.75	-20.59	-37.16, -4.02
NHW	0.84	0.84	0.81	-3.52	-18.54, 11.50
NHB	1.32	0.91	1.18	-10.84	-49.04, 44.47
USH	0.94	1.02	0.92	-2.28	-44.13, 22.42
Mortality (x 100,000)					
Racial/Ethnic Group	1992-1996	1997-2000	2001-2004	% change*	CP 95% CI
PR	0.57	0.57	0.64	12.59	-40.59, 65.86
NHW	0.19	0.19	0.19	0.87	-10.02, 11.74
NHB	0.21	0.30	0.34	57.99	-45.07, 0.00
USH	0.33	0.27	0.26	-22.56	-0.96, 116.86

* Estimation of the percent change was done utilizing 15 decimals points.

Table 2 - Overall age-standardized, age-specific incidence and mortality rates (x 100,000) for penile cancer: 2000 -2004.

	Total number of cases				Age Specific Rate (x 100,000)				Relative Risk (RR)[a]		
	PR	NHW	USH	NHB	PR	NHW	USH	NHB	PR/NHW	PR/USH	PR/NHB
Incidence											
< 60 yrs	71	88	56	14	0.9	0.2	0.3	0.2	4.71 (3.44-6.44)	3.13 (2.21-4.44)	5.93 (3.34-10.52)
60 - 70 yrs	60	87	28	14	8.4	2	4.1	2.7	4.14 (2.98-5.75)	2.03 (1.30-3.18)	3.12 (1.74-3.93)
> 70 yrs	85	220	26	22	12.9	4.7	5	5.3	2.78 (2.16-3.57)	2.59 (1.67-4.02)	2.46 (1.54-3.93)
Overall Age-standardized	216	395	110	50	2.8[b]	0.8[b]	1.1[b]	0.9[b]	3.33 (2.80-3.95)[c]	2.59 (1.99-3.43)[c]	3.04 (2.21-4.36)[c]
Mortality											
< 60 yrs	18	183	67	34	0.2	0	0.1	0	5.01 (3.09-8.13)	3.14 (1.87-5.28)	5.2 (2.94-9.20)
60 - 70 yrs	7	184	31	31	1	0.5	0.9	0.7	2.14 (1.02-4.55)	1.08 (0.47-2.44)	1.4 (0.61-3.17)
> 70 yrs	21	450	48	52	3.2	1	1.8	1.4	3.11 (2.01-4.82)	1.75 (1.05-2.92)	2.24 (1.35-3.71)
Overall Age-standardized	46	817	146	117	0.6[b]	0.2[b]	0.3[b]	0.3[b]	3.32 (2.38-4.43)[c]	1.89 (1.30-2.68)[c]	2.51 (1.71-3.55)[c]

[a]Relative risk with 95% confidence interval.

[b]Age-standardized to the world standard population.

[c]The ratio of two age-standardized rates (using the world standard population) with 95% CI.

than NHW (SIR: 4.71; 95%CI=3.44, 6.44), up to three times higher incidence than USH (SIR: 3.13; 95%CI=2.21, 4.44) and approximately 6 times higher incidence than NHB men (SIR: 5.93; 95%CI=3.34, 10.52).

Penile Cancer Mortality

The annual mortality of penile cancer (per 100,000) ranged from 0.19 in NHW to 0.63 in PR men (Table-1). During the period 2000-2004, Puerto Rican men had a significant higher mortality than their USH, NHB and NHW counterparts ($p<0.05$), with mortality up to three times higher than NHW men (SIR: 3.32; 95%CI=2.38, 4.43). When comparing the age-specific mortality in the same period, higher mortality were also observed among men in PR as compared to the other studied racial/ethnic groups in all age groups, except the 60-70 age group in which men in PR compared with their USH and NHB counterparts (Table-2).

Socioeconomic Indicators and Penile Cancer Incidence and Mortality in PR

As shown in Table-3, men in PR in the lowest socioeconomic deprivation index (SEP1) had 70% higher incidence of penile cancer as compared with those PR men in the highest socioeconomic deprivation index (SEP5). However, the association was marginally significant (SRR: 1.70; 95%CI=0.97, 2.87). When evaluating each of the eight socioeconomic indicators, only low educational attainment (<12 years of education), was statistically associated with higher incidence of penile cancer ($p<0.05$). That is, men in PR with a low educational attainment had up to 2 times higher incidence of penile cancer as compared with those with high educational attainment (SRR: 2.18; 95%CI=1.42, 3.29).

When evaluating the effect of the SEP index in penile cancer mortality, a higher mortality was also observed among those in the lowest socioeconomic deprivation index (SEP1); however, this observed increment was not statistically significant (SRR: 1.61; 95%CI=0.38, 5.21). No statistical association was observed in the other socioeconomic indicators components ($p>0.05$).

DISCUSSION

This analysis supports racial and ethnic differences in the incidence and mortality of penile cancer in the US and PR. The available data also permit the assessment of potential sub-group differences within the broad Hispanic/Latino category and showed important variations between the incidence and mortality for PR and USH. Our study showed that although penile cancer is a relatively uncommon tumor, the incidence of this tumor is up to three times higher in PR as compared with other racial/ethnic groups in the continental US. Mortality was also significantly higher in Puerto Rican men as compared to any other racial/ethnic group.

Epidemiological studies have identified risk factors such as smoking and being uncircumcised as risk factors for penile cancer (1). Tobacco smoking, particularly current smoking, has been reported in a number of studies to be linked to increased risk of penile cancer, although other studies have failed to found support for this association (14). Smoking rates overall have been declining in the last decade (15), with lower median prevalence of adult current smoking in PR (12.2%) as compared to the US (19.8%), a pattern that does not support the higher incidence of penile cancer in PR as compared to the US. Although decreasing trends have been observed in population-based studies US, when differences by Hispanic origin have been examined, respondents of Puerto Rican and Cuban origin have been found to be significantly more likely to smoke (16) as compared to other Hispanic origin sub-groups.

On the other hand, low rates of circumcision in PR and USH have been reported in population-based studies. For example, the National Health and Nutrition Examination Survey (NHANES 1999-2004) reported that 79% men in the US are circumcised (17), with prevalence estimates lower among Mexican American men (42%). Population-based studies in PR have estimated the prevalence of circumcision among men is only 30.6% (18), highlighting a possible mechanism in which penile cancer rates might be higher in this population, although further

Table 3 - Age-standardized (x100,000) incidence and mortality rates for Penile Cancer by SEP index: Puerto Rico 2000-2004.

		SEP 1[a,b]	SEP5[a,c]	SRR (95% CI)[d]
Incidence				
SEP Category		2.6	1.5	1.70 (0.97, 2.87)
SEP Components	Less than 12 years of education[e]	3.8	1.7	**2.18 (1.42, 3.29)**
	English Proficiency	2.2	1.5	1.48 (0.83, 2.53)
	Median Family Income	2.5	1.7	1.50 (0.87, 2.46)
	No Cars	1.9	1.8	1.02 (0.65, 1.60)
	No Telephone	2.7	1.6	1.67 (1.00, 2.70)
	Poverty	2.5	1.7	1.48 (0.85, 2.48)
	Unemployed	2.4	1.6	1.47 (0.84, 2.47)
	White Collar	19.2	9.3	2.06 (0.98, 4.08)
Mortality				
SEP Category		0.5	0.3	1.61 (0.38, 5.21)
SEP Components	Less than 12 years of Education	2.7	1.8	1.53 (0.15, 9.45)
	English Proficiency	0.5	0.3	1.34 (0.31, 4.43)
	Median Family Income	6.2	2.0	3.14 (0.63, 14.68)
	No Cars	3.0	2.4	1.27 (0.28, 6.47)
	No Telephone	8.0	2.1	3.84 (0.93, 15.19)
	Poverty	0.5	0.4	1.15 (0.28, 3.50)
	Unemployed	3.4	2.0	1.72 (0.17, 10.43)
	White Collar	0.9	0.5	1.86 (0.74, 4.24)

[a] Rates are per 100,000 and age-adjusted to the 2000 PR.
[b] SRR=SEP=1 is the low socioeconomic position (highest socioeconomic deprivation).
[c] SEP=5 is the high socioeconomic position (lowest socioeconomic deprivation), reference group.
[d] SRR=SEP1/SEP5 indicates the standardized rate ratio with 95% confidence intervals (Tiwari method).
[e] Statistically significant ($p<0.05$)

research is needed in this area. There is support in the literature that the effect of circumcision is mediated by avoidance of phimosis. In many countries, such as Denmark, circumcision rates have not changed but penile cancer incidence rates are falling. This may be due to better hygiene over the years with lower rates of phimosis. In PR, determining the importance of circumcision in the incidence of penile cancer among men will be necessary to document, as there is evidence that male circumcision reduces the risk of heterosexually acquired HIV infection (19) and clearance of HPV infection, including infection with oncogenic types (20).

Past infection with HPV is also a known risk factor for penile cancer which has been detected in approximately 50% of penile cancer cases (21). As HPV infection and lifestyle sexual partners are significantly associated with oncogenic HPV infection (22,23), the number of lifetime sexual partners on penile cancer is also important to understand the role of higher number of sexual partnering and increased risk of penile cancer. In PR, a population-based study of men and women ages 18-64 reported that 80% of men initiated their sexual activity before age 18 and almost half of the men interviewed (47.8%) had more than 7 sexual partners in their lifetime (21). Although this estimates are lower than a population-based study in the US, these results showed an early age of sexual initiation and a high prevalence of multiple sexual partners in PR, which document the importance of developing interventions that promote safe sex practices among men in this population, which will also partially protect them against HPV infection.

Clinical risk factors have also been identified as strong predictors of penile cancer. The most important clinical risk factor for invasive penile cancer is the history of phimosis (2). Studies have found that history of phimosis was reported in approximately 25%-60% of patients with penile cancer (24). A clinical study realized in PR reported that phimosis was present in (85.7%) of the pathologic reports reviewed between 1979-1989 in a hospital of the western region of PR (25). Other risk factors that were explored in this study and showed significant association with penile cancer were history of Sexually Transmitted Infections (STIs), and leukopakia.

Our study also showed that the rate of penile cancer in PR has been declining. Although other studies have attributed similar declines to the availability of prophylactic HPV vaccination (7), attributing the impact of the decline of penile cancer incidence to HPV vaccination is unlikely since population uptake of the vaccine among Puerto Rico young men is very recent and low (6%) (26) and just recently (2009) vaccination was approved for men. Other studies have attributed this decline in penile cancer incidence due to better hygiene over the years with lower rates of phimosis. Also, the impact of how other risk factors (hygiene, decrease in tobacco consumption or potential increasing circumcision rates) might have an impact on this decline cannot be ascertained utilizing this data set.

On the other hand, it is important to highlight that although a significant reduction was observed in incidence rates, an increased trend in penile cancer mortality was noted among men in PR. Although this increase in mortality was not significant, an evaluation of the clinical characteristics of cancers diagnosed with respect to extent of disease and treatment provided will be important to ascertain in future studies. Within this context, it is important to evaluate the effect of socioeconomic position on penile cancer incidence. Socioeconomic disparities in cancer have been widely reported previously (27). However, which cultural, economic or social factors might be influencing higher risk among Puerto Rican men is unknown. It can be hypothesized that health insurance coverage, access to appropriate early detection and treatment could be influencing this higher rate. Also, low educational attainment might influence the high incidence of penile cancer among PR men, due to inadequate hygiene practices, as has been highlighted (28). From this latest study the importance of education on the prevention of urogenital cancers is highlighted, as a key element to decrease the burden of penile cancer in this population.

Finally, it is important to highlight that this data showed higher incidence and mortality of penile cancer among men younger than 60

years old, which contrasts with the reported epidemiology of penile cancer elsewhere (29). These findings highlight the opportunity to strengthen prevention efforts in Puerto Rican men. A high incidence of penile cancer and mortality within younger cohorts in Puerto Rican men might be possible due to a possible convergence of early and high sexual activity, including lifetime number of female sex partners, low circumcision rates, high prevalence of STIs and HIV in Puerto Rico, particularly among the medically underserved.

Our study has some limitations that need to be considered. Incomplete information regarding stage at diagnosis, histologic type (i.e., squamous versus melanoma versus adenocarcinoma versus urethral type), grade and sub-site of penile cancer cases in PR limits our ability to consider the impact of staging on penile cancer trends. Also, even though PR is an Hispanic population, Hispanics in the US constitute a heterogeneous group of people from a variety of Hispanic origins that show substantial variability in cancer rates. Even though the male Hispanic population residing in the US described in our study is not directly comparable to the Puerto Rican male population living in PR, racial/ethnic group comparisons identify significant disparities in the burden of penile cancer incidence and mortality and permit us generate further hypotheses about the role of environmental, genetic, social, and lifestyle factors on penile cancer occurrence.

CONCLUSIONS

In summary, this study found higher rates of penile cancer in men in PR as compared to any other studied racial/ethnic group in the US. Race/ethnic group differences can involve complex interactions between behavioral and biological processes and perhaps even social factors. The presence of this heterogeneity by race/ethnic groups needs to be acknowledged in the quantification and investigation of race/ethnic differences in penile cancer research.

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CONFLICT OF INTEREST

None declared.

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EDITORIAL COMMENT

This is a well done epidemiologic comparison among PR and American men regarding penile cancer. As expected, low educational level is a determinant for higher incidence and mortality due this malignancy. Penile cancer is a health concern in poor regions worldwide. The findings of this study are result of an elegant and complex statistical job. Unhapily it was not possible to control by stage the mortality analysis, because tumor stage information were lacking in this large retrospective data base.

Although the authors became impressed due the higher incidence and mortality rates in men younger than 60 years old, this situation is not so uncommon in developing countries. In a national survey, sponsored by Brazilian Urological Society, Favorito et al. showed, almost 20% of penile cancer affecting men under 45 years old, being, 3.53% under 26 years and 3.88% under 35 years and 12%, under 45 years old (1), reinforcing the idea that penile cancer is a mutilant disease, which can affect men in the most productive phase of their lives. As the authors stressed in the discussion issues about behavioral aspects and lifestyle characteristics as risk factors for penile cancer, in future studies in PR populations, questions about penile traumas (2), [references 2 and 17] and about sex with animals (3), might be added.

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ORIGINAL ARTICLE

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Downregulation of C3 and C4A/B complement factor fragments in plasma from patients with squamous cell carcinoma of the penis

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ABSTRACT

Purpose: To investigate the use of ClinProt technique to identify cancer markers in plasma of patients suffering from squamous cell carcinoma of the penis (SCCP).

Materials and Methods: Plasma of 36 healthy subjects and 25 patients with penile carcinoma who underwent surgical treatment between June 2010 and June 2011 was collected and analyzed by the ClinProt/MALDI/ToF technique. Then the peptides were identified from the C8 MB eluted fraction of patients' and control subjects' plasma by LIFT MS/MS.

Results: A cluster of 2 peptides (A=m/z 1897.22 ± 9 Da and B=m/z 2021.99 ± 9 Da) was able to discriminate patients from control subjects. Cross validation analysis using the whole casuistic showed 62.5% and 86.76% sensitivity and specificity, respectively. The cluster also showed very high sensitivity (100%) and specificity (97%) for SCCP patients that died due to the disease. Furthermore, patients with lymph node involvement presented sensitivity and specificity of 80% and 97%, respectively. These two peptides were identified by the proteomic approach based on a MALDI-TOF/TOF as fragments of C3 (m/z 1896.17) and C4a/b (m/z 2021.26) complement proteins.

Conclusions: The results showed that as the disease progresses, the fragments C3 and C4 A/B are less expressed in comparison with healthy subjects. These results may be useful as prognostic tools.

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Penis; Penis Cancer; Carcinoma, Squamous Cell; Plasma; Proteomics; Complement C3b Inactivator Proteins

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INTRODUCTION

Squamous cell carcinoma of the penis (SCCP) is a rare disease in developed countries, but in emergent countries it can account for 10% of male neoplasms (1,2). Its etiology is not fully understood, but there is a strong association with poor hygienic conditions, phimosis and human papillomavirus (HPV) infection (2,3). Metastases from penile carcinoma usually spread through penile lymphatic vessels to regional nodes, especially the superficial and deep inguinal nodes, and subsequently to the iliac nodes within the pelvis. Tumor involvement of inguinal lymph nodes is the best indicator of long-term survival in patients with invasive SCCP (1). Twenty to fifty percent of

patients with penile carcinoma present inguinal involvement at diagnosis and physical examination is a not a reliable predictor or lymph nodes status (2). Therefore, reliable staging information can only be acquired through surgical procedures with subsequent histologic examination of the inguinal lymph nodes. The pathologic factors with known prognostic value, other than the presence of lymph node metastasis, include tumor thickness, grade, histologic type, lymphovascular embolization, presence of koilocytosis and stage (4-6). On the clinical nodal status there is a dilemma: a significant number of patients with palpable lymph nodes do not have metastasis, in the other hand, 20% of patients with clinically non-suspicious nodes present micrometastasis at pathological examination. Thus, prophylactic bilateral inguinal lymph node dissection is considered unnecessary in up to 80% of penile carcinoma patients with clinically negative regional lymph nodes (7).

While DNA is the information archive, proteins do the work for the cell. It has been shown that direct gene expression is only responsible for a small part of the complexity of a living organism (8). Proteomics is the large-scale study of proteins, and is associated traditionally with displaying a large number of proteins from a given cell line or organism. Curiously, there is no strict linear relationship between genes and the protein complement or 'proteome' of a cell. Proteomics is complementary to genomics, because it focuses on the gene products, which are the active agents in cells. There are two strategies for finding protein biomarkers in tissues or in biological fluids. Several possible biomarkers have been identified using a gel-based approach in bi-dimensional electrophoresis (with and without stable isotopic labeling) and mass spectrometry (9,10). However, since this approach is very laborious and time-consuming, it is not practicable in clinical chemistry laboratories. Other methods based on the biological fluid proteome prefractionation have been recently made available (11,12). The successful discovery of a proteomic profile correlated to an altered state by the ClinProt method has been reported in various human diseases, such as oral, bladder, nasopharyngeal and neck cancer (13-15). Recently, we have used this approach to find possible ccRCC biomarkers in urine and serum (12,16). In view of these results, we investigated the possibility of using the ClinProt technique to find possible plasma diagnostic markers that can better distinguish healthy subjects from patients affected by SCCP.

MATERIALD AND METHODS

Chemicals

The C8-Hydrophobic kit, α-cyano-4-hydroxycinnamic acid (HCCA) and Protmix1 were purchased from Bruker Daltonics, GmbH (Bremen, Germany). acetonitrile from Merck KGaA (Darmstadt, Germany) and methanol from Sigma-Aldrich, Inc. (St. Louis, MO).

Patients and Blood Sample Collection

Twenty-five patients with penile cancer being treated by the National Cancer Institute and Mário Kröeff Hospital were enrolled in this study after permission of the hospital's ethic committee. In addition, we used blood samples from thirty-six healthy subjects (blood donor volunteers) who underwent circumcision in Santa Veronica Hospital. Informed consent was obtained from all patients. After diagnosis of penile cancer, 5 ml of blood was collected before surgery and stored in a BD vacutainer with EDTA. About 2.5 ml of plasma was obtained after centrifugation at 1500 g for 10 min. within 4 hours of collection. The samples were frozen on dry ice and sent to Milano Bicocca University in Italy. All the samples were stored at -80 ^{o}C until being sent to Italy.

The mean age of the patients and controls was 63.56 (range, 38-90) and 60 years (range, 23-83), respectively. Follow-up was evaluated in all patients. Median follow-up was 9 months (range 1 to 36). The data collected from the patients' medical records are shown in Table-1. Due to the small number of patients, no stratification according to risk factors for penile cancer was performed.

Study Design

The entire data set, composed of 36 controls and 25 penile cancer patients, was randomly split into two groups. The first group (training data set: 28 controls and 17 penile cancer patients) was

used for the identification of signals related to peptides expressed differentially in penile cancer patients compared with controls (pattern recognition). The second group (test data set: 8 controls and 8 penile cancer patients) was used for preliminary pattern validation of the cluster.

Sample Purification

ClintProt was applied to analyze the plasma samples collected from SCCP patients. Healthy subjects and SCCP patients were randomly split into two groups for the training and the test experiments. Because of the high complexity of spectrum profiles, two algorithms were tested for biomarker discovery. After the basic statistical analysis, the two algorithms were used for the selection of a signal cluster that was able to differentiate patients from controls. Peptides were extracted from the plasma by ClintProt C8 magnetic beads according to the kit instructions. Plasma aliquots (40 µL each) were mixed with 5 µL of magnetic beads and 40 µL of the kit buffer, allowing peptides and proteins to bind to hydrophobic C8 surface of the magnetic micro particles. The supernatant was removed after 1 minute of incubation and the beads were washed twice with 45 µL and once with 15 µL of the recommend washing solution. Peptides were then eluted with 10 µL of 50% acetonitrile. The procedure was automatically carried out using the ClinProt robot (Bruker Daltonics, GmbH, Bremen, Germany), thus reducing variability.

Mass Spectrometry Analysis

Aliquots of eluted peptides (5 µL) were mixed with 10 µL of HCCA matrix solution (6.2 g/L HCCA in methanol/acetonitrile/water 50/40/10). About 1 µL of this mixture was spotted four times onto a MALDI-ToF MTP 384 target plate ground steel F (Bruker Daltonics, GmbH, Bremen, Germany). All preparation steps were performed automatically by a robot (ClinProt robot, Bruker Daltonics, GmbH, Bremen, Germany). Mass spectra were acquired by an UltrafleXtreme MALDI TOF/TOF MS instrument (Bruker Daltonics, GmbH, Bremen, Germany) operated in positive-ion linear or reflectron mode, recording m/z values from 1000-10000 Da. External calibration was performed using a set of peptide/protein standards (ProtMix1). MALDI-TOF acquisition parameters were as follows: total of 1200 shots (200 x 6), laser power about 70% (linear mode) and 40% (reflectron mode), laser movement hexagonal.

Data Analysis

Data analysis was performed using the ClinProtTools 2.1 software package (17) (Bruker Daltonics, GmbH, Bremen, Germany). ClinProTools was used for multiple spectra comparison and protein pattern identification with the following workflow: (i) spectra normalization to their total ion current; (ii) spectra recalibration using the prominent peaks; (iii) baseline subtraction and peak detection; and (iv) calculation of peak areas for each spectrum. Peak detection was done with signal-to-noise ratio of 5 and peak areas were calculated using endpoint level integration type. Spectra were also "top hat" baseline subtracted with a minimum baseline width of 10% and processed in a range of 1-10KDa. Only 1 of the 8 spectra obtained from the plasma of each subject by MALDI-TOF was used for the next statistical analysis, with the help of "Support Spectra Grouping" and "Enable Similarity Selection" options. The program, after calculation of the mean spectrum of each subject's data set, selects the spectrum that is most similar to the average for subsequent statistical analysis. Basic statistical analysis, genetic algorithm (GA) (18), and Support Vector Machine (SVM) (19) were then used for the selection of signal clusters that were able to differentiate controls from penile cancer patients. These signals were preliminarily tested for their diagnostic capability using the two different data sets separately. The receiver operating characteristic curve analysis and area under curve (AUC) calculation were performed by the ClinProTools 2.1 software to determine the diagnostic efficacy of each marker. The cut-off value ($P < 0.001$) corresponding to the highest accuracy (the lowest false negative and false positive results) was also calculated, along with specificity and sensitivity.

Peptide Identification by MALDI-TOF/TOF

The peptides were identified from the C8 MB eluted fraction of patients' and control sub-

Table 1 - Patient characteristics, histopathologic findings, pathologic staging, type of surgery and follow-up.

Pts.	Age	Race*	Grade	Vascular Invasion	Stage TNM	Surgery	Follow-up (months)	Deaths by Disease (months)
1	41	mulatto	MD***	negative	pT2N2MX	Partial amputation + bilateral inguinal limphadenectomy	12	
2	41	mulatto	MD	negative	pT3N0Mx	Partial amputation + bilateral inguinal limphadenectomy	12	
3	62	black	MD	negative	pT2N0Mx	Total amputation + bilateral inguinal limphadenectomy	36	
4	62	white	MD	negative	pT1N0Mx	Partial amputation.	2	
5	38	white	WD**	negative	pT1N2Mx	Partial amputation + bilateral inguinal limphadenectomy	1	
6	45	white	MD	negative	pT1N1Mx	Partial amputation + bilateral inguinal limphadenectomy	12	
7	51	white	MD	negative	pT2N0M0	Partial amputation + bilateral inguinal limphadenectomy	24	
8	57	black	WD	negative	pT3N2Mx	Partial amputation + bilateral inguinal limphadenectomy	18	
9	54	mulatto	MD	negative	pT2N0Mx	Partial amputation + bilateral inguinal limphadenectomy	30	
10	73	white	WD	positive	pT2N3Mx	Partial amputation + right inguinal limphadenectomy	11	11
11	76	black	MD	negative	pT2N0Mx	Partial amputation + bilateral inguinal limphadenectomy	5	5
12	57	white	MD	negative	pT3N0Mx	Partial amputation + bilateral inguinal limphadenectomy	7	

13	80	white	WD	negative	pT2N0Mx	Partial amputation + bilateral inguinal limphadenectomy	24	
14	84	white	MD	negative	pT2NxMx	Partial amputation.	3	3
15	86	white	MD	negative	pT3NxMx	Total amputation.	8	
16	64	white	MD	negative	pT3N3Mx	Partial amputation + bilateral inguinal limphadenectomy	5	5
17	80	white	MD	positive	pT4NxMx	Emasculation	8	8
18	90	white	PD****	negative	pT1NxMx	Partial amputation.	1	
19	83	white	WD	negative	pT2N3Mx	Partial amputation + bilateral inguinal limphadenectomy	8	8
20	55	mulatto	MD	negative	pT3N2Mx	Emasculation + bilateral inguinal limphadenectomy	4	
21	61	mulatto	MD	negative	pT2N0Mx	Total amputation + bilateral inguinal limphadenectomy	9	
22	71	white	MD	positive	pT2N0Mx	Partial amputation + bilateral inguinal limphadenectomy	1	
23	56	mulatto	WD	negative	pT2N0Mx	Partial amputation + bilateral inguinal limphadenectomy	4	
24	60	black	WD	negative	pT2NxMx	Total amputation.	1	
25	62	white	WD	negative	pT2N3Mx	Partial amputation + bilateral inguinal limphadenectomy	24	

* In Brazil, race definition is not accurate due to miscegenation. The column represents the self defined skin color of the patients
** WD: well differentiated
*** MD: moderately differentiated
**** PD: poorly differentiated

jects' plasma by LIFT MS/MS. For peptide identification, the LIFT-TOF/TOF spectra were recorded in the UltrafleXtremeTM MALDI-TOF/TOF MS instrument. The fragment masses were analyzed after their ion reflector detection. Analyses were performed using the following acquisition settings: ion source 1, 7.5 kV; ion source 2, 6.7 kV; lens 3.6 kV; reflector, 29.5 kV; reflector 2, 13.95 kV; lift 1, 19 kV; lift 2, 3.15 kV; pulsed ion extraction 80 ns.

Raw MS/MS data were processed with the FlexAnalysisTM 3.3 software (Bruker Daltonics, Germany). Database searching was performed by an in-house Mascot search engine (Version: 2.3.02) using the following parameters: human Swissprot (accessed Feb. 2012- 20,317 sequences) database, no enzyme and fixed and variable modifications. MS and MS/MS tolerances were generally set at 1 Da. Only identifications with a score higher than Mascot identity thresholds were accepted.

RESULTS

The pathological findings of the SCCP are shown in Table-1. Most of the patients (n = 14) were at pT2 level, whereas the remaining were at pT1 (n = 4), pT3 (n = 6) and pT4 (n = 1) levels. Histopathology confirmed invasive SCCP in all patients. Of the 25 cases, 8 (32%) were well differentiated, 16 (64%) moderately differentiated and 1 (4%) poorly differentiated. Amputation and inguinal lymphadenectomy was performed in 20 patients. Of these patients, 11 (55%) were N0, 1 (5%) was N1, 4 (20%) were N2 and 4 (20%) were N3.

ClintProt was applied to analyze the plasma samples collected from SCCP patients and a cluster of two peaks was identified. Comparison of spectrum profiles obtained from the data set used in the training phase showed several ions differentially expressed in the two studied populations (Table-2). On the basis of the GA and SVM results, a cluster of two statistically different signals ($P < 0.05$), at m/z 2021.99 ± 9, 1897.22 ± 9, were identified as able to differentiate the populations. Preliminary statistical analysis was carried out for each marker and for the cluster of signals by the receiver operating characteristic curve analysis. The AUC of peak A at m/z 1897.22 ($P < 0.0001$) was 0.85, which corresponds to a moderately accurate test, according to the criteria suggested by Swets (20). The AUC of peak B at m/z 2021.99 ($P < 0.0001$) was 0.91, which corresponds to a highly accurate test. The combination of the two peaks indicated an improvement in the performance compared to the single signals, with specificity and sensitivity of 100% and 80%, respectively. This pattern was subsequently tested for its ability to differentiate SCCP patients from controls by external validation using data obtained from a second group of normal subjects and patients. Sensitivity and specificity were at 63.6% and 100%, respectively. Cross-validation analysis using the whole casuistic showed 62.5% and 86.76% sensitivity and specificity, respectively. The cluster of signals was also evaluated using the entire set of patient data grouped according to deaths due to disease and lymph node involvement. The results showed 100% sensitivity and 97% specificity for patients who had died of disease. Among patients with lymph node involvement, the sensitivity and specificity was 80% and 97%, respectively. Patients without lymph node involvement showed 54% sensitivity and 97% specificity (Table-3). Both peeks A and B, included in the diagnostic cluster, were under-expressed ($p < 0.05$) in patients compared with healthy subjects (Figure-1). Some of the peaks observed in the plasma protein profile could be identified by LIFT MS/MS (Table-4). In particular, signals at m/z 1897.22 (m/z 1896.17 in reflector mode) and at m/z 2021.99 (m/z 2021.26 in reflector mode) were identified by the proteomic approach based on MALDI-TOF/TOF as fragments of C3 (m/z 1896.17) and C4a/b (m/z 2021.26) complement proteins (Table-4).

Preliminary evaluation of the diagnostic efficacy was determined with an internal validation. The cluster showed a very high specificity value in an external validation test with plasma samples collected from different patients and controls (Table-3).

DISCUSSION

The area of proteomics has begun to revolutionize the study of medicine in the post genomic era, by allowing researchers to study the

Table 2 - Selection of peaks differently expressed (P < 0.05) between controls (n = 28) and penile cancer patients (n = 17) used for the training phase.

Mass	P*	P†	Ave 1‡	Ave 2§	Std D1¶	Std D2‖
6430.52	0.0195	0.00859	50.6	10.16	47.79	13.46
1897.14**	0.0283	0.00859	23.23	5.38	24.34	4.23
1865.76	0.165	0.246	8.89	1.56	13.01	1.71
6628.81	0.28	0.246	129.73	55.2	138.31	41.66
1016.3	0.422	0.546	486.1	391.81	153.1	123.32
2021.91**	0.422	0.000456	70.76	1.09	164.61	4.08
1112.17	0.422	0.0747	17.16	37.25	8.67	30.63
9420.36	0.422	0.395	23.38	14.85	13.94	10.8
1211.9	0.422	0.0423	4.53	-0.18	11.52	1.01
1229.15	0.422	0.246	6.18	10.52	5.46	5.43
3216.09	0.422	0.57	11.2	6.93	8.58	4.44
9377.51	0.422	0.5	8.02	5.25	5.81	2.94
7763.75	0.491	0.615	17.25	12.1	11.69	6.12
4100.59	0.491	0.184	2.33	-0.66	8.87	1.32
3315.24	0.518	0.71	22.33	15.47	16.59	8.47
1767.61	0.518	0.948	24.21	4.21	65.65	2.97
8764.21	0.518	0.684	6.15	2.64	7.19	5.36

* P value by t test and ANOVA; values lower than .0.05 indicate statistical relevance.
† P value calculated with the Wilcoxon/Kruskal–Wallis test; values lower than 0.05 suggest statistical relevance.
‡ Average area of peaks for control subjects.
§ Average area of peaks for SCCP patients.
¶ Standard deviation of peaks for control subjects.
‖ Standard deviation of peaks for SCCP patients.

** Marked peaks represent signals selected for the diagnostic model

Table 3 - Cluster evaluation: diagnostic efficacy of pattern based on deaths from disease and lymph node involvement.

Group	Sensitivity	Specificity
6 patients dead by disease	100%	97%
9 patients with positive lymph nodes	80%	97%
11 patients with negative lymph nodes	57%	97%

Figure 1 - Signal intensity of 2 peaks (1897.22 and 2021.99 m/z) discriminating control (class 1) and tumor (class 2).

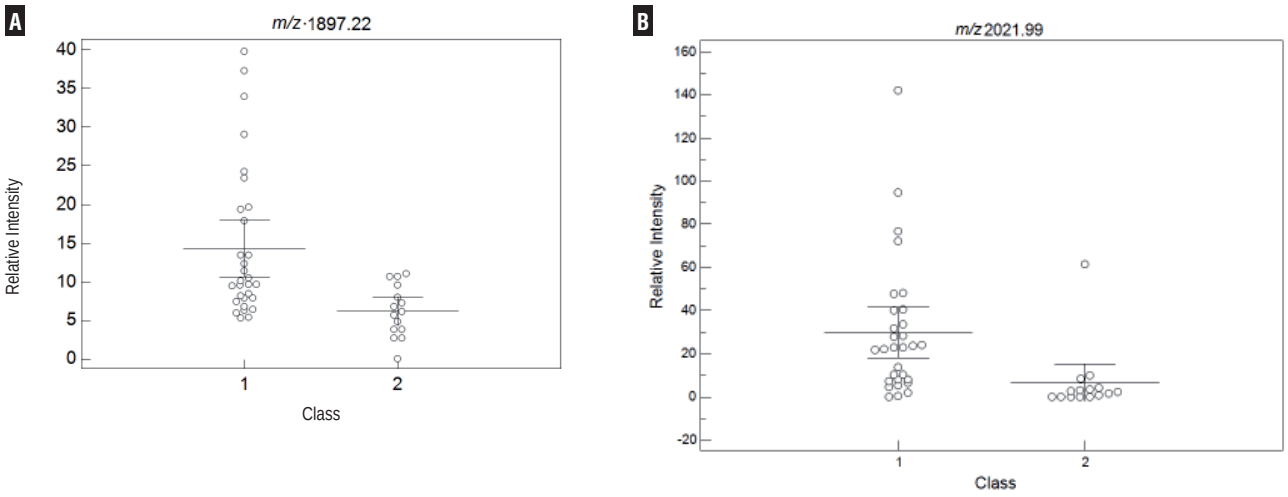

Table 4 - Identified peptides in the C8 MB eluted fraction of patients' and control subjects' plasma by LIFT MS/MS. All peptide identification scores were above Mascot identity threshold. Table reports the m/z values of the ions observed in MALDI linear and reflectron mode.

Reflectron mode m/z	Linear mode m/z	Calc. mass (Da) in all pre-fractionated plasma samples	Peptide sequence	Accession (UNIPROT)	Description	Peptide score
1896.17	1897.22	1895.024	NGFKSHALQLNNRQIR	CO4A_HUMAN CO4B_HUMAN	Complement C4-A OS=Homo sapiens GN=C4A PE=1 SV=1 Complement C4-B OS=Homo sapiens GN=C4B PE=1 SV=1	61
2021.26	2021.99	2020.097	SSKITHRIHWESASLLR	CO3_HUMAN	Complement C3 OS=Homo sapiens GN=C3 PE=1 SV=2	72

role that proteins plays in health and disease. By applying this knowledge, innovative discoveries in early cancer detection have been made, including kidney, prostate, ovarian, and bladder cancer. To our knowledge, this is the first study of penile cancer proteomics.

Although several markers have been evaluated, currently the clinical application of these markers is limited. HPV positive tumors show a variable prognostic outcome. In a previous article we reported the analysis of 80 consecutive cases of patients with penile cancers and HPV status was not significantly associated with the presence of regional metastases (21). Up to now, P53 status may correlate with survival in T1 disease but further studies are required to establish the link to lymph node spread (22) Therefore, it is still necessary to find biomarkers for early detection of metastases, prognosis and follow-up of this disease (22).

Before the use of proteomics in penile carcinoma, a critical assessment of its diagnostic and prognostic value will be required. There is a controversy about whether and when patients should undergo lymphadenectomy. The presence and the extent of metastasis to the inguinal region is the most powerful prognostic factor for survi-

val in patients with SCCP. The principal aim in the management of this disease is correct staging of patients without invasive procedures, since a presence of metastatic lymph nodes is an independent prognostic factor for survival (6). Therefore, research into potential biomarkers and biological targets is very important.

The C3, C4a and C4b complement fragments are involved in the classic and the alternative complement cascade pathway. These peptides were under-expressed ($p < 0.05$) in SCCP patients compared with healthy subjects. During disease progress, under-expression of C3, C4a/b fragments become more evident. More interestingly, we have found very high sensitivity (100%) and specificity (97%) for under-expression of these fragments in SCCP patients that have succumbed to the disease. Besides this, patients with lymph node involvement present sensitivity and specificity of 80% and 97%, respectively. In the case of patients without lymph node involvement, we obtained a sensitivity rate of 57% and specificity of 97%.

Degradation of complement system convertases was observed for patients with breast cancer (BCP). For C3, the degradation was solely on the C3d, C3g, C3α´1 and C3β. Convertases C3, which occupies a central position in the system, displays differential degradation in the BCP (5-fold more peptides than in the healthy subjects). For C4, the degradation was solely on the C4b and C4β. The degradation of the front portion of the C4b fragment was observed solely for the tested BCP (23).

The contribution of the complement system to the control of tumor growth has been neglected for a long time, since the main emphasis has been put on cell-mediated immune response against cancer (24).The innate immune system is the first line of defense, comprised of cells and mechanisms that defend the host from infection in a non-specific manner. Taneja et al. (25) studied the plasma protein profile in patients with hepatitis E. In that study, the levels of complement proteins C3, C3f, C4, and bradykinin and kininogen were found to be lower in the plasma of hepatitis E patients compared to healthy controls. Any perturbation, such as a viral infection in the liver, should trigger a strong innate response as the first line of host defense, but the opposite was found to occur, with these proteins being in downregulation. The exact mechanism of this reduction is not understood at this time.

In our patients, the downregulation of C3 and C4 could be caused by HPV and/or EBV infection, viruses that are highly prevalent in SCCP lesions (26). Viral proteins counteract the immune response (27). This could explain the progression of the disease along with C3 and C4a/b under--expression. It is a hypothesis to be tested in the future.

The over-expression of C regulatory proteins (CRPs) in tumor cells is one way these cells protect themselves from C attack. CD46, CD55 and CD59 are thought to be the most important membrane C regulatory proteins (mCRPs), expressed both on normal and tumor cells. Tumor cells can also evade C attack by binding soluble C inhibitors from serum such as factor H (fH). It is also interesting to note that fH or a related protein is a marker for bladder cancer, suggesting a link between C resistance and escape from immune surveillance (28). CD55 has been identified as a tumor-associated antigen and a high expression level of CD55 in colorectal cancer tissue is correlated with a significant decrease in survival (29). Also, lower CD46 has been found to be inversely related with high levels of C3 deposited in renal and cervical cancer tissue (30).

In addition, in a previous study (31) we detected lower activity of natural killer cells (NK) in patients with cancer of the penis. NK cells are also part of the innate immune response and were first identified for their ability to kill tumor cells without deliberate immunization or activation. Subsequently, they were also found to be able to kill cells that are infected with certain viruses and to attack preferentially cells that lack expression of major histocompatibility complex (MHC) class I antigens. The innate immune response of patients to cancer therefore may be involved, since natural killer (NK) cell activity can be significantly decreased in SCCP patients compared to control groups (31).

Despite increasing evidence suggesting that a variety of mechanisms are responsible for tumor-related tolerance and suppression, little is

known about the timeline and reciprocal connection between these processes. The widely accepted theory of immuno-editing describes a rational, time-structured evolution of the relationship that occurs between tumor and host immune system (32,33). Tumor escape in cancer patients appears to be a cumulative process that involves tumor--derived soluble factors (TDSFs), induction of regulatory elements of various cell lineages and different anatomical environments (34). In a simplified view, tumor-induced immune subversion results from two main activities, namely the induction of tolerance toward tumor antigens and the functional suppression of the effector lymphocytes that normally counteract tumor growth (32-36).

CONCLUSIONS

Our results suggest that a proteomic approach based on magnetic beads is a useful method to discover possible clinical biomarkers. We demonstrated the capability of selected signals to differentiate SCCP patients from normal subjects. The peptides identified from the C8 MB eluted fraction of patients' and control subjects' plasma correspond to fragments of the C3 (m/z 1896.17) and C4 (m/z 2021.26) complement protein. The results showed that when the disease progresses, they are more under-expressed. These fragments are mainly downregulated in patients with metastatic involvement. The innate immune response of patients could be suppressed. Downregulation of C3 and C4A/B represents a promising prognostic tool for SCCP.

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CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Locally advanced penile carcinoma: classic emasculation or testis-sparing surgery?

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ABSTRACT

Purpose: The study evaluates the clinical and pathological findings of 16 patients with locally advanced penile carcinoma (PC) submitted to emasculation, and discusses questions related to the usefulness of bilateral orchiectomy.

Materials and Methods: Between 1999 and 2010, 172 patients with PC were treated. Sixteen (9%) underwent emasculation. Data were retrieved from the institution's database including age, ethnicity, date of surgery, residential setting, level of schooling, time to diagnosis, type of reconstruction, complications, tumor stage and grade, vascular and perineural invasion along with invasion of corpus cavernosum, corpus spongiosum, testicles, scrotum and urethra.

Results: A total of 16 patients (average: 63.1 years) with locally advanced PC were included. All were illiterate or semiliterate rural dwellers and 87% were white. The time to diagnosis was 8-12 months. The mean follow-up time was 31.9 months (1-119). By the time of the last follow-up, only seven patients (43.75%) were alive. Tumors were pT4 (n = 6), pT3 (n = 8), pT2 (n = 2), Grade I (n = 5) and Grade II (n = 11). The histopathological examination revealed invasion of the urethra (n = 13), scrotum (n = 5) and testicles (n = 1). The surgical margin was positive in one patient. Six patients (37.5%) had vascular invasion and 11 (68.7%) had perineural invasion. Currently, only one of the former is alive.

Conclusions: The finding of focal microscopic testicular infiltration in only one of 32 testicles, even in the presence of clinically apparent scrotal invasion, suggests that emasculation without bilateral orchiectomy is a safe treatment option for patients with locally advanced PC.

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Orchiectomy; Penile Neoplasms; Carcinoma, Squamous Cell; Penis

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INTRODUCTION

Penile carcinoma (PC), a potentially fatal malignancy, is rare in developed countries (1). In the United States, for instance, it accounts for only 0.4% of all male malignancies (2), while in less developed regions, especially in Asia, Africa, and South America, the figure may be as high as 10% (3). The incidence typically ranges from less than 1/100,000 in the US and Europe to more than 4/100,000 in Uganda and Brazil (4).

Brazil has one of the highest incidences of penile cancer in the world. The disease represents 2.1% of male neoplasms in the country, reaching 5.7% in the Northeast (5) where the human development index is very low. Not surprisingly, the region accounts for more than half (53.02%) of the cases registered in Brazil. In comparison, whe-

reas 2 or 3 new cases of PC are observed every month at the Ceará Cancer Hospital in Fortaleza (Northeastern Brazil) (6), a British practicing urologist may not see more than one new case every other year.

The causes of penile cancer are not always clear, but major risk factors include poor penis hygiene, phimosis, smegma retention, smoking and sexually transmitted diseases (7,8). In a recent Brazilian multicenter study, the practice of sex with animals was also identified as a risk factor for this malignancy (9).

Penile carcinoma metastasizes stepwise via the lymphatic system: from the inguinal nodes to the pelvic nodes to the distant nodes (10). The presence and extent of lymph node metastases are the most important predictors of survival (4). Histological grade and vascular and perineural invasion have been correlated with poor prognosis and unfavorable outcomes (11).

Surgical management of malignant PC depends on the grade and stage of the disease (12). The National Institute for Clinical Excellence (U.K) recommends managing PC at centers with a catchment area of 4 million. Each center receives approximately 25 new cases annually. Advanced cases of PC are referred to a supra-regional center for multidisciplinary curative and palliative treatment, including reconstructive surgery (13).

At our service, PC patients with proximal corpus cavernosum invasion and scrotal skin erosion are routinely referred to emasculation, including total penectomy (complete penis amputation), scrotectomy and bilateral orchiectomy (14).

The present study evaluates the clinical and pathological findings of 16 patients with locally advanced penile squamous cell carcinoma submitted to emasculation and discusses questions related to surgical technique, patient follow-up and the usefulness of bilateral orchiectomy.

MATERIALS AND METHODS

Between 1999 and 2010, 172 patients with penile invasive squamous cell carcinoma were submitted to surgical treatment at the Ceará Cancer Hospital in Fortaleza, Brazil. Of these, 16 advanced cases (9.3%) underwent emasculation (Table-1).

Complete preoperative, operative and postoperative patient data were retrieved from the institution's prospective database and from a review of the respective medical records. The information included patients' age, ethnicity, date of surgery, residential setting (rural or urban), level of schooling, time from appearance of first lesion to definitive diagnosis, type of reconstruction, duration of follow-up and date of last follow-up visit. The median follow-up time was calculated as the median observation time among patients and the overall survival time was estimated using the Kaplan-Meier method.

Specimens were obtained from all patients and evaluated by a single pathologist with regard to tumor stage and grade, inguinal lymph node status, vascular and perineural invasion, invasion

Table 1 - Cases of penile squamous cell carcinoma submitted to surgical treatment at the Ceará Cancer Hospital between 1999 and 2009.

Surgical technique	Cases (n)	Percent (%)
Wide local excision or circumcision	5	3%
Partial penectomy	125	73%
Total penectomy	26	15%
Emasculation	16	9%
Total	172	100%

of corpus cavernosum, corpus spongiosum, testicles, scrotum and urethra.

All 16 patients were submitted to emasculation, including total penectomy, scrotectomy and bilateral orchiectomy. The technique consisted of an elliptical abdominoscrotal incision with large skin margin and partial preservation of the posterior surface of the scrotum. With the corpus cavernosum and corpus spongiosum exposed, the urethra was dissected and mobilized. The corpus cavernosum was disinserted from the ischial tuberosity followed by en bloc removal of the penis, scrotum and testes. The urethra was tunneled without angulation, spatulated and externalized in the perineum, as described by Campbell (15).

The surgical wound was closed with a scrotal flap in 9 patients. Seven patients required two forms of reconstruction: 1) a vertical rectus abdominis myocutaneous flap was made through a midline abdominal incision, preserving the posterior layer of aponeurotic and inferior epigastric vessels. The skin grafts were rotated to achieve complete wound closure; 2) an eliptical incision was made in the medial aspect of the thigh, starting 10 cm below the ischium, in order to preserve the medial circumflex femoral arteries, followed by wound closure with a gracilis myocutaneous flap. Both reconstruction techniques were performed with the assistance of a plastic surgery team.

Four patients who had previously undergone partial penectomy required emasculation due to tumor recurrence (at 6,2,9 and 13 months, respectively). The previous pathological findings of these patients included: Patient 1 = pT2 GII, margin 4 mm; Patient 2 = pT3 GII, positive surgical margin; Patient 3 = no information available, patient treated at another institution; Patient 4 = pT2 N2 Mx GI.

Tumors were graded with the most commonly used classification system: grade I = well differentiated; grade II = moderately differentiated, and grade III = poorly differentiated (16).

At the moment of recurrence, tumors were large with clinical evidence of scrotal erosion and proximal invasion of the corpus cavernosum and the urethra.

Eleven patients underwent lymphadenectomy due to inguinal lymph node involvement. Pelvic lymphadenectomy was performed in four patients. The pathological nodal status was defined as: N0 = no evidence of lymph node metastasis; N1 = metastasis in a single inguinal lymph node; N2 = metastasis in multiple or bilateral superficial lymph nodes and N3 = unilateral or bilateral metastasis in deep or pelvic lymph nodes. The excised tumors were clinically and pathologically staged according to the guidelines of the International Union Against Cancer (2002 TNM UICC) (17).

The study was previously approved by the Institutional Ethics Committee and the principles of the Helsinki Declaration were followed in all study procedures.

RESULTS

The 16 patients undergoing emasculation were aged 63.1 years on the average (range: 40-78). Fourteen patients (87%) were white and two (13%) were black. All were illiterate or semiliterate rural dwellers (Table-2). The time from the appearance of the first penile lesion to the definitive diagnosis was 8-12 months.

Radical bilateral inguinal lymphadenectomy was performed in 11 patients 3-4 weeks after emasculation. Due to extensive loco-regional disease, in five patients treatment was limited to surgical debulking for palliative and hygiene purposes, followed by reconstruction (Figure-1).

The pathological nodal status of the 11 patients undergoing radical inguinal lymphadenectomy was N0 (n = 7), N2 (n = 3) and N3 (n = 1). The average number of inguinal lymph nodes was 9.7 (2-19). Among the 4 patients who underwent pelvic lymphadenectomy, the average number of lymph nodes removed was 11.7 (4-20). No patient had positive pelvic lymph nodes. One of the 4 patients with positive inguinal lymph nodes presented a large coalescent mass; the remainder had an average of 2.5 metastatic lymph nodes (2-4).

In most patients (n = 9), the surgical wound was closed with a scrotal flap. The remainder (n = 7) required reconstruction with either a vertical rectus abdominis myocutaneous flap (n = 4) or a gracilis myocutaneous flap (n = 3).

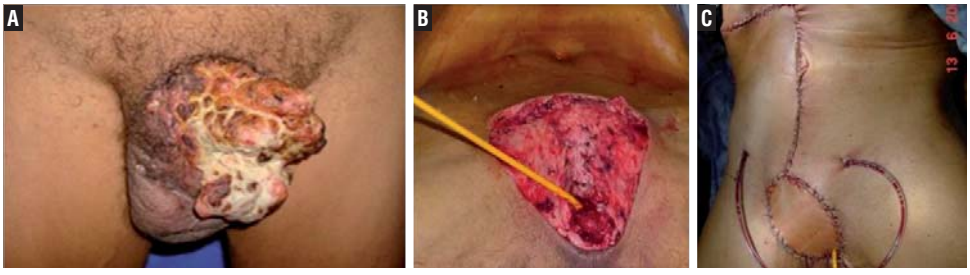
Tumors were mostly T3 or T4 (6=pT4, 8=pT3, 2=pT2). Five tumors were well differentia-

Table 2 - Demographic information, date of surgery, type of reconstruction and last follow-up visit of 16 patients with penile squamous cell carcinoma submitted to emasculation at the Ceará Cancer Hospital in Fortaleza, Brazil, between 1999 and 2010.

Patient	Age	Race	Level of schooling	Date of surgery	Type of reconstruction	Status on last follow-up visit*
I	64	White	Illiterate	2001/05	Gracilis	Alive DF 2011/04
II	78	White	Illiterate	2004/01	VRAM	Dead 2005/01
III	64	White	Illiterate	2004/06	VRAM	Dead 2004/12
IV	66	White	Semiliterate	2004/06	Gracilis	Alive DF 2011/04
V	70	Black	Semiliterate	2004/11	Scrotum	Alive DF 2011/04
VI	52	White	Illiterate	2005/06	VRAM	Dead 2006/10
VII	53	White	Semiliterate	2005/01	Gracilis	Dead 2005/11
VIII	67	White	Illiterate	2006/07	Scrotum	Dead 2009/03
IX	54	White	Semiliterate	2006/10	VRAM	Dead 2007/01
X	49	White	Semiliterate	2007/02	Scrotum	Alive DF 2011/04
XI	40	Black	Illiterate	2007/04	Scrotum	Dead 2008/02
XII	55	White	Semiliterate	2007/10	Scrotum	Alive DF 2011/04
XIII	77	White	Semiliterate	2008/01	Scrotum	Dead 2008/02
XIV	73	White	Semiliterate	2008/11	Scrotum	Alive DF 2011/04
XV	70	White	Illiterate	2010/02	Scrotum	Alive DF 2011/04
XVI	78	White	Illiterate	2009/11	Scrotum	Dead 2010/07

*Last follow-up visit: April 2011
DF = disease-free

Figure 1 A-C: Patient with locally advanced penile squamous cell carcinoma submitted to emasculation and reconstruction with a vertical rectus abdominis myocutaneuos flap. Ceará Cancer Hospital, Fortaleza, Brazil.



ted (Grade I) and 11 were moderately differentiated (Grade II). No tumor was classified as Grade III and no patient presented distant metastases.

Six (37.5%) patients had vascular invasion and 11 (68.7%) had perineural invasion. Corpus cavernosum/spongiosum invasion was observed in all 16 cases (Table-3). No lymphovascular invasion was observed in patients with Grade-I tumors. On the other hand, patients with Grade-II tumors presented lymphovascular and perineural invasion (n = 6), or perineural invasion only (n = 4). A single patient with Grade-II tumor presented no lymphovascular or perineural invasion.

The histopathological examination revealed invasion of the urethra (n = 13), scrotum (n = 5) and testicles (n = 1). The surgical margin was positive in one patient.

The time of follow-up was 31.9 months on the average (range: 1-119). By the time of the last follow-up visit (April 2011), only 7 patients (43.75%) were alive. One of six patients with vascular invasion was alive. A Kaplan-Meier overall survival curve is shown in Figure-2.

Table 3 - Pathological findings of 16 patients with penile squamous cell carcinoma submitted to emasculation at the Ceará Cancer Hospital in Fortaleza, Brazil, between 1999 and 2010.

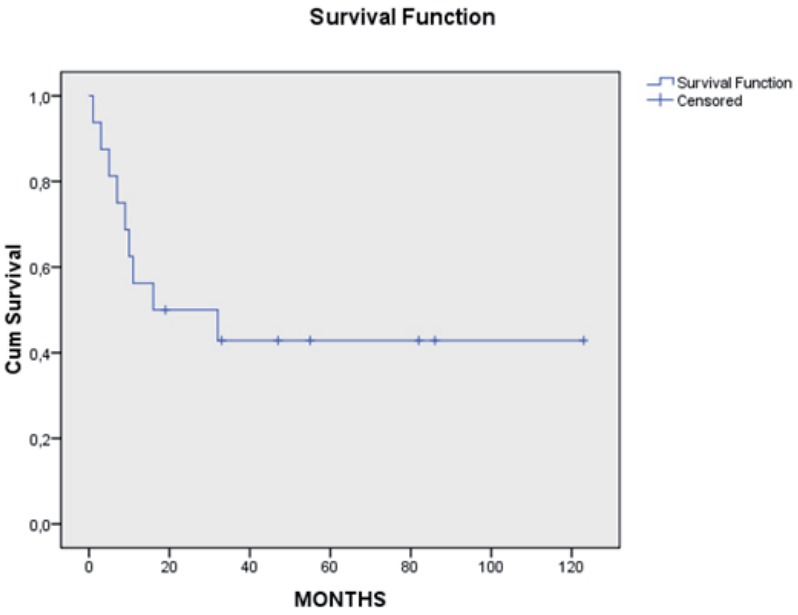
Patient	Stage	Grade	Vascular	Sp/Cav	Perineural	Testis	Scrotum	Urethra	Margin	pN
I	pT4	II	-	+	+	-	+	+	-	pN0
II	pT3	II	+	+	+	-	-	+	-	pNx
III	pT3	II	-	+	+	-	-	+	-	pN0
IV	pT3	I	-	+	-	-	-	+	-	pN0
V	pT3	I	-	+	-	-	-	+	-	pNx
VI	pT4	II	+	+	+	-	+	+	+	pNx
VII	pT4	II	-	+	-	-	+	-	-	pN2
VIII	pT4	II	-	+	+	+	-	+	-	pN0
IX	pT2	II	+	+	+	-	-	-	-	pNx
X	pT2	I	-	+	-	-	-	-	-	pN0
XI	pT3	II	+	+	+	-	-	+	-	pN2
XII	pT3	II	+	+	+	-	-	+	-	pNx
XIII	pT3	II	+	+	+	-	-	+	-	pN3
XIV	pT4	I	-	+	-	-	+	+	-	pN0
XV	pT3	I	-	+	+	-	-	+	-	pN0
XVI	pT4	II	-	+	+	-	+	+	-	pN2

(+) = invasion
(-) = without invasion
Sp/Cav = Corpus spongiosum and corpus cavernosum
pN = pathological nodal status

DISCUSSION

The study was motivated by the question of whether bilateral orchiectomy is necessary in patients with locally advanced penile squamous cell carcinoma and scrotal erosion. Emasculation conventionally involves

Figure 2 - Kaplan-Meier survival curve of 16 patients submitted to emasculation. Data expressed as percentage of live patients over time. Median overall survival was 16 months.



the amputation of penis and testicles and all or part of the scrotum (14).

Although penile amputation can in many subtle and insidious ways give patients an altered sense of masculine identity (18), the crucial question in face of locally advanced PC is not the mutilation per se, but the severity and mortality of the disease.

In our series of PC patients, 9% (16/172) were submitted to emasculation. In comparison, Ornellas et al. referred only 2% (15/688) of their PC patients from Rio de Janeiro to emasculation during a period of 46 years (14). However, it seems likely that due to the geographical location of our institution (Northeastern Brazil), a socioeconomically challenged region, PC is more prevalent and emasculation is more often required.

In addition, diagnosis and follow-up of patients from the rural zone can be very difficult. Some patients wait until after the rainy season or harvest to seek medical care, giving the disease a seasonal character.

Although earlier studies concluded the incidence of PC peaks at 80 years of age (19), more recent investigations have shown the peak to occur in the sixth decade of life (20). In our series, patients were 40-78 years old (mean: 63.1) upon diagnosis.

Favorito et al. reported that 19.41% of their patients were less than 45 years old, 3.52% were under 35 and 3.88% were under 26 (5). In comparison, in our initial sample (n = 172), 18.7% were under 45 years old, 7% were under 35 and 1.7% were under 26.

PC affects mainly poor, uncircumcised men with poor hygiene and limited access to public health care. The proportion of whites (87%) among the patients emasculated in this study matched findings published by Favorito et al. (75.61%) (5). All our patients were illiterate or semiliterate rural dwellers.

The 2002 TNM classification system was adopted throughout the study since all emasculated patients were treated after 2001. Most tumors were pT3 or pT4. Six patients were classified as pT4 due to invasion of the scrotum (n = 5) or the testicles (n = 1), and two were classified as pT2. One pT2 patient was alive at the time of the last

follow-up visit 46 months after surgery. The other surviving patients in our series were staged as pT4 (n = 2) and pT3 (n = 4), suggesting that tumor stage is not the only factor determining survival (14). The two patients staged as pT4 were followed for 119 and 29 months, respectively.

Most cases of PC reported in the literature are low-grade (21). Our cases were staged as Grade I (well differentiated; n = 5) or Grade II (moderately differentiated; n = 11). No tumor was classified as Grade III. Although all Grade-I patients are currently alive, the presence of tumors of both grades (I and II) among the surviving patients suggests a lack of correlation between grade and survival. Johnson reached a similar conclusion in a study of 153 cases (22). On the other hand, Ornellas and Cubillas found histological grade to be a significant adverse prognostic factor for lymph nodes metastasis and survival (16,23).

Clinically, the scrotum appeared to be infiltrated in all 16 cases, but only 5 were confirmed by the pathologist. The single case of microscopic focal testicular infiltration in the absence of scrotal invasion was even more surprising. This infiltration may have been a retrograde extension from the vas deferens--a very rare occurrence (24).

The fact that microscopic focal testicular infiltration was confirmed in only 1 of the 32 testicles in our sample, even in the presence of clinically apparent scrotal invasion, suggests that emasculation without bilateral orchiectomy is a reasonably safe treatment option for patients with locally advanced penile squamous cell carcinoma. As an added benefit, patients not submitted to orchiectomy are spared the serious physiological and psychological adverse effects caused by the procedure, especially those associated with testosterone deprivation, such as metabolic syndrome, anemia, dyslipidemia, arterial hypertension, fatigue, irritability, depression, mood alterations, osteoporosis, bone-related events, muscular atrophy and sleep apnea (25).

One patient had positive surgical margins, proving the choice of surgical technique to be appropriate. The patient survived for 32 months after surgery.

Vascular invasion seems to be a major prognostic factor in patients with PC. Several studies using multivariate analysis have shown a strong correlation between venous and lymphatic embolizations and nodal metastasis (23,26-28).

In a study of 196 patients, Ornelas and coworkers identified 39 (19.9%) cases of lymphovascular embolization (23). In the present study, 6 cases (37.5%) of vascular embolization by cancer cells were detected by the pathologist. The difference in the respective incidences may be due to the greater number of advanced cases in our series.

Only one of our 6 patients with vascular invasion is currently alive. The mean postoperative survival time for the group was 16 months. This finding matches results published by Ornelas et al. who reported higher 5-year survival rates in patients without lymphovascular embolization (23).

Cubilla (16) and Ornellas (23) demonstrated that vascular invasion is a more important predictor of metastasis than perineural invasion. In our study, perineural invasion was observed in 11 patients (68.7%), three (27.3%) of whom are currently alive. The longest postoperative survival observed in our series (119 months) was a patient with perineural invasion. A recently published paper by Ayalla, Cubilla and Guimaraes et al. correlates higher histological grades, deeper anatomical infiltration and vascular and perineural invasion with higher rates of nodal metastasis and mortalily (29).

Nodal status is an important prognostic factor for PC (4). Thus, in our sample, four of the seven patients staged as N0 are currently alive, while no patient staged as N2/N3 was alive at the time of the last follow-up.

CONCLUSIONS

Almost ten percent of our patients with locally advanced penile squamous cell carcinoma were referred to emasculation at the Ceará Cancer Hospital (Fortaleza, Brazil) over a period of 11 years. The fact that microscopic focal testicular infiltration was confirmed in only 1 of the 32 testicles in our sample, even in the presence of clinically apparent scrotal invasion, suggests that emasculation without bilateral orchiectomy may be a reasonably safe treatment option for patients with locally advanced penile squamous cell carcinoma,

thereby avoiding the severe health problems associated with testosterone deprivation. Further studies are required to support these findings.

CONFLICT OF INTEREST

None declared.

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EDITORIAL COMMENT

This is an interesting study, showing several clinical epidemiological and demographic aspects of patients with agressive locally advanced penile cancer, some of them under 50 years old, treated by a skilled team from a tertiary cancer center located in a highly prevalent penile cancinoma area.

Despite its reduced casuistry (16 cases), it brings us a very important new information, that probably has been noted in the daily practice, but was not present in the scientific literature: In the vast majority of debulking surgeries for locally advanced penile cancer, the testis are spared by the disease, probably due the anatomical barriers, as dartos muscles, albuginea and vaginal layers, then they do not deserve to be removed.

Many of this patients will die early due the disease progression. However more than a third of them can be cured or will develop later recurrences (in this present series 7 are alive for long time).

Testosterone plays several important roles in male physiology. If the castration is avoided in this clinical scenario, the long term survivors, will not develop several serious health conditions due androgen deficiency like: metabolic syndrome, anemia, dyslipidemia, arterial hypertension, fatigue, irritability, depression, mood alterations, osteoporosis, bone-related events, muscular atrophy, sleep apnea and others. And for the anedoctal minority of cured subjects seeking for penile reconstruction, the libido persistence will be warranted.

As shown, vascular invasion was a unfavorable anatomopatological pattern, probably this patients deserve an adjuvant chemotherapy or can be candidates for future clinical trials.

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ORIGINAL ARTICLE

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The accuracy of pathological data for the prediction of insignificant prostate cancer

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ABSTRACT

Introduction: The widespread screening programs prompted a decrease in prostate cancer stage at diagnosis, and active surveillance is an option for patients who may harbor clinically insignificant prostate cancer (IPC). Pathologists include the possibility of an IPC in their reports based on the Gleason score and tumor volume. This study determined the accuracy of pathological data in the identification of IPC in radical prostatectomy (RP) specimens.

Materials and Methods: Of 592 radical prostatectomy specimens examined in our laboratory from 2001 to 2010, 20 patients harbored IPC and exhibited biopsy findings suggestive of IPC. These biopsy features served as the criteria to define patients with potentially insignificant tumor in this population. The results of the prostate biopsies and surgical specimens of the 592 patients were compared.

Results: The twenty patients who had IPC in both biopsy and RP were considered real positive cases. All patients were divided into groups based on their diagnoses following RP: true positives (n = 20), false positives (n = 149), true negatives (n = 421), false negatives (n = 2). The accuracy of the pathological data alone for the prediction of IPC was 91.4%, the sensitivity was 91% and the specificity was 74%.

Conclusion: The identification of IPC using pathological data exclusively is accurate, and pathologists should suggest this in their reports to aid surgeons, urologists and radiotherapists to decide the best treatment for their patients.

ARTICLE INFO

Key words:
Prostate cancer; Prostate; Gleason score; Diagnosis

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INTRODUCTION

The detection of patients with nonpalpable prostate cancer has increased since the advent of prostate-specific antigen (PSA) screening. Modified prostatic biopsy schemes have also contributed to higher detection rates (1). Many of these earlier, smaller cancers are low volume (< 0.5 cm^3), low grade and clinically insignificant tumors. Approximately one third of patients with stage T1c cancer have potentially clinically insignificant tumors, and approximately 5% of radical prostatectomy (RP) patients have small cancers that are difficult to identify histologically (2).

Epstein et al. initially created a set of four criteria to predict insignificant prostate cancer prior to definitive therapy: PSA density 0.1-0.15, low or intermediate cancer grade, core involvement of less than 3 mm, and involvement of only one needle biopsy core. These criteria identified

79% of tumors with a volume ≤ 0.5 cm^3 that were organ confined and did not qualify as high-grade lesions at the time of RP (3).

However, the Epstein criteria, which were later updated (4), are insufficient, and 20% of patients who fulfill these criteria may have unfavorable pathological cancer characteristics at RP (5). The validity of these criteria has been questioned. Jeldres et al. demonstrated that the Epstein criteria may not be applicable to European men because prostate cancer was underestimated in 24% of these patients (1).

The aim of this study was to assess the prediction of insignificant prostate cancer based on the biopsy findings of patients who underwent RP and exhibited insignificant cancer. The biopsy features from these subjects were evaluated, and patients whose biopsies had similar parameters were selected. The biopsies were correlated with the respective RP specimens to identify the lesion characteristics and the clinical significance of the tumor.

MATERIALS AND METHODS

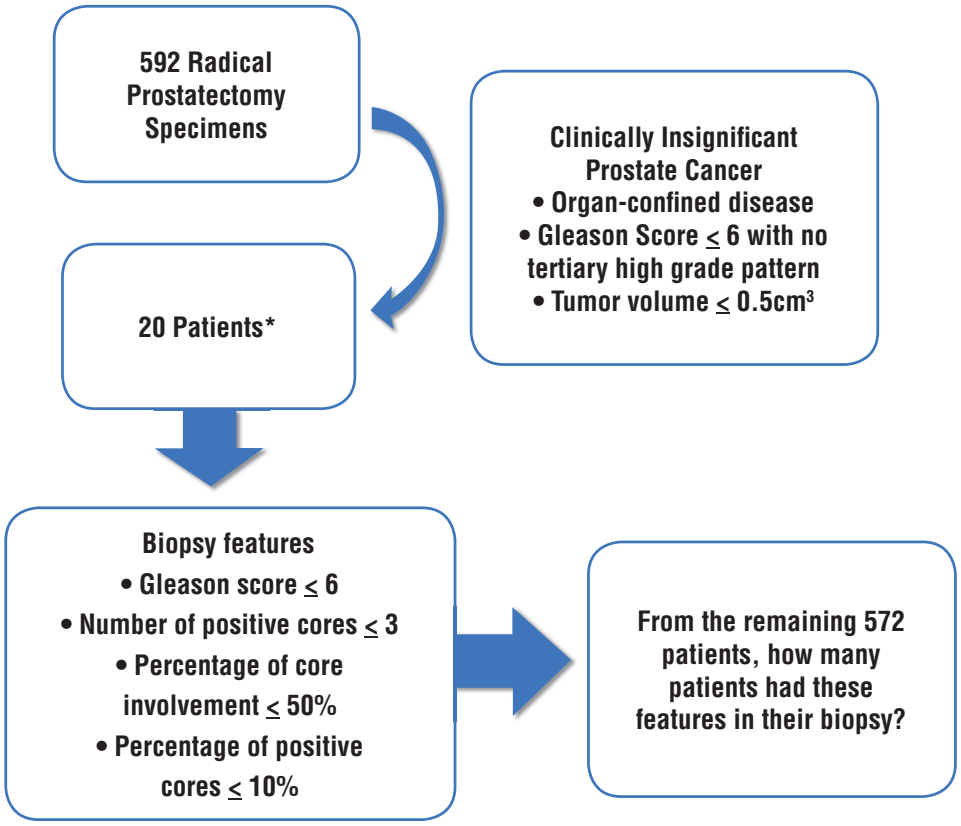
A total of 592 patients underwent transrectal ultrasound-guided (TRUS) prostate biopsy followed by radical prostatectomy for prostate cancer from January 2001 to December 2010. A single surgeon (MS) treated all patients and a single uropathologist (KRML) examined all biopsies and surgical specimens. The surgical specimens were fixed in 10% buffered formalin, the entire surgical margin was stained with India ink, the left and right lobes were separated, 3 mm transverse serial sections were taken from each lobe, and the entire gland was submitted for histologic examination. Sections of the bladder neck, prostatic apex, seminal vesicles, and pelvic lymph nodes were also submitted to exam. The Gleason score (GS) was used for histologic grading (6). The tumor volume was evaluated as described by Humphrey et al. (7). Briefly, a grid was placed below the slides, on which the area involved by the tumor had been previously sketched out. The percentage of tumor on a slide was determined by dividing the number of squares involved by tumor by the number of squares occupied by the whole section on the slide. Tumor volume was defined as the mean percentage of tumor in the prostate gland (the percentage of tumor on each slide divided by the number of slides from the prostate gland). Extraprostatic involvement was defined as tumor infiltration of the adipose tissue, the neurovascular plexus, or the parenchyma of the seminal vesicles. The TNM 2010 system was used for tumor staging and patients were classified as pT2 when tumor was confined to the organ and pT3 when EPE or seminal vesicles were infiltrated by tumor. PM was considered when tumor glands were inked with India ink.

Twenty-two (3.7%) of the 592 patients exhibited insignificant tumor in their RP, which was defined as an organ-confined adenocarcinoma, Gleason score (GS) ≤ 6 with no tertiary high grade Gleason pattern, and a tumor volume ≤ 0.5 cm^3 (Figure-1). The biopsies from these patients were analyzed, and twenty cases (91%) presented the following features: adenocarcinoma (GS) ≤ 6, one to three positive cores that consisted of less than 50% tumor and a total percentage of positive fragments ≤ 10%. These features, which are similar to the Epstein criteria regarding pathological findings and are referred during the paper as “our criteria”, served as the parameters for the selection of patients with potentially insignificant prostate cancer. In order to evaluate the importance of pathological findings in defining these tumors, our criteria were purposely created based solely on the biopsy features and did not include PSA or any imaging method. These criteria were used to review the biopsy and radical prostatectomy results and define the sensitivity, specificity, and positive and negative predictive value of these parameters for the identification of insignificant prostate carcinoma in our population.

RESULTS

Twenty-two (3.7%) of the 592 patients exhibited clinically insignificant prostate cancer in their RP with a mean GS of 5.9 (range 5 to 6) and a tumor volume less than 0.5 cm3 that was organ-confined. Twenty patients (91%) also had potentially insignificant prostate cancer in their biopsies. These biopsy features, which served as

Figure 1 - Selection of patients who had clinically insignificant prostate cancer in radical prostatectomy as well as features of insignificant tumor in biopsy.



* 22 patients had clinically insignificant prostate cancer; however, 2 patients had unfavorable features in their biopsy.

our criteria mentioned above, exhibited a mean GS of 5.9 and mean positive cores of 6%. This group was considered real positive (Table-1). The remaining 2 patients (9%) had GS of 7 in the biopsy, 3 cores positive for tumor, a higher percentage of a single core (40% and 70%) and 4% and 6% positive cores (in 13 and 14 cores, respectively). We consider this group as false negative, since we would not expect them to have insignificant tumor in the RP. The RPs of additional patients who exhibited similar prostate biopsy characteristics of the real positive group were re-examined. A total of 149 patients (26%) had biopsies that met our criteria and were characterized as probable IPC. The mean GS was also 5.9, the mean percentage of tumor in a single core was 23.3%, and the mean percentage of cores that were positive for tumor was 5.4% (range 1.5 to 10%), which represented one to two positive cores in a mean of 15 biopsies. However, their RPs revealed clinically significant carcinomas, including patients with pT3 disease (4.7%) as well as patients with intermediate and high grade tumor (56 patients with GS 7 and 16 with GS > 7). This group was considered false positive, which means they could have been considered as having IPC based on their biopsy features, despite having tumor with adverse pathological characteristics. The real negative group

Table 1 - Comparison between biopsy and RP of the patients regarding clinical significance of the tumor.

Number of patients (%)	Biopsy	Radical Prostatectomy 1	
20 (3.4%)	insignificant	insignificant	Real Positive
149 (25.2%)	insignificant	significant	False Positive
2 (0.34%)	significant	insignificant	False Negative
421 (71.11%)	significant	significant	Real Negative

1 Gold Standard

was composed of 421 men whose biopsy and RP revealed significant prostate cancer. All patient data are detailed in Table-2.

The sensitivity of our criteria for the identification of clinically insignificant prostate cancer in RP was 91% with 74% specificity. The positive predictive value was only 12%, and the negative predictive value was 99.5%. Therefore, if our criteria were used to predict significant cancer, the probability of a patient exhibiting significant cancer would be almost certain at level of accuracy of 91.4%.

DISCUSSION

Several studies have questioned the efficacy of diagnosing limited cancer by needle biopsy, and the possibility of predicting tumor extent at RP based on biopsies. The applicability and validity of the criteria and nomograms that are commonly used to predict insignificant prostate cancer have also been discussed. The current study designed a set of novel criteria that were based on our own data and restricted to morphological aspects without the consideration of clinical stage or tumor markers. Although clinical findings, such as PSA level and PSA density, were not used, the features of the biopsies from patients with insignificant tumors at RP were similar to the biopsy criteria in the literature, such as a GS ≤ 6 and limited tumor extent on biopsy. The current study does not propose criteria or models for the prediction of insignificant tumor. Conversely, this study clarified the use of precise morphological findings of prostate biopsy in the identification of insignificant prostate cancer.

Our data for the prediction of organ-confined tumors are consistent with the literature. Bastian et al. updated the Epstein criteria, which are the most widely used criteria for the prediction of clinically insignificant prostate cancer, and demonstrated concordance with pathologically organ-confined disease and a favorable grade (GS 6) in 83.9% of patients (4). Although 91.6% of these patients had organ-confined disease, 7.6% had a GS of 7 or higher.

The validity of the Epstein criteria has been questioned in European men. In a study that evaluated 366 patients who fulfilled the contemporary Epstein criteria demonstrated a similar rate of organ-confined disease (91.7%). But the percent of patients with a GS of 7 was substantially higher; 24% of patients had a GS of 7 at RP, which yielded lower overall accuracy (76% vs. 84%) (1). Unfortunately, these authors did not mention the number of patients with clinically insignificant tumor at RP. According to our criteria, 95.3% of the tumors were organ-confined, but 48.3% were GS ≥ 7 (37.6% patients were GS 7 and 10.7% were GS 8 or 9), which is substantially higher than the reported rate in previous studies.

Jeldres et al. argued that the differences between biopsy and radical prostatectomy with respect to GS contributed to the observed error rate of the Epstein criteria (1). A potential upgrading of the GS occurs in 24.3% to 28.2% of patients (8,9). The grade assignment is also harder to predict because of the small amount of tumor that is analyzed on biopsy. High-grade prostate cancer is the most important predictor of prognosis (10)

Table 2 - Demographics and data from prostate biopsies and radical prostatectomies from 592 patients whose biopsy and surgical specimens were examined in our laboratory from January 2001 to December 2010.

Insignificant biopsy and insignificant RP (N = 20)										
	Biopsy							Radical Prostatectomy		
	Age	#cores	GS	#positive cores	%cores	Greatest%	PNI%	GS	Tumor volume (cm^3)	pT2(%)
Mean	60.4	16.6	5.9	1.3	6	20.5	0	5.9	0.43	100
Median	59.5	16.5	6	1	6	20		6	0.40	
Min	39	7	5	1	3	10		5	0.40	
Max	74	24	6	3	10	50		6	0.50	
Insignificant biopsy and significant RP (N = 149)										
	Age	#cores	GS	#positive cores	%cores	Greatest%	PNI%	GS	Tumor volume (cm^3)	pT2(%)
Mean	59.5	15	5.9	1.6	5.4	23	0	6.6	3.10	95.3
Median	60	14	6	1	5.1	20		6	2.70	
Min	39	6	4	1	1.5	5		5	0.40	
Max	76	30	6	3	10	50		9	22.0	

Significant biopsy and insignificant RP (N = 2)									
	Age	#cores	GS	#positive cores	%cores	Greatest%	PNI%	GS	Tumor volume (cm^3)	pT2(%)
Mean	57	13.5	7	3	5.4	55	0	6	0.4	100

Significant biopsy and significant RP (N = 421)									
	Age	#cores	GS	#positive cores	%cores	Greatest%	PNI	GS	Tumor volume (cm^3)	pT2(%)
Mean	61.3	14.3	7.1	5.1	8.5	66.3	22.6%	7.2	6.08	76.7
Median	61	14	7	4	7.5	70		7	5.00	
Min	41	6	5	1	1.2	2		4	0.33	
Max	79	30	10	18	33.3	100		10	29.0	

even when the tumor is organ confined, and it is the most informative predictor of biochemical recurrence (11).

Epstein et al. created their criteria to predict insignificant tumor in patients with stage T1c, and it accurately predicted 73% of insignificant tumor (3). The current study predicted only 12% of the clinically insignificant tumors, but our samples were not limited to stage T1c patients. Despite this low positive predictive value, the negative predictive value was 99%, which indicated that ninety-nine percent of the tumors that were identified as significant and required active therapy were indeed significant tumors. This result addresses the concern of overdiagnosis, which occurs at a rate of approximately 56% (12,13).

The retrospective assessment of biopsies from patients with insignificant or significant tumors at RP revealed that all of the parameters that are usually used to estimate tumor extent, such as the number of positive cores, maximal involvement of a single core and the percentage of positive cores, were similar in both groups. These results suggest an absence of rules for insignificant tumor behavior on needle biopsy.

Numerous difficulties exist in the use of prostatic needle biopsies to predict limited cancer at RP, and even the smallest cancer focus on a needle biopsy does not guarantee a clinically insignificant tumor. Small amounts of carcinoma (total linear extent less than 3 mm) do not predict insignificant tumors (14,15). Patients who are diagnosed based on a single focus less than 3 mm (GS $\leq$ 6) have only a 30% chance of harboring an insignificant tumor (16). Samaratunga et al. examined 58 patients with a single minute focus of a GS 6 on biopsy, and only 10 patients (17%) had insignificant tumor at RP (17). Forty-eight patients had significant tumor, 8 patients had extraprostatic extension and 32% of the patients had a GS > 6. These authors concluded that a minute focus of prostate cancer on a needle biopsy is not indicative of insignificant carcinoma in most cases. Interestingly, this study was performed without PSA screening. However, these authors demonstrated that a larger prostate size was significantly correlated with potentially insignificant cancer. These patients likely presented earlier for PSA testing because of symptoms, and an elevated PSA level due to the benign enlargement might lead to biopsies at an earlier stage. Cupp et al. demonstrated only an 8% risk of insignificant cancer using tumor volume at biopsy (14), which is similar to our results. The percentage of tumor extension in millimeters relative to the total extension of all of the cores was 5% and a GS less than 7.

In contrast, Allan et al. evaluated 54 PSA--screened patients with limited adenocarcinoma (< 0.5 mm) on biopsy; the majority of these patients exhibited potentially insignificant cancer, but only one-third warranted definitive therapy (2). Potentially clinically insignificant tumors were present in 67% of the patients in this study, and 44% of these patients had small tumors at RP (less than 0.1 cc). These authors also reported that a PSA density cutoff of 0.15 or less was correlated with clinically insignificant tumor, and the association of these criteria with limited cancer on biopsy predicted patients with insignificant tumors with a greater than 80% accuracy.

The Epstein criteria are not perfectly accurate, but no alternatives for prediction of clinically insignificant prostate cancer are available (1). Kattan et al. derived several nomograms for the prediction of pathologically confirmed insignificant prostate cancer with an accuracy of 64 to 79% (18). However, this series included 13 to 20% of Gleason patterns of 2 as part of the GS, which is much lower than the GS consensus from 2005. Nakanishi et al. improved the accuracy of the existing tools to 73%, especially in patients with a single positive core at biopsy (19).

Chun et al. developed a nomogram to predict the probability of insignificant prostate cancer in a cohort of 1132 men and revealed a predictive accuracy of 90% (5). However, a strikingly important proportion of patients who were qualified with a high probability of insignificant tumor using the Chun et al. nomogram (63%) and the Kattan et al. nomogram (45%) harbored aggressive prostate cancer at RP. Chun et al. concluded that the nomogram studies were similar to the original Epstein et al. criteria in their ability to predict pathologically confirmed insignificant prostate cancer. Clinicians may expect a 80% accuracy when organ-confined prostate cancer is predicted

or a 76 to 79% accuracy when pathologically confirmed insignificant prostate cancer is predicted.

Do these data suggest that a significant number of patients might be left undertreated? Is active surveillance a dangerous choice that could jeopardize the curability of prostate cancer in some men? The answer to these questions appears to be no.

A review of active surveillance revealed that the majority of patients stay on active surveillance, and once a patient requires active treatment, that patient presents with curable prostate cancer (12). The detection of prostate cancer progression in a patient who is selected for active surveillance remains a continuing challenge, and the PSA level remains important during the decision process. The progression of Gleason grade and the increased percentage of cancers per core are also indicators for the cessation of active surveillance.

Duffield et al. have also studied the RP findings of patients in whom active surveillance has failed (13). These authors relied solely on subsequent biopsy pathology to determine progression, and more extensive disease was observed in surveillance biopsies during the first 2 years of follow-up in the majority of cases.

Despite their high accuracy rates, currently available models for the prediction of insignificant prostate cancer are incorrect in 10 to 20% of cases. The addition of novel markers is required, and current imaging techniques, such as multiparametric magnetic resonance, may have a potential role.

Tumor location is problematic for sampling adequately. Anterior tumors are difficult to assess and sample clinically, and the amount of these tumors in biopsies is lower than the amount of tumor from equivalently sized posterior tumors (20,21). These tumors appeared smaller on biopsy, but they were also undersampled. Takashima et al. analyzed the anatomical patterns of disease distribution in nonpalpable tumors and demonstrated that these tumors were localized predominantly in the anterior half of the prostate at the apical to midprostate level (21). These authors suggested that additional cores from the anterior apical site could enhance the detection rate of prostate cancer. Miyake et al. evaluated the significance of additional cores in the dorsal apex and demonstrated a significant increase (9.3%) in the cancer detection rate, particularly for early stage disease (22).

In conclusion, we have shown that the biopsy data exclusively are accurate to diagnose IPC, and pathologist should suggest this possibility in their reports helping urologists, oncologists and radiotherapists to choose the better treatment for each patient.

CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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The significance of biological, environmental, and social risk factors for prostate cancer in a cohort study in Brazil

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ABSTRACT

Purpose: To evaluate the significance of several risk factors for prostate cancer in a cohort of Brazilian men.

Subjects and methods: Men ≥ 40 years-old participating in a prostate cancer screening program between December 2006 and April 2011 in the city of Curitiba, Brazil, were evaluated to determine the prevalence, relative risk (RR) and 95% CI of prostate cancer according to age, race, ethnicity, family history of prostate cancer, educational level, and history of vasectomy, increased blood pressure, diabetes mellitus, and urethritis.

Results: In 2121 men included in this study, prostate cancer prevalence was 0.6% for men between 40-49 years versus 2.0% (adjusted RR = 2.58), 7.7% (adjusted RR = 5.76), and 8.4% (adjusted RR = 4.88) for men 50-59 years, 60-69 years, and ≥ 70 years, respectively ($p < 0.05$ to all). The prevalence of cancer was 5.1% in blacks versus 3.3% in whites (adjusted RR = 1.56, $p > 0.05$); 6.1% in African descendants, in comparison to 3.0% in non-African descendants (adjusted RR = 3.17, $p < 0.05$); 5.1% in men with a positive family history, compared to 2.5% in those with no family history (adjusted RR = 1.55, $p > 0.05$); and 4.8% in participants with incomplete elementary school level or lower, compared to 2.2% in men with complete elementary school level or higher education (adjusted RR = 1.85, $p > 0.05$). Men with/without history of vasectomy, increased blood pressure, diabetes, and urethritis had a prostate cancer prevalence of 0.8%/3.0% (adjusted RR = 0.23, $p > 0.05$), 3.8%/2.2% (adjusted RR = 1.16, $p > 0.05$), 3.7%/2.6% (adjusted RR = 1.39, $p > 0.05$), and 2.6%/2.6% (adjusted RR = 0.99, $p > 0.05$), respectively.

Conclusions: Risk factors associated with an increased prevalence of prostate cancer in this cohort included increasing age and African ethnicity.

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INTRODUCTION

Prostate cancer is one of the most common visceral malignant neoplasms in men worldwide. The clinical incidence and mortality of prostate cancer vary widely between different geographic regions, with the lowest rates in Asia, and the highest in North America and Scandinavia (1,2).

Although the specific causes of prostate cancer are not completely known, epidemiological evidence suggests that biological, environmental, and social risk factors play a role in the initiation and progression of this disease (1,2).

Differences in exposure to these factors among various populations may be responsible for geographic variations in prostate cancer rates. Studies of the potential risk factors associated with prostate cancer are important to identify the significance of each factor in specific populations for counseling, to determine entry into screening programs, and as a means of comparison between different regions or countries.

Little has been published about the risk factors for prostate cancer in the Brazilian population. In this prospective study, we evaluated the importance of several acknowledged risk factors for prostate cancer in a cohort of Brazilian men.

MATERIALS AND METHODS

All men attending a prostate cancer education program conducted in the city of Curitiba (PR) as part of the city employees' Health Care System were invited to join this survey. Inclusion criteria were men ≥ 40 years of age willing to undergo prostate cancer screening. The study protocol was reviewed and approved by the Institutional Ethics Committee on Human Research (registry number 2253.147/2010-06).

Subjects accepting to participate this study were interviewed and examined by a single Urologist. Participants were recommended to follow-up annually, and data was collected prospectively between December 2006 and April 2011.

Clinical evaluation consisted of complete medical history, digital rectal examination (DRE), and serum PSA determination. Participants with prostate nodularity suspicious for cancer, and/or a PSA level ≥ 4.0 ng/mL were indicated for ultrasound-guided 12-core prostate biopsy. We excluded subjects with prior history of prostate cancer, those who did not return to follow-up, and those that elected not to undergo biopsy.

Outcomes of interest included prevalence, relative risk (RR) and 95% confidence intervals (95%CI) of prostate cancer according to age (50-59 versus 40-49, 60-69 versus 40-49, or ≥ 70 versus 40-49 years), race (black versus white, or brown versus white), ethnicity (African descendants versus non-African descendants), family history of prostate cancer (yes versus no), education (incomplete elementary school level or lower versus complete elementary school level or higher), and personal history of vasectomy, increased blood pressure, diabetes mellitus, and sexually transmitted urethritis (yes versus no, to all). Univariate (non-adjusted) analysis was calculated using the Fisher's Exact Test or Pearson's Chi-square, whichever appropriate. Multivariate (adjusted) analysis was performed using logistic regression models (IBM SPSS Statistics, version 20.0.0). Statistical significance was set when $p < 0.05$ or when the 95%CI did not include the null hypothesis (95%CI ≠ 1.00).

RESULTS

From 2499 men initially enrolled in this cohort, we excluded 323 participants that did not return to follow-up, and 22 men with history of prostate cancer. Indications for prostate biopsy were present in 244 (11.3%, t. 2154) subjects, of whom 33 (13.5%, t. 244) elected not to undergo biopsy and were also excluded. Finally, 2121 men with a mean follow-up of 21.5 (range 1 to 52) months were included in the analysis (Table-1), 58 of whom (27.5% of all biopsies [t. 211], and 2.7% of all participants [t. 2121]) were diagnosed with prostate cancer.

Age

Participants with 40-49 years, 50-59 years, 60-69 years, and ≥ 70 years had a prevalence of prostate cancer of, respectively, 0.6%, 2.0%, 7.7%, and 8.4%. Compared to men aged between 40-49 years, the prevalence of prostate cancer was 3.2 times higher in those 50-59 years (non-adjusted RR 3.19, $p = 0.009$; adjusted RR 2.58, $p = 0.049$), 12.4 times increased for those 60-69 years (non-adjusted RR 12.42, $p < 0.001$; adjusted RR 5.76, $p < 0.001$), and 13.5 times greater for those ≥ 70 years-old (non-adjusted RR 13.54, $p < 0.001$; adjusted RR 4.88, $p = 0.019$) (Table-2).

Race

The prevalence of prostate cancer in black participants was 5.1% (non-adjusted RR 1.56, $p = 0.082$; adjusted RR 1.56, $p = 0.599$), and it was 2.6% in brown individuals (non-adjusted RR 0.80,

p = 0.479; adjusted RR 0.75, p = 0.427), compared to 3.3% in white men (Table-2).

Ethnicity

The prevalence of cancer in African descendants was 6.1%, in comparison with 3.0% of that in non-African descendants (non-adjusted RR 2.06, p = 0.070; adjusted RR 3.17, p = 0.024) (Table-2).

Family history

The prevalence of prostate cancer in participants with a positive family history was 5.1%, in contrast to 2.5% of those with negative family history (non-adjusted RR 2.01, p = 0.036; adjusted RR 1.55, p = 0.289) (Table-2).

Education

The prevalence of prostate cancer in participants with incomplete elementary school level or lower was 4.8%, compared to 2.2% in men with complete elementary school level or higher education (non-adjusted RR 2.17, p = 0.004; adjusted RR 1.85, p = 0.062) (Table-2).

Vasectomy

In the group of participants with prior history of vasectomy, 0.8% was diagnosed with prostate cancer, in comparison to 3.0% of those with no history of vasectomy (non-adjusted RR 0.26, p = 0.016; adjusted RR 0.23, p = 0.150) (Table-2). Most individuals with a positive history of vasectomy were aged < 60 years (90.7%, n. 235/t. 259). In 137 (52.9%, t. 235) men aged 40-49 years, the mean interval between vasectomy and screening was of 8.1 (± 6.0) years. The 98 (37.8%, t. 235) participants 50-59 years had a mean interval between vasectomy and screening of 13.2 (± 6.8) years. In the 24 (9.2%, t. 235) men ≥ 60 years of age, the mean interval between vasectomy and screening was 17.6 (± 6.5) years.

Increased blood pressure

The prevalence of prostate cancer in men with arterial hypertension was 3.8%, compared with 2.2% of those with no history of hypertension (non-adjusted RR 1.74, p = 0.031; adjusted RR 1.16, p = 0.677) (Table-2).

Diabetes mellitus

Between participants with self-reported diabetes, the prevalence of prostate cancer was 3.7%, compared with 2.6% of men with no history of diabetes (non-adjusted RR 1.39, p = 0.108; adjusted RR 1.39, p = 0.563) (Table-2).

Sexually transmitted urethritis

Prostate cancer was diagnosed in 2.6% of men with a history of urethritis, and in 2.6% of those with no history of disease (non-adjusted RR 1.02, p = 0.959; adjusted RR 0.99, p = 0.904) (Table-2).

DISCUSSION

Prostate cancer has been known as a disease of the elderly men (2). Age is known as the most significant risk factor for prostate cancer (1,2). Diagnosis is rare before the age of 50 but, after this age, incidence and mortality both increase almost exponentially (2). In this study, overall prostate cancer prevalence increased from approximately 1:200 (0.6%) men 40-49 years, and 1:50 (2.0%) men 50-59 years, to 1:13 (7.7%) men 60-69 years, and 1:12 (8.4%) men ≥ 70 years of age.

Black men have the highest reported incidence of prostate cancer in the world, with an increased risk in several populations including North American, Caribbean, Scandinavian, and Britain (3-7). Although the risk of prostate cancer in the present study was not significantly increased for black versus white men (adjusted RR 1.56, 95%CI 0.64-3.82), similarly to several other Brazilian studies (8-11), a meta-analysis evaluating the prevalence of prostate cancer in black versus white men in Brazil, including the results of our study, demonstrated that the pooled risk of prostate cancer was significantly increased in blacks (RR 1.55, 95%CI 1.32-1.82), suggesting that race is a significant risk factor for prostate cancer in Brazilian men, and that the individual results of most published studies may have been limited by a low sample, a reduced proportion of black men, or a small rate of prostate cancer in each study group (12).

Stratified by ethnicity, African descendants showed a significantly increased prevalence of prostate cancer compared to non-African

Table-1- Demographic characteristics of study population.

Characteristic	Working definition	Number	Percent (%)
Age			
40-49 years	Age at last follow-up or, in the presence of prostate cancer, age at diagnosis	805	38.0
50-59 years		859	40.5
60-69 years		350	16.5
≥ 70 years		107	5.0
Race			
Black	Dark skin, and typical hair texture and facial characteristics	176	8.3
Brown		574	27.1
White	Those who did not fit the Black or White category	794	37.4
Missing	White to pale pink skin color Missing data	577	27.2
Ethnicity			
African descendants	Any African ancestry in the family	82	3.9
Non-African descendants	No African ancestry in the family	1421	67.0
Missing	Missing data	618	29.1
Family history			
Yes	Prostate cancer in a first-degree relative	158	7.4
No	No history of prostate cancer in first-degree relatives	1947	91.8

Unknown	Those adopted or that missed contact with the family	11	0.5
Missing	Missing data	5	0.2
School level			
Incomplete elementary school or higher	Those who did not study or that did not complete elementary school level	437	20.6
Complete elementary school or higher	Those who completed elementary school or higher educational level	1488	70.2
Missing	Missing data	196	9.2
Vasectomy history			
Yes	Past surgical history of bilateral vasectomy	259	12.2
No	No history of vasectomy	1859	87.6
Missing	Missing data	3	0.1
Increased blood pressure			
Yes	History of increased blood pressure or use of antihypertensive drugs	707	33.3
No	No history of increased blood pressure or use of antihypertensives	1411	66.5
Missing	Missing data	3	0.1
Diabetes mellitus			
Yes	History of sustained hyperglycemia or use of antidiabetic medications	219	10.3
No	No history of hyperglycemia or use of antidiabetics	1899	89.5
Missing	Missing data	3	0.1

Table-2 - Prostate cancer prevalence, relative risks (RR) and 95% confidence intervals (CI) of prostate cancer according to age, race, ethnicity, family history, school level, vasectomy, increased blood pressure, diabetes mellitus, and urethritis.

Variables	Prostate cancer prevalence		Univariate analysis		Multivariate analysis	
	Cases/total	%	RR	95%CI	RR	95%CI
Age (years)						
40-49	5 / 805	0.6	1.00	-	1.00	-
50-59	17 / 859	2.0	3.19 (*)	1.18-8.60	2.58 (*)	1.00-6.63
60-69	27 / 350	7.7	12.42(*)	4.82-31.99	5.76 (*)	2.20-15.12
≥ 70	9 / 107	8.4	13.54 (*)	4.62-39.66	4.88 (*)	1.30-18.35
Race						
White	26 / 794	3.3	1.00	-	1.00	-
Brown	15 / 574	2.6	0.80	0.43-1.49	0.75	0.36-1.56
Black	9 / 176	5.1	1.56	0.74-3.27	1.56	0.64-3.82
Ethnicity						
Non-African descendants	42 / 1421	3.0	1.00	-	1.00	-
African descendants	5 / 82	6.1	2.06	0.84-5.07	3.17 (*)	1.16-8.62
Family history						
Negative	49 / 1947	2.5	1.00	-	1.00	-
Positive	8 / 158	5.1	2.01 (*)	0.97-4.17	1.55	0.58-4.14

Education						
Complete elementary school or higher	33 / 1488	2.2	1.00	-	1.00	-
Incomplete elementary school or lower	21 / 437	4.8	2.17 (*)	1.27-3.71	1.85	0.97-3.54
Vasectomy						
No	56 / 1859	3.0	1.00	-	1.00	-
Yes	2 / 259	0.8	0.26 (*)	0.06-1.04	0.23	0.03-1.70
Increased blood pressure						
No	31 / 1411	2.2	1.00	-	1.00	-
Yes	27 / 707	3.8	1.74 (*)	1.05-2.89	1.16	0.61-2.23
Diabetes mellitus						
No	50 / 1899	2.6	1.00	-	1.00	-
Yes	8 / 219	3.7	1.39	0.67-2.89	1.39	0.57-3.34
Urethritis						
No	32 / 1231	2.6	1.00	-	1.00	-
Yes	16 / 606	2.6	1.02	0.56-1.84	0.99	0.50-1.94

(*) $p < 0.05$

descendants in multivariate analysis (adjusted RR 3.17, 95%CI 1.16-8.62). This result is comparable to the only other Brazilian survey that used African ancestry as a criteria to classify men in different racial/ethnic groups while evaluating the risk of prostate cancer (13).

Ample epidemiologic evidence suggests that prostate cancer has both a familial and genetic component (1). In one meta-analysis, the pooled risk of prostate cancer was higher in men who had a father or brother affected by prostate cancer, compared to a control population with no affected relatives (RR 2.5, 95%CI 2.2-2.8) (14). Another meta-analysis showed an increased risk of prostate cancer for any affected family member (RR 2.04, 95%CI 1.64-2.55), a higher risk for disease for affected first-degree relatives (RR 2.24, 95%CI 2.08-2.41), and an elevated risk of cancer in affected second-degree relatives (RR 1.91, 95%CI 1.58-2.30) (15). In the present research, although there was a marginal increased prevalence of prostate cancer in men with affected first-degree relatives in univariate analysis (non-adjusted RR 2.01, 95%CI 0.97-4.17, $p < 0.05$), the risk of cancer became similar between men with and those without family history of prostate cancer in multivariate regression (adjusted RR 1.55, 95%CI 0.58-4.14). These results may have been limited by the lack of knowledge or forgetfulness of the diagnosis of prostate cancer in a family member (measurement bias), or by unknown intervening factors (confounding bias).

Existing data suggest that the incidence of different types of cancer, including that of the prostate, increases with decreasing socioeconomic status (2). Socioeconomic factors may influence the risk of developing prostate cancer indirectly, influencing dietary factors, occupational exposure, access to health care, the type of care available, and even the attitudes and concerns over health matters exhibited by the various populations (confounding bias) (2,16). Our data showed that men with incomplete elementary school or lower school level had a higher risk of prostate cancer compared to those with complete elementary school or higher education (non-adjusted RR 2.17, 95%CI 1.27-3.71). However, since lower school levels were more prevalent in participants $\geq$ 60 years (41.4%), compared to those < 60 years of age (17.9%), as well as in black (31.4%) versus white individuals (20.4%), both of whom have a potentially increased risk of prostate cancer, we must acknowledge that the association between cancer of the prostate and lower education may be biased by age, race, or other unidentified confounding variables. The absence of statistically significant difference in multivariate analysis (adjusted RR 1.85, 95%CI 0.97-3.54) supports this hypothesis.

Several studies have associated a history of vasectomy with an increased risk for prostate cancer, although an equal number of studies provide evidence to the contrary (2). One meta-analysis reported an elevated risk of prostate cancer after vasectomy (RR 1.37, 95%CI 1.15-1.62), with a linear trend suggesting a 10% increase for each additional 10 years since vasectomy up to 30 years (RR 1.32, 95%CI 1.17-1.50) (17). Nevertheless, the results of two other meta-analyses showed no difference in the risk of prostate cancer between subjects with and without a history of vasectomy, regardless of the time since surgery (18,19). Our study demonstrated an inverse relation between vasectomy and prostate cancer (non-adjusted RR 0.26, 95%CI 0.06-1.04, $p < 0.05$). However, most participants with a history of vasectomy were 40-49 years-old, suggesting that the inverse association may be the result of a lower prevalence of prostate cancer in the younger men most frequently undergoing vasectomy (confounding bias), as well as a shorter time distance since vasectomy, not long enough for prostate cancer to develop (susceptibility bias). The inverse association between vasectomy and prostate cancer may also be influenced by detection bias. It is possible that men undergoing vasectomy were more likely to be examined with serum PSA level or DRE, which led to the earlier discovery of prevalent prostate cancers, resulting in a spuriously reduced occurrence of prostate cancer in later years.

The association between blood pressure and prostate cancer has been poorly investigated. In a prospective study, Martin et al. found that hypertension was associated with an increased risk in developing prostate cancer (RR 1.05, 95%CI 1.01-1.10) (20). A small case-control study in Bra-

zil also showed a significantly greater occurrence of arterial hypertension in men with prostate cancer (21). Our findings demonstrated an increased univariate risk of prostate cancer in men with history of increased blood pressure (non-adjusted RR 1.74, 95%CI 1.05-2.89). However, given the frequent occurrence of these conditions in ageing men, a large proportion of patients can be expected to suffer from such an association (confounding bias), which may explain the loss of statistical significance in multivariate analysis (adjusted RR 1.16, 95%CI 0.61-2.23).

A lower risk of prostate cancer among diabetics has been suggested by several studies, including two separate meta-analysis (22,23). Although not significant, our results showed an overall increased risk of 39% in the risk of prostate cancer between diabetics and nondiabetics (adjusted RR 1.39 95%CI 0.57-3.34). Potential confounding factors (such as diet, body weight, and physical activity), inaccuracies in the diagnosis of diabetes (measurement bias), and failure to account for time since the diagnosis (susceptibility bias) may have limited our findings. Furthermore, age-adjusted risk of prostate cancer in diabetics > 60 years-old, allegedly with a longer time of disease, was decreased in comparison with men in the same age group without diabetes (non-adjusted RR 0.86, 95%CI 0.37-2.01), potentially supporting an inverse association between prostate cancer and long time-history of diabetes.

Chronic inflammation leading to cellular hyperproliferation to replace damaged tissue contributes to the development of infection-associated cancers of the colon, esophagus, stomach, bladder, and liver. Accumulating epidemiologic, histologic, and genetic evidences suggest that a similar process may underlie the development of prostate cancer (1,16,24). Notwithstanding, the results of our study demonstrated no association between a past history of urethritis and the development of prostate cancer. A potential source of bias in studies evaluating self-reported history of sexually transmitted diseases (STDs) is measurement bias, given the difficulty in identifying oligosymptomatic infections or undiagnosed outbreaks of the disease (under diagnosis bias), and the possible acknowledgement of other urological conditions, such as balanoposthitis or urinary tract infections, as if they were STDs (overdiagnosis bias). Furthermore, given the social connotations of STDs, patients may not be forthcoming about their history of disease (social convenience bias) or may not recall being diagnosed with STDs (recall bias).

Besides the limitations described specifically for each potential risk factor, another shortcoming of our study is the relatively small sample of participants ≥ 70 years, given that the study population was built mainly of active municipal employees from an established private Health Care System. The subjects in this study also had a potentially lower prevalence of black race, as well as an increased percentage of participants with higher educational levels, compared to the overall population. Notwithstanding, the frequency of hypertension and diabetes in our sample was similar to the estimated prevalence of hypertension and diabetes in the population of the same age living in the city of Curitiba (25), which partially validates our findings.

CONCLUSIONS

The risk factors associated with an increased prevalence of prostate cancer, in our sample of Brazilian men, include increasing age, and African ethnicity.

ABBREVIATIONS

PSA = Prostate Specific Antigen
RR = Relative Risk
95%CI = 95% Confidence Interval
STDs = Sexually transmitted diseases

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CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Application of Yang-Monti Principle in Ileal Ureter Substitution: Is It a beneficial Modification?

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ABSTRACT

Purpose: Ureteric substitution using the Yang-Monti principle was reported as a modification of simple ileal ureter replacement. In this study, we evaluate its safety, surgical outcome and impact on renal function.

Materials and Methods: Sixteen patients underwent ileal ureter replacement using the Yang-Monti principle to overcome long ureteric defects. Exclusion criteria included patients with elevated serum creatinine > 1.8 mg/dL, inflammatory bowel syndrome or irradiated bowel. Antireflux implantation into the bladder was performed in 12 patients while 4 patients with intact healthy lower ureters underwent distal ileal-ureteral anastomosis. Follow-up protocol was carried out for up to 3 years in 9 patients.

Results: No intra-operative or postoperative mortality or significant complications occurred. There were minor complications in the form of urinary leakage that necessitated prolonged ureteric stenting in one patient, superficial wound infection in another one and 3 patients developed treatable urinary tract infection without late harmful effects. During follow up, no excess mucus production or metabolic abnormalities were encountered. All patients had preserved renal function (improved in 13 patients and stabilized in 3) without any evidence of urinary obstruction.

Conclusions: The reconfigured ileal segment for ureteric substitution is a safe technique with an excellent outcome. It uses short ileal segments for reconstruction of an ileal tube of adequate length and optimal caliber that permits easy antireflux implantation into the bladder so it is not associated with excess mucus production or metabolic abnormalities and offers a durable preservation of renal function without urinary obstruction.

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INTRODUCTION

Long ureteric defects may occur as a result of iatrogenic ureteral injuries, recurrent pelvi-ureteric junction obstruction, chronic inflammatory diseases such as bilhariziasis, retroperitoneal fibrosis and ureteral carcinoma. Several techniques were described to compensate this defect by intrinsic urinary tissue such as direct anastomosis, psoas hitch, Boari flap, transuretero-ureterostomy and renal autotransplantation.

In some occasions the ureteral defect is too long to the degree that extra urinary tissue is needed to overcome it. Accordingly, other alternatives have been tried such as artificial ureteral substitutes e.g. Gore-Tex tube graft (1) which showed disappointing results in contrast to the promising ones that were achieved with the use of pedicled bowel grafts (2). The most commonly used technique is that of ileal ureter replacement which was first described by Schoemaker in 1906 (3) and gained wide acceptance later on. One of

the modifications of this technique was the application of Yang-Monti principle (4,5) which allowed the creation of a long tube from short bowel segment after its reconfiguration. It was applied for ureteral replacement first in dogs (6) then clinically (7) in few reports. In this study, we report our experience with ileal ureter substitution using the Yang-Monti principle with follow-up data for up to 3 years.

MATERIALS AND METHODS

The current study was performed from October 2005 till February 2010. Patients with long ureteral defects that could not be repaired using the intrinsic urinary tissue were included in this study. Elevated serum creatinine > 1.8 mg/dL, inflammatory bowel syndrome, irradiated bowel or a non functioning renal unit were considered as exclusion criteria.

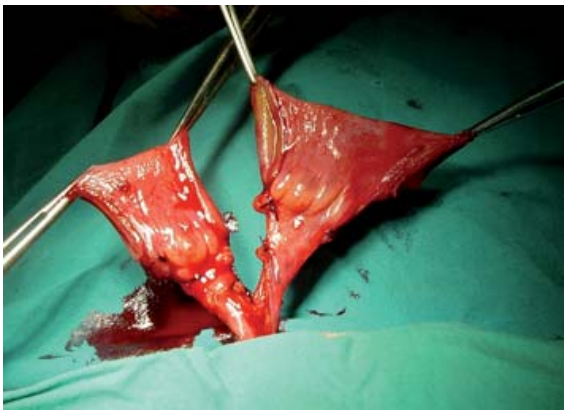
All patients underwent preoperative evaluation in the form of careful history taking, medical examination and laboratory investigations which included complete urine analysis, serum creatinine, sodium, potassium and chloride and PH level estimation. Radiological investigations to visualize the upper urinary tract included renal ultrasonography, intravenous urography or magnetic resonance urography. Renal isotope scanning was performed to estimate the split renal function.

The study was approved by the ethical committee of Ain Shams University. All patients were consented for approval of surgery and underwent preoperative colonic preparation for 48 hours.

Technique

Isolation of 5 - 7.5 cm of the terminal ileum on their vascular bed was performed according to the length required to compensate the ureteral defect. Then, according to the Yang-Monti principle, the isolated ileal segment was further subdivided into 2-3 equal parts (each 2.5 cm in length) with preservation of the individual blood supply (Figure-1). The continuity of the ileum was reestablished. Each ileal segment was incised at its longitudinal axis close to the mesenteric border. Unfolding of the incised segments with suturing

Figure 1 - Isolation of 2 ileal segments on their mesenteric pedicle.



of their adjacent ends were performed and resulted in the formation of an intestinal plate 2.5 cm wide and 12-18 cm long (according to the number of isolated ileal segments) (Figure-2). This plate was tubularized around 16F Nelaton catheter using 4/0 absorbable sutures (Figure-3). The proximal end of the ileal tube was anastomosed to the ureter or renal pelvis while the distal end was anastomosed

Figure 2 - Unfolding of the incised ileal segments with suturing of their adjacent ends.

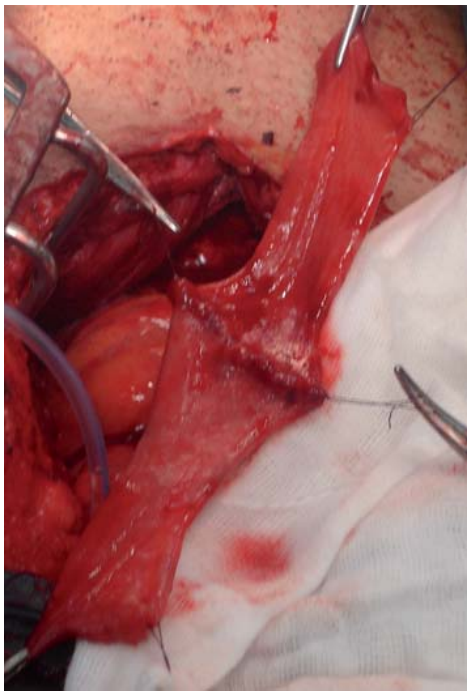
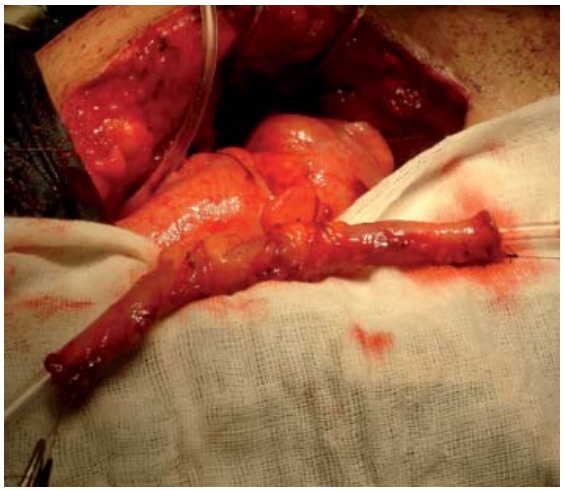
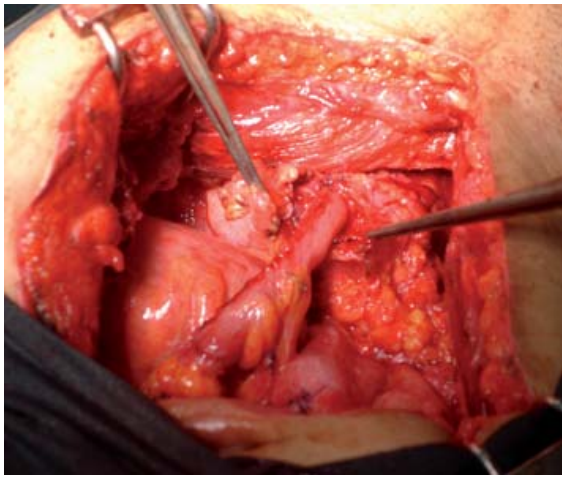


Figure 3 - Tubularization of the incised ileal segments.



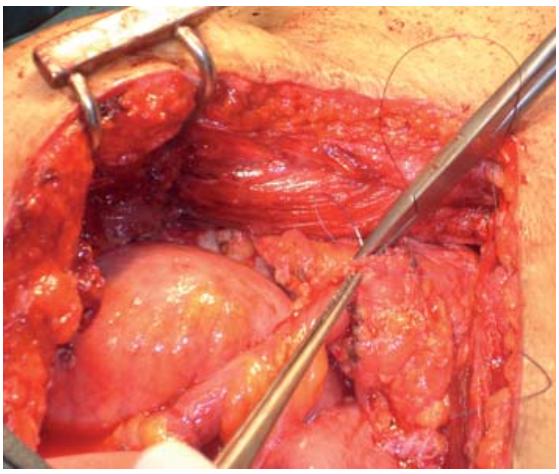
either to the bladder using a non refluxing Lich--Gregoir technique (Figures 4 and 5) or to the spatulated cephalic end of the lower ureter in cases with the presence of an intact healthy lower ureter. At the end of the procedure, a ureteric silicon stent and a urethral catheter were left for 3 weeks and 10 days respectively.

Figure 4 - Anastomosis of the ileal tube to bladder mucosa.



Follow-up was carried out at 3 months postoperatively then annually thereafter and included estimation of serum creatinine, sodium, potassium and chloride and PH, isotope renal scanning and imaging of upper urinary tract. Ascending cystography was performed only for patients who underwent ileal tube implantation into the bladder at 6 months postoperatively.

Figure 5 - Reapproximation of detrusor layer to create a submucosal tunnel.



Statistical methods

IBM SPSS statistics (V. 19.0, IBM Corp., USA, 2010) was used for data analysis. Data were expressed as "Mean ± SD" for quantitative parametric measures. Paired t-test was used for comparison between 2 dependent groups. The probability of error at 0.05 was considered significant.

RESULTS

The study included 16 patients (13 males and 3 females). Their age ranged from 23 - 57 years (mean: 35 ± 8 years). They presented with loin pain secondary to ureteric stricture, lumbar urinary fistula or pyelonephritis. All patients had a long ureteral defect which was secondary to bilharziasis in 2 cases or iatrogenic injury in 14 cases [12 after urologic procedures, one post hysterectomy and one post surgical excision of sigmoid tumor (for whom percutaneous nephrostomy and antegrade study were performed)].

Intraoperatively, we used 2 - 3 ileal segments (2 in 13 and 3 in 3 patients) to create a tube about 12 - 18 cm in length and of a reasonable caliber (16 CH) to overcome the ureteral defect.

Antireflux implantation of the ileal tube into the bladder was performed in 12 patients while 4 patients with intact healthy lower ureter underwent distal ileoureteral anastomosis. No intraoperative complications occurred.

Postoperative follow-up showed no intestinal complications or significant morbidity. With application of The Clavien-Dindo Classification of Surgical Complications (8), early postoperative complications were minor in the form of prolonged urinary leakage in one case (6%) (grade I), superficial wound infection in another one (6%) (grade I), and uncomplicated UTI in 3 patients (18%) (grade II) that were managed properly without late harmful sequelae. No metabolic abnormalities occurred in any patient during early postoperative period.

Follow-up was carried out for up to 3 years (ranged 6-36 months). At 3 months postoperatively, improvement of serum creatinine among all patients with no electrolyte disturbance or metabolic acidosis were noticed. No complaint of excess mucous production was noticed. Imaging of the upper urinary tract showed improvement of renal dilatations in 12 patients and stable renal configuration in 4. We noticed transient stagnation of the dye at the distal ileoureteral anastomosis without upper tract dilatation followed by complete evacuation of the dye into the bladder among the four cases with distal ileal anastomosis to the lower ureter. Radioisotope scanning showed split renal function improvement in 13 patients and stable in 3 with no evidence of urinary obstruction in anyone (Table-1). Ascending cystography revealed first degree reflux in one patient. Follow-up was continued for 3 years in 9 patients who showed maintained renal function without metabolic complications or recurrent urinary tract infections (Table-2).

Table 1 - Preoperative and 3 months postoperative results of laboratory investigations and split renal function.

	N	Preoperative (mean ± SD)	3 months postoperative (mean ± SD)	t	P
Mean serum creatinine (mg/dL)	16	1.13 ± 0.24	1.006 ± 0.16	4.226	0.001
Split renal function	16	37.69 ± 1.92	41.13 ± 1.54	-16.893	0.0
Mean serum sodium	16	138.06 ± 2.59	137.94 ± 1.65	0.251	0.8
Mean serum potassium	16	4.21 ± 0.42	4.17 ± 0.35	1.385	0.18
Mean serum chloride	16	103.38 ± 2.89	104.19 ± 3.58	-1.8	0.09
PH	16	7.39 ± 0.01	7.39 ± 0.00	-2.334	0.034

DISCUSSION

Nowadays, iatrogenic ureteral trauma showed an increased incidence as one of the causes of ureteric stricture (9,10). This could occur as a result of direct injury or focal ischemia to the ureter (11). Most of our cases (14/16) had a history of a complicated iatrogenic ureteral injury or stricture with an inserted percutaneous nephrostomy to preserve renal function till time of surgical repair and to perform an antegrade study. All cases had a ureteric defect that was too long to be repaired using internal urinary tissue and so the decision of ileal ureter substitution was taken to compensate this defect.

Ileal ureter replacement became now an established procedure for ureteric reconstruction especially after the urologists gained more experience in bowel surgery (12). Several studies reported encouraging results regarding surgical ou-

Table 2 - 3 months and 3 years postoperative results of laboratory investigations and split renal function.

	N	3 months postoperative (mean ± SD)	3 years postoperative (mean ± SD)	t	P
Mean serum creatinine (mg/dL)	9	1.011 ± 0.2	0.989 ± 0.2	0.8	0.44
Split renal function	9	41.56 ± 1.66	40.78 ± 1.39	-0.8	0.44
Mean serum sodium	9	137.22 ± 1.39	138.56 ± 1.74	-0.66	0.52
Mean serum potassium	9	4.10 ± 0.35	4.12 ± 0.32	0.0	1
Mean serum chloride	9	104.89 ± 3.4	105 ± 3.46	-0.13	0.9
PH	9	7.397 ± 0.0	7.395 ± 0.00	0.8	0.44

tcome and renal function (2,10), however common drawbacks were reported mostly attributed to the absorbing and secreting criteria of the involved ileal segment such as hyperchloremic metabolic acidosis and excess mucus production and also to the wide caliber refluxing ileal ureter with subsequent progressive dilatation, functional obstruction and recurrent UTI (10,13). So, to overcome these complications and to improve the surgical outcome, the Yang-Monti principle was applied as a modification of original simple ileal ureter replacement, which allowed the reconstruction of long tube from a reconfigured short bowel segment and the ability of antireflux implantation of the reconfigured ileal tube into the bladder. The criticism to this idea was the fear of impaired urine transport through the reconfigured ileal tube, however a dynamic unidirectional flow of urine was reported using cineradiographic studies in a study that explained this by a decrease in tube diameter and reorientation of the 2 muscle layers of the ileal tube after its reconfiguration (7).

Intraoperatively, we used 2 ileal segments in 13 patients. Each ileal segment provided a tube of about 6 cm length after reconfiguration. 3 patients needed 3 ileal segments to compensate the ureteric defect. We found that the addition of a third ileal segment can be done successfully without complications with better results than performing the anastomosis under tension with a risk of subsequent stenosis. On the other hand, extra length of the ileal tube can lead to redundancy and functional obstruction, so adjustment of the ileal tube length is advisable to prevent such complications.

We found that this technique is safe as it was not associated with mortality or significant morbidity. Postoperative complications were minor in the form of prolonged urinary leakage in one case for 3 weeks that was managed conservatively by temporary urinary diversion through the preoperative nephrostomy tube and postponing removal of ureteric stent for 5 weeks with no late harmful effects, superficial wound infection in one patient that was managed by repeated dressing and secondary suturing of the skin and subcutaneous tissue under local anesthesia and uncomplicated urinary tract infections (UTI) in 3 patients that was treated by proper antibiotics. Other authors also reported absence of any mortality or significant morbidity following this procedure (7,14).

During follow-up, no complaint of excess mucus production was observed which was also noticed by other authors that used the same procedure (7,15). This observation is considered an advantage of this modification and most probably due to the marked reduction in the size of the secreting surface area in comparison to simple ileal ureter that may be associated with mucous obstruction in some cases (13). Moreover, hyperchloremic metabolic acidosis did not occur in our

series, however it was reported by various studies using simple ileal ureter in varying percentages (9,10). Absence of metabolic disorders among our cases can be explained by proper selection of patients (serum creatinine ≤ 1.8 mg%), reduction of the size of absorbing surface area and the antireflux implantation of the reconfigured ileal tube which decreased the contact of urine with the ileal mucosa.

The importance of antireflux implantation of the simple ileal ureter remains a controversial issue; some authors recommended it (13) and found that refluxing anastomosis was harmful (16) while others reported no difference in outcome as the natural isoperistaltic waves of simple ileal ureter can prevent reflux from reaching the kidney (7) and an ileal loop longer than 15 cm was considered a safe guard against the harmful effect of reflux on the kidney (17). Other authors tried to prevent reflux by construction of an intussuscepted distal nipple valve, however complications occurred in the form of desussception of the valve and stone formation at the intussuscepted segment (18). In our series, we applied antireflux implantation of the ileal tube in 12 patients with excellent results in reflux prevention which was facilitated by the reasonable caliber of the reconfigured ileal tube (16 CH) and the asymmetrical incision of the ileal segment that led to creation of an ileal tube with ends free of mesentery. Follow-up of our cases revealed no graft dilation, recurrent UTI or metabolic acidosis so we thought that antireflux implantation of the reconfigured ileal tube is advisable as it is now easy to perform, can prevent pressure transmission to the kidney and progressive graft dilation with subsequent functional obstruction, decreases the incidence of recurrent UTI and metabolic acidosis and subsequently attributes to preservation of renal function.

Four cases with intact lower ureter were included in our series. Intraoperatively, we had to choose between preservation of the lower ureter with distal ileoureteral anastomosis or to sacrifice it, add another reconfigured bowel segment and perform ileovesical anastomosis. We chose the preservation of the lower ureter as we found it healthy, had good vascularity, non refluxing and of adequate length (about 7 - 8 cm) so, we performed ureteric spatulation and end to end anastomosis to the ileal tube which was facilitated by the reasonable caliber of the reconfigured tube. Follow-up imaging of these cases showed transient stagnation of dye at the distal anastomosis followed by complete evacuation of the dye into the bladder without renal or graft dilatation. Renal isotope scanning showed improvement of split renal function without urinary obstruction in any patient so we found that distal ileoureteral anastomosis can be done successfully with no worry about organic or functional obstruction in carefully selected cases. This was facilitated by the diminished caliber of the ileal tube after its reconfiguration which added another advantage to Yang-Monti modification.

The main target of any technique for ureteric substitution is to preserve the renal function. Follow-up of our patients by isotope renal scanning and upper tract imaging revealed improvement of renal function and renal configuration in most patients while the rest showed stabilized renal function without evidence of urinary obstruction or progressive renal or graft dilation in any patient and this outcome was durable and maintained all over the follow-up. Other authors that used the Monti principle also reported preserved renal function during their short term follow-up (7,14). We thought that this excellent functional outcome was obtained as a result of gaining the proponents of this procedure that led to absence of organic or functional urinary obstruction, easy antireflux implantation into the bladder or distal end to end anastomosis to lower ureter and less incidence of recurrent UTI which were aided by proper selection of cases.

CONCLUSIONS

In conclusion, we advice the use of Yang--Monti principle in ileal ureter replacement as it consumes a short bowel segment with less mucus production and absence of metabolic abnormalities. It also leads to creation of a long tube of reasonable diameter with easy antireflux implantation. Moreover, it is a safe technique without significant morbidity that permits a durable preservation of renal function. Distal ileoureteral

anastomosis can be performed successfully in carefully selected cases.

CONFLICT OF INTEREST

None declared.

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EDITORIAL COMMENT

The authors present one more series confirms that the use of intestinal segments reconfigured by the Yang-Monti technique as alternative ureteral replacement.

Their results confirm those published in the literature. Alternatives to the technique used, but always based on the principle of Monti, are modified spiral Casale (1) and the use of intestinal segments in series to achieve the same purpose as Ghoneim (2). The use of the variation of Casale allows to obtain intestinal with conduits and vascular pedicle free ends in its middle portion and does not require performing a circular anastomosis for joining two segments, as is customary when using the technique of Yang-Monti original.

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EDITORIAL COMMENT

Initially, the use of reconfigured ileal tube was described for the replacement of an unavailable cecal appendix, used as efferent conduit for catheterization in continent urinary cutaneous diversions according to Mitrofanoff (references 4 and 5). Pope and Koch were the first to describe the replacement of the right ureter with a single 2 cm segment of reconfigured ascending colonic segment, resulting in a tube of 12 cm (1). The use of ileal segments between the kidney and the bladder has several side effects, well described in the text, and they are avoided with the use of the proposed technique, due to the minimal surface of intestinal mucosa exposed to urine. The reconfigured ileal segment allows the use of correct caliber and extension for each case; a 2.5 cm segment of ileum results in a tube of 6 cm. Usually a double tube (12 cm) associated with a psoas hitch is sufficient to replace the lost segment. Anti-reflux technique and interposition of the ureteral inferior and superior stubs are feasible due to the small caliber of the tube. The concern of the loss of the peristaltic movements of the tube due to the reconfiguration (transforming longitudinal muscular fibers in transverse fibers and vice-versa) has no ground, since dynamic image studies showed that there is a unidirectional flow of urine, with preservation of the peristaltic capacity (reference 7). The loss of long ureteral segments has a high morbidity and is becoming more frequent due to the large use of ureterorrenoscopy. In the present, as concluded by the authors, the transverse tubularization technique of ileal segments is efficient, easy to perform and with low morbidity, to replace long ureteral losses, and is superior to the use of natural ileal segments.

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ORIGINAL ARTICLE

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Percutaneous Nephrolithotomy for Staghorn Stones in Patients with Solitary Kidney in Prone Position or in completely Supine Position: a Single-center Experience

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ABSTRACT

Purpose: To evaluate the effectivity and safety of percutaneous nephrolithotomy (PCNL) in the treatment of solitary kidney with staghorn stones in prone position or in completely supine position.

Materials and methods: We retrospectively reviewed the records of 18 patients with staghorn stones in a solitary kidney treated with PCNL. 12 patients underwent PCNL in prone position (group A). 6 patients underwent PCNL in completely supine position (group B). Demographic data, number of accesses, operating time, stone free rate, hemoglobin values, hospital stay and complications were studied. Serum creatinine, systolic and diastolic blood pressure, and new onset hypertension were determined preoperatively and postoperatively at 3 months.

Results: No blood transfusions were required and no abdominal or thoracic organ injuries were reported in both groups. The mean operative time was 104 minutes (range:72-145 minutes) and 128 minutes (range:80-170 minutes), respectively. The I stage stone free rate was 91.7% and 83.3%, respectively. There was no new onset hypertension by the end of follow-up in both groups. Both groups showed a similar fall in serum creatinine at 3 month follow-up period ($p = 0.004$ and 0.029, respectively). Systolic blood pressure showed a statistically significant improvement in group B ($p = 0.034$).

Conclusion: PCNL is safe and has an acceptably high stone free rate in patients with solitary kidneys in both prone and completely supine position. At short-term follow--up, systolic blood pressure had improved in PCNL in supine position.

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Nephrostomy, Percutaneous; Kidney; Urinary Bladder Calculi; Prone Position; Supine Position

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INTRODUCTION

Staghorn calculi are branched stones that occupy a large portion of the collecting system, typically flling the renal pelvis and branching into several or all of the calices (1). Treatment of staghorn stones in patients with solitary kidney is one of the most challenging problems in urology (2). In this study, we evaluated the safety and efficacy of PCNL in the treatment of staghorn stones both in prone position and in completely supine position in a solitary kidney. To the best of our knowledge, this study is the first series reported in the literature.

MATERIALS AND METHODS

Clinical data

We retrospectively reviewed the records of 18 patients with solitary kidneys who had un-

dergone PCNL for stone disease in our department between March 2004 and October 2011. Eight patients had a previous contralateral nephrectomy (44.4%), 4 patients had an anatomy solitary kidney (23.3%), and 6 patients had nonfunctional contralateral kidneys (33.3%). Non-functional contralateral kidneys were confirmed by nephro-dynamic imaging. Twelve patients underwent PCNL in prone position. Six patients underwent PCNL in completely supine position. Patient demographic characteristics, including gender, age, body mass index (BMI), history of shock wave lithotripsy (SWL), mean maximum stone diameter, hydronephrosis and previous kidney surgery (open and/or PCNL) were recorded. All surgeries were performed by the same surgeon. The operation style was decided after the common discussion of the surgeon and the patient. Informed consent was obtained from patients before operation. The study protocol was approved by Institutional Review Board of the First Hospital of Jilin University.

Preoperatively, widespectrum antibiotics were administered to patients with bacteriuria by experience (Cefuroxime Sodium or Ciprofloxacin Lactate), or patients were treated according to the antibiogram results. Operation could be done if the results of urinalysis and urine culture follow up testing became negative. Serum creatinine (Cr), systolic blood pressure, diastolic blood pressure, and new onset hypertension were determined preoperatively and postoperatively at 3 months.

Equipment and instruments

18-gauge coaxial needle (COOK Inc.), Zebra guide wire(Boston Scientific Corporation), fascial dilators(COOK Inc.), X-Force N30 Nephrostomy Balloon Dilation Catheter (BCR Inc.), F9 Olympus ureteroscope (Germany), F20 Storz nephroscope (Germany), Cybersonics Double-catheter system (America), Lumenis 60w holmium lithotripter (USA), Fluoroscopic table (Siemens), Aloka 5 multicolor ultrasound instrument with transducer frequency 3.5 MHz.

Technique of PCNL

The entire procedure was performed on the fluoroscopic table with the patient under general anesthesia.

Prone position (Group A)

After placing the patient in lithotomic position, retrograde ureter catheterization with a 5-French open-ended ureter catheter was performed. All other procedures were completed in the prone position. Under the guide of ultrasound, the coaxial needle was placed in the desired calyx. The working channel was then dilated by using the plastic dilator system or X-Force Nephrostomy Balloon Dilation Catheter to either F18 or F26. And then, the F9 ureteroscope or the F20 nephroscope was placed directly into the kidney through the established tract. The Lumenis 60w lithothiptor or Cybersonics Double-catheter system was used to fragment the renal stone.

Completely supine position (Group B)

The patients were placed in a completely supine position with the flank to be operated raised and slightly rotated by a single underlying 3-liter water bag. The procedure was the same to prone position.

Stone clearance was determined by a combination of fluoroscopy and ultrasound. At the end of the procedure, a double J tube was placed within the ureter. And a clamped 14F or 20F Foley catheter was placed as a nephrostomy tube and it was opened within 24 hours. We rechecked KUB or ultrasound 1 or 2 days post-operation. And the nephrostomy tube was removed if there was no extravasation and larger residual stones at approximately 3 days post-operation. We routinely removed the double J tube about 1 month post-operation in the Outpatient Clinic.

Patients were considered stone-free when no stone > 4 mm was visualized. Residual fragments > 5 mm in diameter were treated with extracorporeal shock wave lithotripsy (ESWL) or second phase PCNL. Both of them were performed 1 week postoperatively.

Statistical analysis

Comparisons between continuous variables were performed using the Student t test while comparisons between categorical variables were performed using a Pearson chi-square test (SPSS 13.0, SPSS Inc, Chicago, IL). $P < 0.05$ was considered to be statistically significant.

RESULTS

The patients in group A were equally matched to those in group B cohort with regards to sex ratio, age, body mass index (BMI), stone location, stone size, existence of hydronephrosis, previous open surgery and ESWL ($p > 0.05$) (Table-1). The main intraoperative and postoperative parameters are summarized in Table-2. The mean operative time was 104 minutes (range: 72-145 minutes) and 128 minutes (range: 80-170 minutes), respectively ($p > 0.05$). The I stage stone free rate was 91.7% and 83.3%, respectively ($p > 0.05$). One patient in group A who had residual fragments required drug therapy. While another patient in group B who had residual fragments underwent ESWL. No blood transfusions were required and no abdominal or thoracic organ injuries were reported in both groups.

There was no new onset hypertension by the end of follow-up in both groups (Table-3). Blood pressure levels in group A were equally matched to those in group B with regard to pre-operation and 3 months after operation. Systolic blood pressure showed a statistically significant improvement in group B ($p = 0.034$), but a non-statistically significant improvement in group A ($p = 0.368$) by the end of follow-up period. There were no statistical improvement in both groups about diastolic blood pressure before and 3 months after operation ($p = 0.275$ and 0.363, respectively). Baseline values of serum creatinine were comparable in the two patients groups ($P = 0.92$ before operation and $P = 0.783$ by the end of follow-up). Both groups showed a similar fall in serum creatinine at 3 month follow-up period ($p = 0.004$ and 0.029, respectively).

DISCUSSION

"Correct position, half operation" is one of the dogmas in surgery. So does in PCNL. The correct position of the patient during PCNL has always been a debated issue, as the precise access to the kidney is facilitated by a careful positioning of the patient and can reduce intraoperative complications (3-5).

PCNL is traditionally performed in the prone position for a safe approach to the kidney.

Table 1 - Patient characteristics.

	Group A (prone)	Group B (completely supine)	P value
Total patients	12	6	
M/F ratio	8:4	4:2	1.0
Mean age, yr(range)	43.8 (30-54)	44.8 (29-52)	0.881
Mean BMI, kg/m^2	24.2 (18.6-28.7)	24.5 (19.0-28.5)	0.804
Side,right/left, n	7:5	3:3	0.737
Mean maximum stone diameter,cm(range)	3.3 (2.3-4.8)	3.6 (2.2-5.0)	0.568
Hydronephrosis yes/no	9:3	4:2	0.710
Previous open surgery (%)	3 (25)	1 (16.7)	0.688
History ESWL (%)	10 (83.3)	5 (83.3)	1.0

M/F = Male-to-Female;
BMI = body mass index;
ESWL = Extracorporeal shockwave lithotripsy

Table 2 - Intraoperative and postoperative parameters.

	Group A (prone)	Group B (completely supine)	P value
No. of access tracts			
Simple	10	6	0.289
Complex	2	0	
Mean operating time, min(range)	104 (72-145)	128 (80-170)	0.077
Stone free rate, %	91.7 (11/12)	83.3 (5/6)	0.596
Mean blood loss(△Hb), g/dL	-2.06 (3.5-0.5)	-2.12 (3.6-0.5)	0.488
Postoperative fever,n(%)	4 (33.3)	0	0.18
Mean hospital stay, d(range)	9.08 (6-11)	9.17 (6-12)	0.43
II stage ESWL, n(%)	0	1	0.146
II stage PCNL, n(%)	0	0	
Major complications, no. of patients	0	0	

Major complications included septicemia, hemorrhage requiring blood transfusion, thoracic or abdominal organ injury, acute pancreatitis.

The prone position has some inherent merits (6-8). For example, a wide surgical field for the selection of the puncture site, an adequate nephroscopic manipulation, and a good distention of the collecting system. However, the prone position is often associated with a limitation in respiratory movements and potential anesthesia danger.

Recently, there have been many reports about PCNL in the supine position (9-11). The potential advantages of supine position in PCNL are as follows. Firstly, the surgeon can work while sitting on chairs during the whole procedures which is more comfortable. Secondly, the supine position has important anesthesiological advantages, such as a low incidence of cardiovascular and respiratory problems. Some authors believe that the incidence of colonic injuries is lower in supine position than that in prone position (11,12). What's more, the supine position allows a simultaneous retrograde approach to the ureter and renal pelvis, with both rigid and flexible scopes, for contemporaneous treatment of ureteral or complex renal stones. However, every coin has its two sides, so does the supine position. The obviously anteromedial movement of the kidney during dilation makes the procedure more difficult. Besides, the superior calyceal puncture is more challenging. A narrow surgical field for the selection of the puncture site is another shortcoming of the supine position.

Valdivia Uria et al. (12) proposed PCNL in a completely supine position with a 3 liter water bag below the ipsilateral flank and with the ipsilateral leg totally extended. In their report on 557 patients, the procedure was successful in 93% of the cases, with a low complication rate and no colonic perforation. We adopted this position in our department.

Treatment of staghorn stones in patients with solitary kidney is one of the most difficulties in PCNL (13). Major complications, although rare, can lead to significant morbidity especially in patients with solitary kidney. This study shows it is safe both in prone position and modified position. No blood transfusions were required and no abdominal or thoracic organ injuries were reported in both groups. The stone free rate of solitary kidney PCNL in group A and group B was 91.7% and 83.3%, res-

Table 3 - Blood pressure levels and serum creatinine before and after PCNL.

	Group A (prone)	Group B (completely supine)	P value
New onset hypertension	0	0	
Systolic blood pressure (mmHg)			
Preoperative	127.5 ± 14.5	132.5 ± 14.1	0.8
Postoperative, 3 months	125.8 ± 10.8	126.7 ± 11.7	0.97
Improvement of blood pressure	1.37 ± 6.15		0.368
Improvement of blood pressure		5.83±4.92	0.034
Diastolic blood pressure (mmHg)			
Preoperative	80 ± 8.53	82.5±11.7	0.283
Postoperative, 3 months	78.8 ± 6.4	80.8 ± 9.7	0.167
Improvement of blood pressure	1.25 ± 3.77		0.275
Improvement of blood pressure		1.67 ± 4.08	0.363
Serum creatinine (mg/dL)			
Preoperative	1.18 ± 0.31	1.24 ± 0.32	0.92
Postoperative, 3 months	1.00 ± 0.17	0.98 ± 0.13	0.783
Improvement of blood serum creatinine	1.37 ± 6.15		0.004
Improvement of blood serum creatinine		5.83 ± 4.92	0.029

pectively. The stone free rate was higher in group A than that in group B, but this was not statistically significant (p = 0.596). Four patients(33.3%) in the group A had postoperative fever. This was in contrast to the group B, of which none of the patients had postoperative fever. The probable reason is that the percutaneous tract is horizontal or slightly inclined downward, spontaneous evacuation of stone fragments during the procedure is easier and the pressure inside the pelvis is low in supine position.

Study of blood pressure in patients with solitary kidney and staghron stonesis few. Berkan et al. (14) observed staghorn stones in 16 patients. The number of patients with hypertension before PCNL was five and by the end of follow-up there was no new onset hypertension. The result is similar with us. We also observed that systolic blood pressure showed a statistically significant improvement in group B. The reason why systolic blood pressure improved after 3 months instead of diastolic blood pressure was unclear.

A few studies have investigated the factors that affect renal function in patients with solitary kidney (15-19). Most of them demonstrated a significant improvement in creatinine or GFR levels from preoperatory levels to about 1-year of follow-up. In this study, we observed that both groups showed a similar fall in serum creatinine at 3 month follow-up period (p = 0.004 and 0.029, respectively).

However, the number of cases in the study was comparatively small, which result in lack of enough confidence on statistical analysis of the data. The reasons of fewer patients are a short

study period and a comparatively lower incidence rate of staghorn stones in patients with solitary kidney. However, we believe that our research will give some inspiration to new studies that should also estimate the preoperative and postoperative GFR and compare them.

CONCLUSIONS

PCNL is safe and has an acceptably high stone free rate in patients with solitary kidneys. At short-term follow-up, systolic blood pressure has improved in PCNL in supine position.

CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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The comparison of standard and tubeless percutaneous nephrolithotomy procedures

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ABSTRACT

Purpose: To compare totally tubeless and standard percutaneous nephrolitotomy procedures on many parameters.

Materials and Methods: Percutaneous nephrolitotomy was performed on 195 patients between June 2009 and May 2012. The data of those patients were evaluated retrospectively. Totally tubeless cases were enrolled to Group 1, and Group 2 consisted of non-tubeless cases (re-entry or Foley catheter).

Results: Group 1 included 85 cases and group 2 a total of 110 patients. Paper tracing values for the kidney stones were 321.25 ± 102.4 mm^2 and 324.10 ± 169.5 mm^2 respectively. Mean fluoroscopy time was 4.9 ± 1.9 min and 5.08 ± 2.7 min, mean operation time was 78.8 ± 27.9 min and 81.9 ± 28.77 min and mean decrease in hematocrit was 2.6 ± 1.6 and 3.74 ± 1.9 respectively. All these comparisons were statistically significant. Length of hospitalization was 1.6 ± 1.1 and 3.5 ± 1.5 days for Groups 1 and 2 respectively. Mean superficial pain score was 5.8 ± 1.6 and 6.7 ± 1.2 respectively for both groups after 1 hour. At 6 hours, the scores changed to 3.87 ± 1.22 and 4.84 ± 1.3 respectively. The analgesic dose was 1.00 ± 0.7 and 1.53 ± 0.6 for the groups respectively at 6 hours. All the statistical differences were significant for these three parameters.

Conclusions: We believe that, because of their post operative patient comfort and decreased length of hospital stay, totally tubeless procedures should be considered as an alternative to standard percutaneous nephrolitotomy.

ARTICLE INFO

Key words:
Kidney Calculi; Nephrostomy, Percutaneous; percutaneous nephrolitotomy; Kidney Tubules, Distal

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INTRODUCTION

Kidney stones have been a frequent, significantly morbid problem. Patients need to undergo invasive surgery and to go through a hard time with long recovery. Until the last 2 decades, open surgery for kidney stones was a must. Because of the morbidity of those operations, new modalities were researched and Percutaneous Nephrolitotomy (PCNL) was described as a treatment method. Initially, Rupel and Brown's removal of the obstructing kidney stone (1), Fenstrom and Johansson published their report on the new stone surgery, which they called Percutaneous Pyelolithotomy (2). The method quickly gained popularity and all patients assigned for open surgery were told to be candidates for percutaneous surgery. After gaining experience with the standard method, surgeons tended to perform this operation with even more decreased morbidity. To achieve that goal,

they got rid of the nephrostomy tube and tubeless PCNL emerged.

We, in this study, aimed to compare the results of the traditional PCNL with the less invasive method, tubeless PCNL, retrospectively.

MATERIALS AND METHODS

Data of 195 patients submitted to PCNL in our clinic between June 2009 and May 2012 were evaluated retrospectively. Two groups were formed: Group 1 included patients submitted to totally tubeless and Group 2 to standard PCNL (with any nephrostomy tube). All procedures were performed by a single surgeon. The groups had no differences between operation technique- including dilatation size and type. Pre and post operative hematocrit values, blood transfusion rates, fluoroscopy durations, post operative complications, length of hospital stay, post operative 1st and 6th hours pain scores and analgesic requirements were compared in tubeless and standard patients. Patients having staghorn calculi, requiring multiple accesses were excluded from the study. Pain scores were performed using the Visual Analog Scale. In group 2, patients were checked for residual fragments by X-ray and in addition to this, renal ultrasound was performed to patients in Group 1 for urinoma, hematoma or clinically significant residual fragments.

Inclusion criteria for the totally tubeless PCNL during the operation were intact collecting system (no perforations), no serious bleeding, having one access, no previous operations or drainage and no serious extravasations determined by retrograde pyelogram at the end of the operation. Achieving stone free patients or patients having clinically insignificant residual fragments (CIRF) (smaller than 4 millimeters,-mm-), no bleeding for 5 minutes after finalization of operation, were also considered as inclusion criteria.

All patients had been evaluated with complete blood count, plasma electrolytes, kidney and liver function tests, coagulation parameters and urine analysis and urine cultures. Patients with urinary tract infections (UTI) were treated with antibiotics and operation was performed after sterile urine cultures.

Measuring the largest edge and the edge perpendicular and multiplying them calculated the stone load. With multiple stones, the measurement was done to all stones and added.

Statistical analysis was done using SPSS for Windows 15.0 and Mann Withney U test. P values < 0.05 were considered statistically significant.

RESULTS

We evaluated the data of 195 cases undergoing PCNL in our clinic. There were 85 patients in Group 1 and 110 in group 2. The groups had similar demographics according to co-morbidities. They are summarized in Table-1. Mean stone burden was 321.25 ± 102.4 mm² in Group 1 and, 324.10 ± 169.5 mm² in Group 2. Even though mean stone load, fluoroscopy duration and operative time was higher in Group 2, the difference was not statistically significant.

Table 1 - Co-morbidites and BMI of both groups.

	Group 1 (n = 85)	Group 2 (n = 110)	p
Hypertension	10 (11.7%)	14 (12.7%)	> 0.05
Diabetes Mellitus	7 (8%)	10 (9%)	> 0.05
Chronic Respiratory Diseases	4 (4.7%)	6 (5.4%)	> 0.05
BMI (Mean ± SD)	23.6 ± 0.18	24.1 ± 0.27	> 0.05

Need for analgesics and VAS scores at 1st and 6th hours were statistically lower for patients in Group 1. Parameters concerning the operation are summarized in Table-2.

In Group 1, for 78 (91.6%) patients and in Group 2, for 96 (87.7%) patients complete stone removal was achieved. 7 (8.2%) cases in Group 1 had prolonged (at least 24 hours) leakage. Six patients were treated by placing a double j stent, the other patient undergone ureteroscopy for distal ureter stone. In group 2, 11 (10%) cases had prolonged leakage. 3 of these cases (2.7%) had to undergo ureterorenoscopy and 7 were treated by retrograde double j stenting. There were no complications postoperatively.

Postoperative outcomes are summarized in Table-3.

All patients were evaluated with urinary X-ray post operatively. In addition, all patients in group 1 were evaluated by renal ultrasonography and no pathology was revealed. In Group 2, one patient suffered from toxic hepatitis, caused by hyper reaction to anesthesia. Postoperative cultures of 3 patients revealed E.coli, but that was not found to be statistically significant. No major complications were seen in both groups.

COMMENTS

The European Association of Urology (EAU) recommends PCNL for kidney stones larger than 2 cm in its guideline on Urolithiasis (3).

Table 2 - Operative parameters of both groups.

	Group 1 (n = 85)	Group 2 (n = 110)	p
Length of hospitalization (days) Mean ± SD	1.6 ± 1.1	3.5 ± 1.5	< 0.05
Length of fluoroscopy (min.) Mean ± SD	4.9 ± 1.9	5.08 ± 2.7	> 0.05
Hematocrit decrease Mean ± SD	2.6 ± 1.6	3.74 ± 1.9	< 0.05
Operative time (min) Mean ± SD	78.8 ± 27.9	81.9 ± 28.7	> 0.05
VAS score (1st hour) Mean ± SD	5.8 ± 1.6 /10	6.7 ± 1.2 /10	< 0.05
VAS score (6th hour) Mean ± SD	3.9 ± 1.22 /10	4.8 ± 1.3 /10	< 0.05
Analgesic dose in first 6 hours Mean ± SD	1 ± 0.7	1.53 ± 0.6	< 0.05

VAS: Visual Analog Scale

In our practice, placing a nephrostomy tube after PCNL is the standard method. In the last years, tubeless PCNL, the procedure without placing a nephrostomy tube, neither internally nor externally was described. By placing a nephrostomy tube, the surgeon obtains adequate urinary

Table 3 - Success rates of both groups.

	Group 1 (n=85)	Group 2 (n=110)	p
Stone free	78 (91.6%)	96 (87.7%)	> 0.05
Clinically insignificant residual fragments	4(4.1%)	6 (5.4%)	> 0.05
DJ	7 (8.2%)	11 (10%)	> 0.05
URS	1 (1.1%)	2 (2.7%)	> 0.05
Residual fragments	1 (1.1%)	3 (2.7%)	> 0.05

DJ: Double-J ureteral stent.
URS: Ureteroscopy.

discharge, hemostasis, tract recovery and a guide for a second operation, if needed. However, because it can cause pain in early hours, it can deteriorate the patient comfort. In the series published by Bdesha, patients with tubeless PCNL were hospitalized for about 2 days, and there was no need for urgent placing of a tube. Hemedra reported a 1.2 gr/dL decrease in hemoglobin and suggested the tubeless procedure in patients with solitary kidney. Goh and Wolf reported decreased morbidity with tubeless operations (4).

After the rapid advancement in PCNL, some surgeons have a tendency on not placing the nephrostomy tube (5). Zilberman et al. reviewed the papers about tubeless PCNL. They reported similar results with tubeless PCNL compared to standard PCNL (6). With this approach, the target is to achieve less hospital days, less pain scores, less analgesics, faster return to normal activities and lower costs.

In addition, there are some indications for tubeless PCNL, such as cases with single tracts, no distal obstruction, no intraoperative complications (such as calyx perforation) and not planning the second look (7,8).

In the study designed by Karami et al. (9), 210 patients had undergone tubeless PCNL. All patients had over 2 cm kidney stones (avg 3 cm) and 21 had staghorn stones. 91.04% of the cases were stone free, and 8.95 % (18 patients) had residual fragments around 7 mm and they all were treated by SWL. 40 patients had minor bleeding, 22 patients (10.9%) needed blood transfusion and 16 patients (7.9%) suffered UTI. For pain management, diclofenac or indomethacin was used; 50 mg of petidine was used for 10 patients. Mean hospitalization time was 3.5 days. The researchers underlined that tubeless PCNL is a safe and economic approach with high patient comfort (9).

In a similar study, Shah et al. (10) randomized patients to tubeless and a small diameter (8F) nephrostomy tube and compared their pain, need for analgesics and days of hospitalization. Tubeless group were placed a 6 F Double J tube. That group had less pain, need for analgesics and days of hospitalization. But 39.4% of the same group suffered pain from Double J.

Tubeless PCNL indications expanded in recent literature. Jung and Bellman used the technique successfully on obese patients (5). Shuh et al. performed on bilateral kidney stones. Jou et al. underlined the fact that over 3 cm or staghorn stones were also candidates for tubeless PCNL (11).

PCNL is a challenging operation; even in the most experienced hands around 1.1-83% complications may emerge. Hemorrhage, cured by intervention (0.6-17%) is the most important complication (12,13). Bleeding may occur during

needle entry, tract dilatation, and nephroscopy. Placing a nephrostomy tube usually avoids this complication. This might mean that in tubeless PCNL it is not possible to perform hemostasis. Even though it is said that diathermia can be used for intrarenal hemorrhage and fibrin injection can be used for parenchymal bleeding, these are used only in experimental studies (14,15). Cormio et al. used Tachosil® for bleeding, and reported less hospital stay for tubeless patients (16). We did not use any additional method for hemostasis. de la Rosette et al. published the data of 96 centers and 5803 patients. They reported 7.8% significant bleeding, 3.4%, renal pelvis perforation and 1.8%. hydrothorax; blood transfusion was administered in 328 (5.7%) patients, and fever > 38.5 °C occurred in 10.5% of patients (17).

In our study, our data tend to be parallel to current literature. Even though it was not statistically significant, duration of operation and fluoroscopy were both longer in Group 2. It was considered to be because of the extra time spent to place the nephrostomy tube. Performing a tubeless PCNL is decided at the end of the operation. By that means, the higher number of patients with decrease in hematocrit in Group 2 was considered on this matter. Mean hospitalization days were higher in Group 1 ($p < 0.05$). Also, pain scores and need for analgesics were higher in Group 2 ($p < 0.05$). All those parameters are about patient comfort and being able to return to everyday activities. We suggest choosing tubeless PCNL when possible.

CONCLUSIONS

Because of the superiority of PCNL over open surgery about safety, easily usability, being able to gain more stone-free rates and patient comfort, it is commonly used on stone disease. Parallel to that fact, post operative patient comfort, shorter hospitalization, less need for analgesics might make tubeless PCNL the new standard. In suitable cases, it can be used safely as standard PCNL.

Despite our study's low patient population and retrospective nature, for cases without collecting system perforation or intraoperative hemorrhage, we suggest the use of tubeless PCNL safely. Prospective randomized studies including larger populations are needed to back up these results.

CONFLICT OF INTEREST

None declared.

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EDITORIAL COMMENT

Several publications have been done in the last decade pro tubeless PCNL including prospective and randomized trials (1-3). The present study does not represent any news, but have a representative number of patients included. Do not put a tube after PCNL it is necessary that no complication have been occurred, like significant bleeding, perforation of the pelvis and residual fragments otherwise place a tube is imperative. Residual fragments are better identified by CT scan than ultrasound or X-ray. The present study showed the same findings which others authors have been published before: less residual fragments, less hospitalization and lower pain scores and need of analgesics.

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ORIGINAL ARTICLE

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Structural analysis of the phimotic prepuce in patients with failed topical treatment compared with untreated phimosis

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ABSTRACT

Objectives: To evaluate histological alterations in prepuce of patients with phimosis submitted to topic treatment with betamethasone in association with hyaluronidase.
Materials and Methods: We studied sixty patients (mean age 4.5), presenting true phimosis and treated with a topical treatment with betamethasone cream (0.2%) + hyaluronidase. The parents of seven of these patients opted for circumcision (control group). The other fifty-three patients were submitted to clinical treatment. The samples were stained with Weigert's resorcin-fuchsin (analysis of the elastic fibers) and Picro-Sirius Red, for analysis of the collagen. The volumetric density of the elastic fibers was determined by stereological methods.
Results: Only eight (15%) of the fifty-three patients submitted to topical treatment presented failure, being indicated for circumcision (histological analysis). We observed an increase of the collagen type III of the patients submitted to topical treatment. The quantification showed a reduction of the volumetric density of the prepuce's elastic fibers of the patients submitted to the cream treatment, when compared to the control group (p = 0.056). The volumetric density of the elastic fibers of the prepuce at the group not submitted to topical treatment showed an average of 14.60% (11.06 to 21.64%); in the group submitted to the cream treatment, the volumetric density of the elastic fibers of the prepuce showed an average of 10.34% (3.45 to 17.9%).
Conclusion: The topical treatment of phimosis with betamethasone 0.2% + hyaluronidase had a success rate of 85%. Patients with failure of the topical treatment with steroid had histological alterations in the prepuce.

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INTRODUCTION

The foreskin is a specialized and innervated mucous-cutaneous tissue that covers and protects the glans. After 3 years of age, cysts of keratin are formed below the foreskin adherences and together with intermittent erections are able to enlarge the phimotic ring, exposing the glans. Around 80 to 90% of the boys that were not circumcised became capable to expose the glans after 3 years of age (1).

Pathological phimosis is characterized by a foreskin fibrotic ring with adherences that does not allow the exposition of the glans (1). This alteration hinders adequate penile hygiene that favors foreskin infections, repeated urinary infections, sexually transmitted diseases and, at adult age, penile carcinoma (2).

Circumcision at childhood must be performed under general anesthesia. Moreover, the procedure itself is not free of risks, presenting complication rates up to 34% (3,4). The most common complications of circumcision are hemorrhage, urethral meatus and preputial ring stenosis, and even glans amputation (5). Besides these problems, circumcision presents considerable costs, which could approach a mean of US$ 1,920.00 per procedure, according to recent reports (6,7).

The clinical treatment of phimosis with topical corticosteroids has been proposed as an alternative to surgery in the beginning of 1990, demonstrating acceptable results (8-10) and low cost (11). Since then, several authors have presented results varying from 67% to 95% of sucess, independently of the patients' age. The most common used topical treatments were betamethasone, clobetasol, diclofenac sodium, mometasone furoate 0.05% and triamcinolone acetate (10,12,13). Recent randomized prospective studies have confirmed that topical use of corticosteroids as treatment for phimosis is superior to placebo (14,15).

Studies on the foreskin structure in patients with phimosis are scarce. Also, to our knowledge, there are no studies on histological alterations of the foreskin after topical corticosteroids treatment. Therefore, the objective of the present work was to evaluate the possible histological alterations in the foreskin of patients with phimosis submitted to topical application of an association of betamethasone 0.2% and hyaluronidase cream.

MATERIALS AND METHODS

The present work received institutional committee review and parental approval. This work was carried out in accordance with the ethical standards of the institutional committee responsible for human experimentation.

We studied 60 patients ranging from 3 to 10 years (mean 4.5), during the period from January 2006 to October 2010. All patients presented true phimosis with foreskin stenosis and attended the pediatric urology ambulatory care to be submitted to circumcision. All 60 patients presented total impossibility of foreskin retraction, which characterizes the type A foreskin anatomy according to Marques (16). The exclusion criteria were patients with less than 3 years of age, patients without true phimosis and patients with clinical suspicion of balanitis xerotica obliterans.

To the parents of the boys referred for circumcision we offered an alternative clinical management of the phimosis. The regimen proposed was a topical application of an association of betamethasone 0.2% and hyaluronidase (150 units of turbid retention) cream on the phimotic ring. The parents were oriented to perform gentle traction of the foreskin until the stenotic ring could be seen. The cream was applied twice a day, for 6 consecutive weeks, associated with appropriate hygiene of the penis. The children had a follow up every 3 weeks in our ambulatory.

The parents of 7 children did not agree with the clinical treatment and opted for circumcision directly. These 7 patients served as the control group and the final treated sample was composed of 53 patients.

The topical treatment was considered successful if the patient was able to expose the glans completely. We considered failure if the glans could not be exposed after treatment or if occurred foreskin infection during treatment. In these cases, circumcision was performed. The patients submitted to topical treatment were followed up for 6 months.

Histological Analysis

In controls and in those cases on which the patients failed treatment and were submitted to circumcision, the foreskin was fixed in a 10% buffered formalin solution, routinely processed for embedding in paraffin and 5-µm thick sections were obtained. The sections were stained with Picro-Sirius Red, for qualitative analysis of the collagen under microscope of polarization and Weigert's Resorcin Fuscin with previous oxidation by oxona, for characterization and quantification of the elastic system fibers.

Quantitative Analysis

From each specimen (foreskin), 5 different sections were randomly selected. From each section, 5 random fields were analyzed, totaling 25 fields (test areas) for each specimen. The data on

elastic system fibers were expressed as volumetric density (Vv - %). The sections were observed with X400 magnification using an Olympus light microscope coupled to a video camera Sony CCD, and the images transferred to a Sony monitor KX14-CP1. The selected histological areas were then quantified using M42 test-grid system on the digitized fields (17,18). (Figure-1). All numerical results are presented as mean ± standard deviation.

The data were analyzed with the Graphpad software. To compare the quantitative data in both groups (controls and treated) and the outcomes, the Student's t-test was used ($p < 0.05$ was considered significant) (19).

RESULTS

From the 53 patients submitted to topical treatment with the association of betamethasone and hyaluronidase cream, 8 (15%) presented failure (6 could not expose the glans and 2 remained with foreskin stenosis) and were referred to circumcision. The foreskin of these patients was submitted to histological analysis and composed our treated group. The others 45 patients presented significant improvement of the phimosis, with total exposure of the glans without foreskin stenosis. After 6 months of follow-up, these patients considered as success did not present recurrence of the phimosis.

We analyzed the alterations on the elastic fibers and collagen of the foreskin. Elastic system fibers were analyzed in the foreskin of the control (n = 7) and treatment failed (n = 8) groups. Figure-2 shows the elastic system fibers analysis in the foreskin. One may note in Figure-2B, in a patient who failed the topical treatment, an apparent lower amount of elastic fibers when compared to controls (Figure-2A). The quantification demonstrated that the mean volumetric density of elastic fibers in the control group was 14.60% (11.06 to 21.64%) and in the group who failed treatment was 10.34% (3.45 to 17.9%); nevertheless, the difference was not significant ($p = 0.056$). Data on the stereological quantification of elastic system fibers in the foreskin are presented in details on Table-1.

The Figure-3 evidences the alterations on the concentration of the collagen of the two groups studied, through the PicroSirius Red stain (with polarization). We can see the alteration on the pattern of the collagen of the patients submitted to the treatment with the cream. In Figure-3B,

Figure 1 - Quantification of elastic fibers: The quantification was obtained using the M42 Test system. Photomicrograph of the prepuce of an 8-year old patient of the control group (without treatment). Weigert stain reduced from X400.

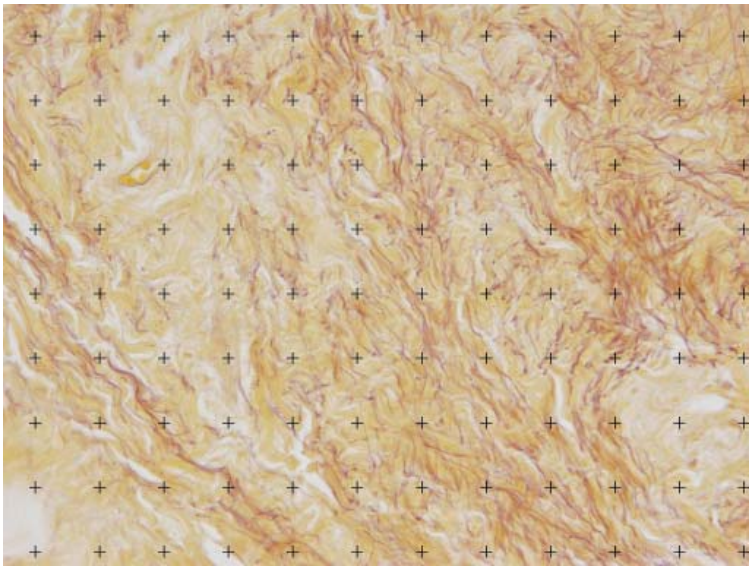
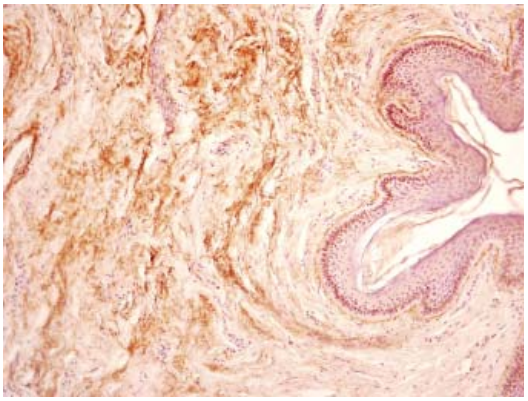


Figure 2 - Analysis of the elastic fibers: A) Photomicrography of the prepuce of a 3-year old patient not treated with topical steroid. It is possible to observe that the elastic fibers (purple) are distributed over the whole field. Weigert stain reduced X400. B) Photomicrography of the prepuce of a 3-year old patient submitted to topical steroid treatment. It is possible to oberserve the elastic fibers (purple). Weigert stain reduced X400.

A



B

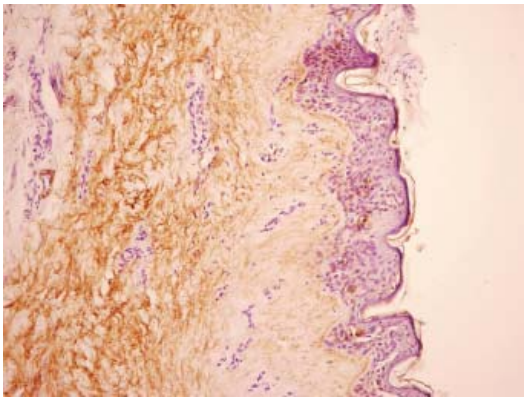


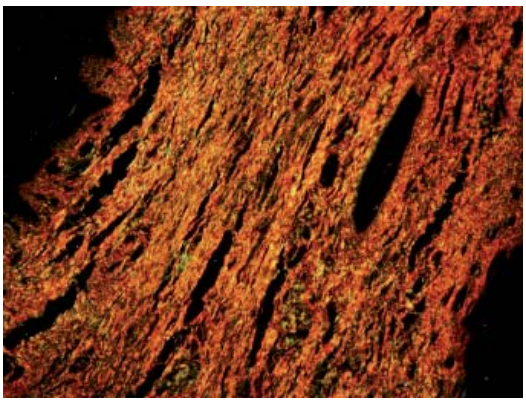
Table 1 - Quantification of elastic system fibers in the foreskin of controls (n = 7) and of patients who failed topical treatment (n = 8) with the association of betamethasone and hyaluronidase cream.

Elastic System Fibers	Mean (%) ± Standard Deviation	Maximum	Minimum	Variance
Controls	14.61 ± 4.106	21.64	11.06	1.552
Failed Treatment	10.35 ± 5.247	17.90	3.45	1.855

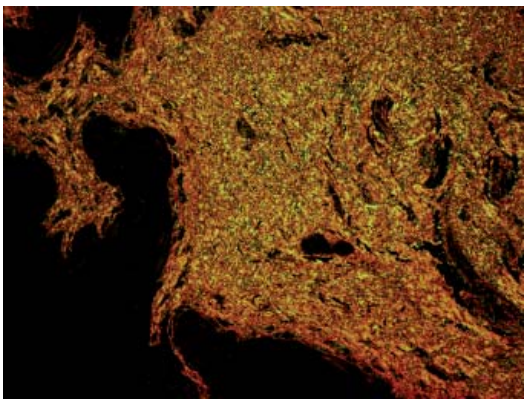
p = 0.056; not significant.

Figure 3 - Qualitative analysis of the collagen: A) Photomicrography of the prepuce of a 4 year old patient without treatment with topical steroid. Collagen type I - red; Collagen type III - green. Picro-Sirius Red stain reduced X 400. B) Photomicrography of the prepuce of a 4 year old patient of the group submitted to topical steroid treatment. It is possible to observe an enlargement of green color (collagen type III), in this group. Picro-Sirius Red stain reduced X 400.

A



B



one may observe the increase of the collagen type III (colored in green), on patients submitted to treatment with topical steroid.

DISCUSSION

The physiological phimosis affects 96% of the newborns and its incidence reduces with age. At 3 years of age, 10% of the patients present phimosis and at 14 years of age, this incidence affects only 1% (20). The natural process of enlargement of the prepuce can suffer alterations when facing episodes of balanoposthitis and lesion of the prepuce by traction of the ring that leads to the formation of a healing fibrosis and the impossibility to expose the glans.

The clinical treatment of the phimosis with topic corticosteroids is well accepted by the parents, since it is a simple procedure, presents low costs and risks, no side effects and a good compliance to the treatment when the guardians of the patients are well oriented (14,15). In our studies we did not have reports of significant side effects and all fifty-three patients submitted to clinical treatment completed the six weeks of treatment.

The success rate of the phimosis topic treatment with corticosteroids is significant, with satisfactory results (67%-95%) (8-10,14,15). The success rate of the topical treatment of the phimosis is lower when smaller concentrations of betamethasone are used (21). We opted for the use of a larger concentration of betamethasone (0.2%) associated with hyluronidase. This larger concentration of corticosteroids does not present a larger rate of side effects and could enhance the success rate of the treatment. After six weeks of treatment, among the fifty-three patients treated, 85% showed significant improvement and were not submitted to surgery, demonstrating the efficiency of the treatment.

Betamethasone is one of the substances that present better rates of efficiency as a topical treatment of phimosis (20,22), therefore the chosen medication in this study. Corticosteroids reduce the arachidonic and hydroxyecosatetranoic acids on the reproductive inflammatory skin disease, inhibiting the liberation of prostaglandins and enlarging the level of dismutase super-oxidative activity, being able to liberate anti-oxidants (20). Side effects, such as the suppression of the hypothalamo-pituitary-adrenal axis or cutaneous atrophy, may ocurr; nevertheless, the doses used in the topical treatment of the phimosis are not sufficient to lead to this type of complications (1).

Hyaluronidase is an enzyme obtained from cattle testicles, which depolymerize hyaluronic acid, a mucopolysaccharide that is present in the interstitial tissue spaces (conjunctive tissue of the preputial adherences). The depolimerization of the hyaluronic acid reduces the inter-cellular viscosity, allowing the tissue to remain available to the dispersion of substances. Besides that, hyaluronidase presents elastic cohesion functions, being applied with the objective of cleaning the adherences and also because it presents the effects of a local anesthesia.

Collagen and elastic fibers are the fibrotic components of the cellular matrix and are related to pathological alterations in different tissues. In our studies, we noticed a reduction of volumetric density of the elastic fibers of the patient´s prepuces submitted to topical treatment, with corticosteroids + hyaluronidase.

The reduction of elastic fibers of the prepuce in patients submitted to topical treatment with the cream was not significant but is typical of the healing process of this treatment (23). The increasing concentration of elastic fibers is related to a larger widening of the tissues. For an easy exposure of the glans, the prepuce needs a larger concentration of elastic fibers (24). The reduction of the elastic fibers in the prepuce of the patients presenting phimosis and submitted to a betamethasone + hyaluronidase treatment is similar to what occurs in the healing process and could be related to a larger difficulty of the exposure of the glans; these patients did not report infections of the prepuce.

Topical treatment with steroids would intuitively reduce tissue scarring/fibrosis, and therefore is the mechanism by which the treatment would be successful. The interesting question to ask would be why did this cohort of patients have a significant reduction in elastic fibers but still fail topical treatment? These alterations of the elastic system could be involved on the therapeutic failu-

re of the cream applied; nevertheless, to confirm this hypothesis, it would be necessary to analyze the prepuces of the patients that received the treatment which obviously, for ethical reasons, is not possible.

The use of the betamethasone + hyaluronidase cream led to an evident alteration in the distribution of the collagen in the prepuces submitted to this topical treatment. There was a reduction of the collagen in the prepuce of the patients that made use of it. Nevertheless, when we analyzed the prepuces with the Picrosirus stain, it could be noticed a clear alteration in the distribution of the collagen, with and increase of collagen type III. The greenish fibers that appear under the Picrosirus stain characterize the collagen type III, a recent collagen which is probably produced by muscular retraction. In the control group there was a predominance of a clear reddish color that characterizes the predominance of the collagen type I. It is very difficult to say, without studying the histology of the patients that made use of the cream and did not need a surgery, if the alterations of the collagen occurred due to induced alterations provoked by the cream or due to tissue alterations of the prepuce resulting from local infections.

CONCLUSIONS

The topical treatment of phimosis with betamethasone 0.2% + hyaluronidase was effective with a success rate of 85%. Patients in whom topical steroid treatment failed had fewer elastic fibers, which characterize the healing processes, as well as an amplification of the collagen type III, a recently found collagen that is associated with muscular retraction. The betamethasone + hyaluronidase cream leads to significant histological alterations in the prepuce.

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CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Is a sequence of tests during urethral pressure profilometry correlated with symptoms assessment in women?

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ABSTRACT

Introduction: Our purpose was, applying a strictly defined protocol for urethral profilometry, 1) to test the repeatability of same session rest maximum urethral closure pressure (MUCP) and 2) to search for correlation between women complaint and the changes in MUCP value (rest and dynamic tests).

Materials and Methods: A population of 140 consecutive women referred for evaluation of lower urinary tract dysfunction was stratified in 4 groups according with the urinary symptoms: stress, urge, mixed incontinence and continent and in each group in 3 age groups (young, middle age and old). The sequence of tests recorded in supine position was: urethral pressure profile at rest bladder empty, after bladder filling at 250 mL (reference test), stress profile, fatigability (before (rest) and after 10 successive strong coughs), then in standing position.

Results: In all groups, there was no significant difference between the two MUCP values at rest bladder filled. In the three incontinent groups, MUCP was higher bladder empty than bladder filled ($p < 0.05$) except in the young sub-group. Stress incontinence led to significant decrease of MUCP during dynamic tests in the young group. MUCP was not modified after fatigability test in women with urge complaint whatever age.

Conclusion: When recorded following a strictly defined protocol, MUCP at rest bladder filled has a good repeatability in individual. However a complex sequence of tests during urethral pressure profilometry remains discussed in middle-age and old age--groups, it allows specifying the stress component of incontinence in young women and the urgency component in all age-groups.

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INTRODUCTION

Controversies about the role of urethral pressure profilometry (UPP) in clinical practice (1-3) are mainly based on the reproducibility of the measurements and on the lack of standardization (4).

Other main controversies are related to the predictive value of the preoperative maximum urethral closure pressure (MUCP) and the success of the surgery of stress incontinence (5-9) and the postoperative quality of life (10). Conversely, De Lancey (11) found that MUCP was the most characteristic parameter of stress incontinence.

In our urodynamic laboratory, we have defined a strict protocol for UPP which is routinely applied in order to rule out many causes of variability. It consists of a well-defined sequence

of tests. UPP is always performed by the same two nurses and the tests always performed following the same sequence. If it is not observed any alteration of the MUCP value at rest, we search for a correlation between the patient complaints and MUCP value changes following dynamic tests.

Is the MUCP value at rest reproducible during a session whatever the patient complaints are? Do the changes in MUCP values during different dynamic tests provide information on the lower urinary tract dysfunction? The aim of this retrospective study was to try to give an answer to these questions.

MATERIALS AND METHODS

Population

The studied population consisted of 140 consecutive women without neurological disease or/and pelvic organ prolapse of grade > 2 (mean age 56.8 ± 15.5 years [21-90 years]) referred for evaluation of lower urinary tract dysfunction (June 2007 to March 2009). Each woman completed a 3-day frequency-volume chart and the ICIQ-UI-SF before the urodynamic testing; this allowed a stratification of the population in four groups according with the symptoms: continent, with stress incontinence, urge incontinence, or mixed incontinence. The continent women complained of urgency or pain.

Stress incontinence is defined as involuntary leakage of urine following effort or exertion, including sneezing or coughing; urge incontinence is defined as the involuntary leakage accompanied by or immediately preceded by urgency, and mixed incontinence as the involuntary leakage associated with stress and urge conditions (12). A second stratification was made according to age: less than 45 years old (before menopause), 45 to 65 years old (menopausal transition) and above 65 years old (long term menopause). In France, the mean age for menopause is 50 years.

This study was conducted in accordance with the Declaration of Helsinki. According to the local practice of our Ethics Committee, there is no formal Institutional Review Board approval required for retrospective studies performed with the usual procedure of investigation.

Urodynamic session

A urodynamic session included the following tests: 1) free uroflowmetry at arrival; 2) UPP with empty bladder (supine position) before filling cystometry and pressure flow study (seating); 3) UPP bladder filled with 250 mL (or less, in cases of detrusor overactivity, at normal desire to void) with saline at room temperature (supine position except for standing recording) and 4) free uroflowmetry. Cystometry (filling rate 50 mL/min.) and UPP were performed using a 7F triple lumen water perfusion catheter. Pressures were zeroed to atmosphere with the transducers placed at the level of the upper edge of the pubic symphysis. All urodynamic studies were performed using a Dorado® unit from Laborie and conducted by two nurses who applied our protocol.

Urodynamic testing was performed according with Good Urodynamic Practices (13) and, during cystometry, women were asked to cough during filling at 100 mL intervals to ensure that pressure signals responded equally.

Urethral protocol: For UPP the puller speed was set to 1 mm/s except for standing recording when the catheter was pulled manually (14). The distal eye-hole was kept in the lateral position and the catheter position observed during the test to avoid change in orientation. The sequence of test was: (1) UPP at rest; (2) Kegel manoeuvre at MUCP; (3) UPP with 3 to 5 successive coughs (stress profile); (4) VLPP; (5) UPP before 10 successive strong coughs and (5') after (fatigability test), then (6) UPP was recorded in standing position.

Recordings

All MUCP values were reviewed independently by 2 investigators. Good agreement occurred in up to 88% of the files. In the remaining 12%, an additional interpretation was made jointly to reach a single conclusion.

Comparisons

MUCP value at rest (empty bladder) (P1) was compared with (P0), (P3), (P5), (P5') and (P6) according with the continence status in the whole population and in the age groups. The relevant MUCP value (P3) was the value obtained after ma-

nual smoothing of the stress profile in order to exclude the spikes due to cough efforts.

Statistical analysis

The numerical data are described by means and standard deviation and categorical data as percentages. The Wilcoxon signed rank test was used for comparison of related samples, analysis of variance and the Chi 2 test to compare unrelated samples. Statistical analysis was performed using SAS, version 5.0 (SAS Institute, Inc., Cary, NC). All statistical results were considered significant at $p < 0.05$.

Reproducibility

The Bland-Altman plot (15) (difference vs. mean plot) was used to show the agreement between the two measurements of MUCP during resting profile (empty bladder).

RESULTS

Demographic data showed that there was no significant difference in age and in percentage of menopause between the 4 symptoms groups; previous pelvic surgery was more frequent in the groups with urge or mixed incontinence (Table-1). There was no significant difference neither in subjective symptoms appraisal in incontinent groups nor in VLPP between stress and mixed incontinence groups (Table-1). The motive for referring was not significantly different in the 3 age-groups.

Analysis of all subjects (Table-2)

Whatever the continence status, there was a good reproducibility during a session of the resting MUCP (empty bladder) (P1 and P5) (t-test and Bland-Altman plots, Figure-1).

In incontinent women, MUCP with filled bladder (P1) decreased significantly when compared to MUCP with empty bladder (P0). MUCP of stress profile (P3), after fatigability test (P5') and standing (P6) were significantly lower than MUCP at rest in women with stress or mixed incontinence. We observed no difference between MUCP values during dynamic testing (P3, P5'and P6) and the resting value (P1) in women with urge incontinence.

In continent women, only two significant decreases of MUCP were observed: during the stress profile ($p = 0.0012$) and after the fatigability test ($p = 0.0005$).

Table 1 - Patients' characteristics. Data are given as mean ± standard deviation.

Group	Continent	Stress Inc.	Urge Inc.	Mixed Inc.
No women	22	34	40	44
Age	54 ± 15 y	55 ± 14 y	57 ± 15 y	59 ± 17 y
Parity	2.2 ± 1.5	1.6 ± 1.2	1.6 ± 1.3	1.9 ± 1.4
% menopausal	59	65	65	71
% previous pelvic surgery	33	44	61	55
VAS / ICIQ-UI SF	5 ± 3	11.2 ± 5.4	13.1 ± 3.8	13.0 ± 4.3
pves (cm H_2O) during Valsalva maneuver	74 ± 25	61 ± 21	70 ± 26	70 ± 25
VLPP (No patients) cm H_2O	85 (No = 1)	70 ± 23 (No = 17)	52 ± 10 (No = 5)	77 ± 27 (No = 17)

Abbreviation: Inc. Incontinence

Analysis per age-group (Tables 3, 4 and 5)

In all continence sub-groups, as in the whole population, there was no significant difference between the MUCP measurements (P1 and P5) at rest (empty bladder).

In the young group (Table-3), MUCP decreased significantly during the stress profile in the SUI sub-group (p = 0.0028) and after the fatigability test for women with SUI (p = 0.0277) or mixed incontinence (p = 0.0330) (that last type of incontinence implying a stress component). There was a good reproducibility of MUCP during all tests for both continent and urge incontinence subgroups.

In the middle age group (Table-4), changes in MUCP were significant with filled bladder (P1) vs. empty bladder (P0) except in the continent subgroup, during stress profile (P3) in all subgroups, after fatigability (P5') in the SUI and mixed incontinence subgroup, and only in the SUI group in standing position (P6).

In the oldest group (Table-5) with SUI, the change in MUCP was significant with filled bladder (P1) vs. empty bladder (P0) (p = 0.0374) and during the stress profile (P3) (p = 0.0103). Changes in MUCP were observed in all tests for the mixed incontinence subgroup but only with empty bladder (P0) (p = 0.0054) and standing (P6) (p = 0.0015) in the urge subgroup. In the continent subgroup, MUCP decreased only after fatigability test (P5').

Figure 1 - Assessment of measurements reproducibility. Bland and Altman plots (15) for MUCP value at rest (empty bladder) in supine position. (P1): first measurement bladder filled, (P5): before fatigability test. (a) continent women, (b) women with stress incontinence complaint, (c) women with mixed incontinence complaint, (d) women with urge incontinence complaint.

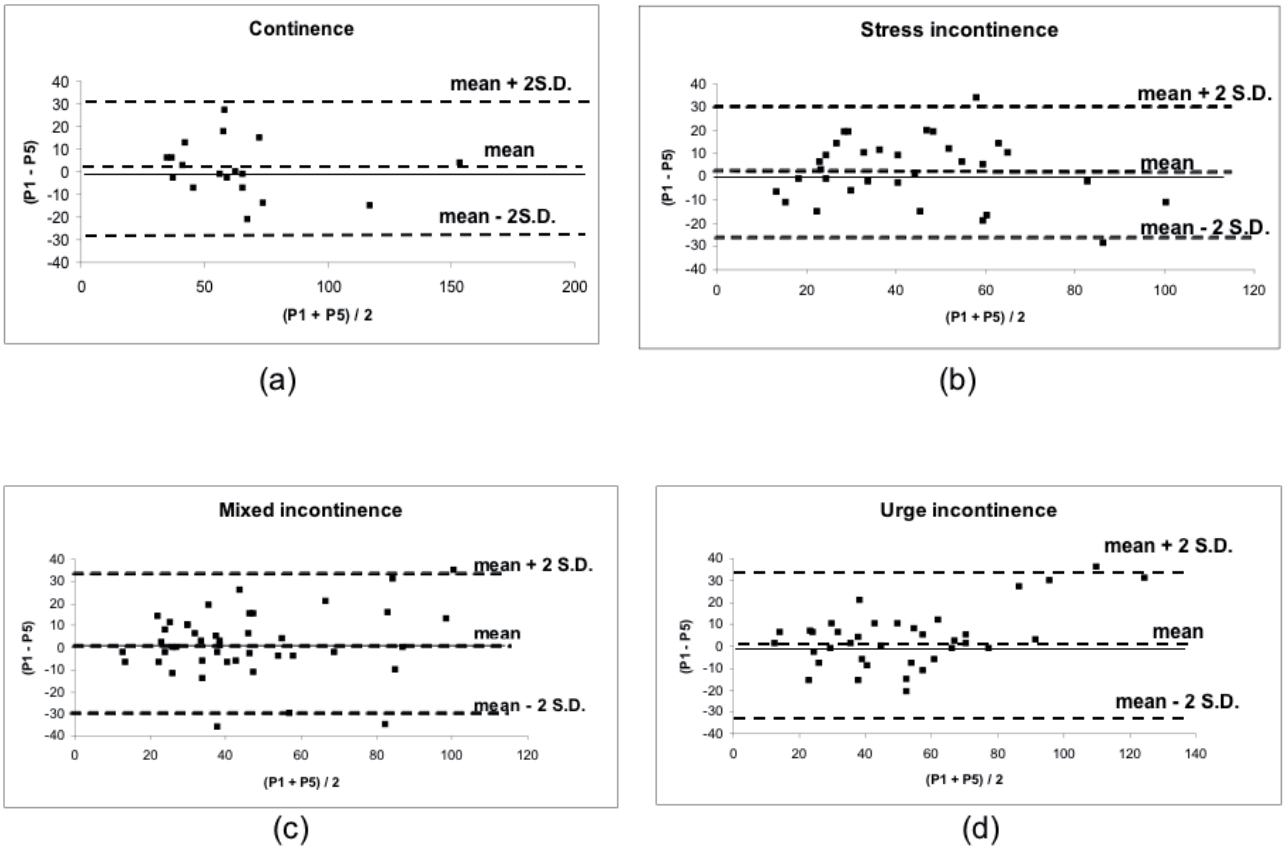

Table 2 - Maximum Urethral Closure Pressure (MUCP) value during Urethral Pressure Profilometry in the whole population. Comparison between the value at rest (bladder filled) (P1) and the value during other tests: P0 bladder empty, P3 during stress profile, P5 before 10 cough efforts, P5' after these cough efforts, and P6 in standing position. P0 to P5': supine position. MUCP values are given as mean ± standard deviation. In P1 value and MUCP values significantly different of P1.

	No	P1	P0	p	P3	p	P5	p	P5'	p	P6	p
Incontinent	118	50.1 ± 30.3	57.9 ± 27.0	< 0.0001	44.6 ± 31.0	< 0.0001	47.7 ± 27.6	n.s.	41.2 ± 24.3	< 0.0001	44.0 ± 28.2	< 0.0001
SUI	34	44.7 ± 21.2	54.1 ± 20.1	0.0001	38.0 ± 20.9	< 0.0001	41.2 ± 24.0	n.s.	34.8 ± 22.3	0.0014	37.0 ± 20.9	0.0019
Mixed Inc	44	46.5 ± 25.2	52.3 ± 24.0	0.0022	40.1 ± 24.8	< 0.0001	44.8 ± 22.8	n.s.	36.9 ± 19.7	< 0.0001	40.4 ± 24.4	0.0082
Urge Inc	40	57.7 ± 39.9	67.6 ± 32.4	0.0064	54.5 ± 41.3	n.s.	52.6 ± 32.0	n.s.	51.7 ± 28.4	n.s.	53.3 ± 35.2	n.s.
Continent	22	69.6 ± 30.5	76.3 ± 29.3	n.s.	62.1 ± 31.7	0.0012	59.1 ± 26.8	n.s.	48.3 ± 24.4	0.0005	69.7 ± 35.2	n.s.

Abbreviations: MUCP, Maximum Urethral Closure Pressure; SUI, stress urinary incontinence; Inc,incontinence; n.s., non significant.

Table 3 - Maximum Urethral Closure Pressure (MUCP) value during Urethral Pressure Profilometry in young age group (< 45 years). Comparison between the value at rest (bladder filled) (P1) and the value during other tests: P0 bladder empty, P3 during stress profile, P5 before 10 cough efforts, P5' after these cough efforts, and P6 in standing position. P0 to P5': supine position. MUCP values are given as mean ± standard deviation. In P1 value and MUCP values significantly different of P1.

< 45 years	No	P1	P0	p	P3	p	P5	p	P5'	p	P6	p
SUI	7	57.9 ± 25.2	63.4 ± 17.5	n.s.	51.4 ± 27.5	00280	57.3 ± 31.0	n.s.	46.0 ± 29.5	00277	53.3 ± 32.2	n.s.
Mixed Inc	9	77.9 ± 30.3	75.8 ± 28.7	n.s.	71.7 ± 26.5	n.s.	69.9 ± 25.9	n.s.	56.8 ± 18.9	00330	64.7 ± 28.2	n.s.
Urge Inc	10	96.9 ± 55.2	96.4 ± 43.9	n.s.	95.5 ± 60.5	n.s.	89.9 ± 44.3	n.s.	73.6 ± 35.1	n.s.	81.7 ± 52.6	n.s.
Continent	8	92.9 ± 37.4	98.1 ± 34.4	n.s.	87.6 ± 34.4	n.s.	84.8 ± 42.8	n.s.	78.8 ± 32.8	n.s.	96.0 ± 39.7	n.s.

Abbreviations: MUCP, Maximum Urethral Closure Pressure; SUI, stress urinary incontinence; Inc,incontinence; n.s., non significant.

Table 4 - Maximum Urethral Closure Pressure (MUCP) value during Urethral Pressure Profilometry in middle-age age group (45-65 years). Comparison between the value at rest (bladder filled) (P1) and the value during other tests: P0 bladder empty, P3 during stress profile, P5 before 10 cough efforts, P5' after these cough efforts, and P6 in standing position. P0 to P5': supine position. MUCP values are given as mean ± standard deviation. In P1 value and MUCP values significantly different of P1.

45-65 years	No	P1	P0	p	P3	p	P5	p	P5'	p	P6	p
SUI	17	48.5 ± 18.2	59.8 ± 21.2	0.0018	40.0 ± 17.7	0.0064	43.8 ± 21.8	n.s.	34.9 ± 21.3	0.0438	38.2 ± 14.2	0.0065
Mixed Inc	19	44.6 ± 16.4	55.5 ± 17.8	0.0013	38.8 ± 16.5	0.0023	45.7 ± 18.7	n.s.	37.7 ± 18.8	0.0038	43.5 ± 19.4	n.s.
Urge Inc	17	50.2 ± 24.5	62.0 ± 20.5	0.0031	45.5 ± 18.4	0.0437	47.3 ± 17.4	n.s.	48.9 ± 24.0	n.s.	52.6 ± 21.6	n.s.
Continent	7	58.9 ± 13.0	67.1 ± 16.1	n.s.	44.3 ± 17.2	0.0431	58.0 ± 17.8	n.s.	55.5 ± 22.7	n.s.	55.2 ± 17.6	n.s.

Abbreviations: MUCP, Maximum Urethral Closure Pressure; SUI, stress urinary incontinence; Inc,incontinence; n.s., non significant.

Table 5 - Maximum Urethral Closure Pressure (MUCP) value during Urethral Pressure Profilometry in oldest age group (> 65 years). Comparison between the value at rest (bladder filled) (P1) and the value during other tests: P0 bladder empty, P3 during stress profile, P5 before 10 cough efforts, P5' after these cough efforts, and P6 in standing position. P0 to P5': supine position. MUCP values are given as mean ± standard deviation. In P1 value and MUCP values significantly different of P1.

> 65 years	No	P1	P0	p	P3	p	P5	p	P5'	p	P6	p
SUI	10	29.0 ± 14.2	37.8 ± 8.0	0.0374	22.5 ± 13.5	0.0103	24.0 ± 6.7	n.s.	24.8 ± 7.2	n.s.	23.3 ± 9.6	n.s.
Mixed Inc	16	31.0 ± 12.7	35.4 ± 13.6	0.0015	23.9 ± 13.2	0.0009	29.5 ± 9.0	n.s.	24.8 ± 10.3	0.0045	23.0 ± 11.8	0.0004
Urge Inc	13	37.4 ± 16.3	52.8 ± 20.8	0.0054	33.0 ± 15.4	n.s.	36.8 ± 16.8	n.s.	32.5 ± 14.7	n.s.	32.4 ± 13.5	0.0015
Continent	7	50.4 ± 10.0	56.3 ± 12.8	n.s.	43.3 ± 13.0	n.s.	49.4 ± 14.1	n.s.	34.9 ± 14.5	0.0277	47.0 ± 6.5	n.s.

Abbreviations: MUCP, Maximum Urethral Closure Pressure; SUI, stress urinary incontinence; Inc,incontinence; n.s., non significant.

DISCUSSION

MUCP is a specific aspect of UPP. The main criticism addressed to it is that it is not an absolute measure of the urethral pressure, since it is an evaluation of the sphincter at rest, that does not provide information about the bladder neck or proximal urethra, that can be variable due to involuntary muscular contractions (irritant effect of the catheter), that the pressure varies with size and type of catheter, rate of perfusion, bladder volume and patient position (4), and that there is a wide variation in MUCP among individuals of similar age (16).

In relation to the causes of urinary incontinence in women, we must underline two urethral components: urethral hypermobility and intrinsic sphincter deficiency. It has been demonstrated that women with MUCP in the lowest quartile are likely to experience failure of mid urethral sling surgery (17) and that MUCP $\leq$ 40 cm H_20 is a risk factor affecting cure after transobturator tape procedure (5).

The conclusion of the ICS standardisation report on urethral pressure measurement (18) is that "the clinical utility of urethral pressure measurement is unclear" and that "there is no doubt that the urethral pressure is of significant importance for the continence mechanism".

Our study searched for the reproducibility of the MUCP value at rest, after filling the bladder, during a UPP session and tried to assess if different static and dynamic tests during that UPP session were correlated with the patient complaint.

During the UPP session, there was no significant change of MUCP value with filled bladder (P1 and P5) whatever the age and the complaint. That first result allowed us to use a complex UPP session and to search for a correlation between the change in MUCP value and the symptoms evoked before urodynamics.

Comparisons of MUCP value between full and empty bladder are still controversial: "some studies report lower mean MUCP with a full bladder, but others show no difference or even an increase in MUCP with bladder filling" (4). Most of these studies are limited to a comparison between "normal" and stress incontinent women without reference to the menopause status. In our population, MUCP value with empty bladder (P0) was almost always higher than MUCP with filled bladder (P1). However, the difference was only significant in incontinent women and, looking at age-groups, only in middle age and oldest subgroups. That variation can be explained by an impairment, occurring early with ageing, of both the sphincteric unit and the support system in women with stress or mixed incontinence; in women with urge incontinence that decrease can be the consequence of a voiding-like reflex mediated by changes in intercellular electrical coupling and localized contractions of smooth muscle due to the stretching of small portions of the bladder wall (19-20).

When we look at the specific condition of stress incontinence, resting MUCP value is known to tend to be lower than in continent women and decreases as a function of age (11-16), but there is no absolute cut-off value with sufficient sensitivity and specificity for that diagnosis and the association between MUCP and the severity of incontinence (1,21,22). In our population, MUCP values at rest (P1) tend to be lower in stress incontinent women except in the middle age group where women with mixed incontinence (which however implies a stress component) have lower MUCP.

When analyzing the results of the dynamic tests, we notice some differences. In standing position, looking at the whole population, a significant decrease of MUCP (P6) was only observed in women with stress or mixed incontinence which could be related to a deficiency of both intrinsic sphincter and peri-urethral muscles. In relation to the age groups, some differences appeared: the MUCP decreased only in women with SUI in the middle age and in women with mixed or urge incontinence in the oldest age. The previous assumption remains valid while the unexpected result for the oldest SUI group could be the consequence of a very low resting MUCP value. MUCP value remained unchanged in the continent group; that last result differs from the conclusion of Henriksson et al. (23) and Dörflinger et al. (24) but agrees with the conclusion of Lose (25).

In the whole population, MUCP decreased during the stress profile (P3) except in women with urge incontinence. In relation to age groups,

that decrease occured always in SUI women, in all women in the middle age group and in women with stress or mixed incontinence in the oldest group. The decrease of MUCP during stress profile (P3) could be related to a urethral hypermobility as it has been demonstrated by Schick et al. (26). The difference between the middle age and the oldest group could be the consequence of a more rigid urethra due to oestrogen deficiency or pelvic surgery or both.

In the whole population, a MUCP decrease after the fatigability test (P5') was observed in the same groups as during stress profile (all except urge incontinence). In young and middle-age groups, a significant MUCP decrease was observed in women with SUI or mixed incontinence (stress component). In the oldest group, the non significant decrease of MUCP resulted from the lower resting value of MUCP as for standing position. The decrease of MUCP following repeated cough efforts (fatigability test) has been described in women with stress urinary incontinence (14) with the hypothesis of an increased fatigue of the peri-urethral muscles. In the whole population, we finded a similar decrease not only in women with stress incontinence but also in women with mixed incontinence due to the stress component.

Thus, this study showed that there was a good reproducibility of MUCP value at rest when a rigorous protocol of testing was applied. What can we conclude about a correlation between the patient complaints and the MUCP value change? In a first approach (i.e. the whole population) decrease of MUCP during dynamic testing was related to stress or mixed incontinence. Interestingly, that results must be cautiously analysed according to the age groups. In the youngest group (non menopausal women), the sequence of tests revealed the stress component of incontinence. In the middle-age group (peri-menopausal women) the sphincter behaviour was modified by the hormonal status and the fatigability test appeared to be the only dynamic test allowing to discriminate urge from stress. In the oldest group (long-term menopause), the low resting value of MUCP seemed to conceal most of the results of dynamic testing.

At last, a negative result (no MUCP decrease) during the fatigability test was observed whatever the age of women with urge incontinence complaint.

Criticism to our protocol could be that it is time consuming or that it can be exhausting. But in fact, our protocol only takes about 10 min. and women do not complain of tiredness.

Limitations of this first study include the fact that this is a retrospective study and the stratifications of the population according with age (rough hormonal status) and symptoms (continence status) were not accurate; it would be completed by the approach of MUCP value changes according with the urodynamic diagnosis.

CONCLUSIONS

When a strict protocol is applied, MUCP at rest has a good reproducibility during a urethral profilometry session. Bladder filling induces a decrease in MUCP value in incontinent women whatever the type of incontinence in middle age and old age.

However, if a complex sequence of tests during urethral pressure profilometry remains discussed in peri- and long-term menopause women, it allows specifying the stress component of incontinence in non menopausal women and the urgency component in all incontinent women.

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CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Prevalence and risk factors for nocturia in middle-aged and elderly people from public health centers in Taiwan

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ABSTRACT

Purpose: To assess the occurrence and the associated risk factors for nocturia among middle-aged and elderly people from public health centers in southern Taiwan.

Materials and Methods: Data were part of our previous cross-sectional study which used a self-administered questionnaire for the assessment of lower urinary tract symptoms. A total of 1011 responders who were at least 40 years of age were enrolled from any of four local public health centers for any reason in Pingtung County, Taiwan. Nocturia, as a dependent variable, was defined as two or more episodes per night. Covariables included age, gender, and chronic illnesses (obesity, hypertension, diabetes, cardiovascular disease, and stroke). Multivariate logistic regression was applied to determine the risk factors associated with nocturia. A p-value of less than 0.05 was considered statistically significant.

Results: About 38.1% (385/1011) of the participants reported having nocturia ≥ 2 episodes/night, and the occurrence of nocturia increased with advanced age. More than half (51.2%, 197/385) participants with nocturia perceived at least "a bit of a problem" on the sleep quality. The multivariate logistical regression showed that the independent risk factors for nocturia were age (OR:1.06, CI:1.05-1.08), hypertension (OR:1.58, CI:1.16-2.16) and diabetes (OR:1.59, CI:1.03-2.45) and obesity (OR:1.47, CI:1.02-2.10), while a borderline effect on nocturia was produced by cardiovascular disease (OR:1.66, CI: 0.98-2.79) and stroke (OR:2.75, CI:0.88-8.64).

Conclusions: Several chronic illnesses coexisted with nocturia. Health care providers need to be aware of an increased risk of nocturia among people with certain chronic illnesses, and provide appropriate health care.

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INTRODUCTION

In 2002, the International Continence Society (ICS) proposed the first standardization of nocturia terminology. Since then, nocturia has begun to be recognized as an entire clinical concern, instead of just a lower urinary tract symptom. Based on the ICS definition, nocturia is defined as an individual needing to wake from sleep to void (1). Nocturia can cause sleep disturbances, lead to poor physical and mental health (2,3), and even increase the mortality risk (4).

Nocturia is age-related and both genders have a similar prevalence (5-7). In previous epidemiologic surveys, the prevalence of nocturia (≥

2 episodes per night) showed great variation, from 10% (5) to 30% (6,7), depending upon the study population and methodology. Despite the variations, the reports revealed that nocturia is a non-specific, but common urinary symptom.

People who suffer from nocturia are usually older and may also have other chronic illnesses. Clinical observations have indicated that some chronic illnesses are associated with nocturia. The Tikkinen et al. study reported that being overweight or obese, having coronary artery disease or diabetes in women and obesity in men, were significantly associated with nocturia in Finland (8). The Gourova et al. study revealed that cardiovascular disease, hypertension, diabetes mellitus/insipidus, and cerebrovascular disease were independent risk factors for nocturia in men aged 55-75 years in the Netherlands (9). The BACH study showed that nocturia was significantly associated with an increasing body mass index, type II diabetes, cardiac disease, and diuretics use (7). However, in a community-dwelling elderly population study conducted in Sweden, there was no correlation between nocturia and a known and treated hypertension, angina pectoris, congestive heart failure or diabetes mellitus (10). The similar finding was also noted by a Dutch study that nocturia was not associated with cardiovascular symptoms, hypertension or diabetes mellitus in men (11). Furthermore, base on frequency-volume chart data, Doorn et al. also found different results that nocturia did not necessarily related to increased mortality risk (12). Thus, some discrepancy exists among previous reports.

From the viewpoints of public health and disease prevention, it is important to identify the risk factors of nocturia so that effective prevention or treatments can be given when needed. In addition, to our knowledge, most published literatures were conducted in the western world (7-11). The data from Asia are relatively limited. Thus, we investigated the prevalence and risk factors of nocturia in middle-aged and elderly people in Taiwan.

MATERIALS AND METHODS

Design and sample

The data were part of our previous cross-sectional study which used a self-administered questionnaire to assess lower urinary tract symptoms (13). For this study, based on a convenient sampling, adults who were 40 or more years of age and had visited any of the local four public health centers for a physical checkup in Pingtung County, Taiwan, were enrolled from March to July of 2010. The inclusion criteria were voluntary participation and being free of severe disabilities related to hearing, seeing, speaking, and walking. People who complained of active urinary tract infections or micturition pain were excluded.

Of the 1190 subjects who were contacted, 135 subjects who did not complete the survey, and 44 subjects who complained of active urinary tract infection or micturition pain were excluded. As a result, 1011 participants (539 women and 472 men) were included in this analysis. The sample was designed to yield results within ± 3% error with 95% confidence level.

Measurement

As an outcome measure, the measure of nocturia was based on the nocturia item in the International Prostate Symptom Score. Since two episodes of nocturia constitute meaningful nocturia affecting well-being and perceived health (14), we defined nocturia as two or more episodes per night. Covariables included age, gender, and chronic illnesses. Chronic illnesses were assessed by asking the participants whether their doctors had told them about the following diseases: hypertension, diabetes, heart disease (congestive heart failure, myocardial infarction, angina, coronary artery bypass, or angioplasty), and stroke. The body weight and height were measured after interview and obesity was recorded if participants had a body mass index of > 27 kg/m^2. In addition, each participant was asked "How much of a problem does nocturia pose to your quality of sleep?" Their responses were (based on "not at all", "a bit of a problem", "quite a problem", and "a serious problem") were also documented.

The study was approved by the Institutional Review Board of a local university in Pingtung, Taiwan, and written informed consent was obtained from all participants. During the interviews, if any participant felt uncomfortable and wanted to discontinue the interview, he or she had the right to do so.

Data analysis

Continuous variables are presented as the mean ± standard deviation (SD), while categorized variables are presented as number ± percentage (%) and were compared using the chi-squared test. We used multivariable logistic regression analysis controlling age, gender, and all chronic illnesses (obesity, hypertension, diabetes, heart disease, and stroke) to explore the significance of the factors associated with nocturia of ≥ 2 episodes/night, and calculated the odds ratio (OR) and 95% confidence interval (95% CI). The Hosmer-Lemeshow χ^2 test was used to test the logistic regression model fit. Statistical analyses were performed using SPSS for Windows (version 17.0; SPSS Inc, Chicago, IL, USA). A p--value of less than 0.05 was considered statistically significant.

RESULTS

The mean age of the 1011 participants (539 women and 472 men) was 61.5 ± 12.0 (medium 61, range 40-93). The prevalence of nocturia (≥ 2 episodes/night) according to the distribution of age and gender is shown in Table-1. Overall, 38.1% (n = 385) of the individuals answered that they arose for urination during the night at least twice. Excepting the sixth decade (47.4% vs. 33.6%, $\chi^2 = 4.76$, p = 0.029), both genders had similar occurrence of nocturia on other age groups. Facing substantial participants with nocturia, about half (51.2%, 197/385) of them perceived at least "a bit of a problem" with their sleep being impaired by nocturia (Table-2).

Table-3 reveals the prevalence of nocturia (≥ 2 episodes/night) in the potential factors and the multivariate logistic analysis for the risk factors of nocturia. The highest percentage of nocturia occurred in participants with stroke (81.0%), followed by diabetes (60.7%), and cardiovascular disease (60.1%). The multivariate logistic regression showed that nocturia was independently associated with increased age (OR 1.06, 95 % CI: 1.05-1.08), hypertension (OR 1.58, 95 % CI: 1.16-2.16), diabetes (OR 1.59, 95 % CI: 1.03-2.45), and obesity (OR 1.47, 95 % CI 1.02-2.10), while cardiovascular disease (OR 1.66, 95 % CI: 0.98-2.79) and stroke (OR 2.75, 95 % CI: 0.88-8.64) had borderline effects on nocturia.

DISCUSSION

Our findings revealed that in the studied sample with a mean age of 60 years, approximately 38% of participantants had nocturia twice or more per night, and half (51.2%, 197/385) of them perceived at least "a bit of a problem" with their sleeping quality. Facing the fact that the percentage of the population who are reaching middle or old age is rapidly rising in Taiwan, health care providers should be more cognizant of nocturia.

In addition to age, which had a strong association with nocturia in this study, several

Table 1 - Prevalence of nocturia (≥ 2 episodes/night) by age and gender.

Age	Both genders		Women		Men		p-value
	N	(%)	N	(%)	N	(%)	
40-49	28/197	(14.2)	18/119	(15.1)	10/78	(12.8)	0.650
50-59	74/273	(27.1)	42/147	(28.6)	32/126	(25.4)	0.556
60-69	97/242	(40.1)	43/128	(33.6)	54/114	(47.4)	0.029*
≥ 70	186/299	(62.2)	90/145	(62.1)	96/154	(62.3)	0.962
Total	385/1011	(38.1)	193/539	(35.8)	192/472	(40.7)	0.112

Chi-squared tests were used.

*indicated p < 0.05.

Table 2 - The impact on sleeping between participants with and without nocturia (≥ 2 episodes/night).

	Total	Non-nocturia (< 2 episodes/night)		Nocturia (≥ 2 episodes/night)		p-value
		n = 626		n = 385		
Sleeping quality		N	(%)	N	(%)	0.000*
Not a problem	702	514	(82.1)	188	(48.8)	
A bit of a problem	231	100	(16.0)	131	(34.0)	
Quite a problem	58	11	(1.8)	47	(12.2)	
A serious problem	20	1	(0.2)	19	(4.9)	

Chi-squared test was used.
*indicated $p < 0.05$.

Table 3 - Prevalence of nocturia (≥ 2 episodes/night) in potential factors and the analysis for the risk factors of nocturia.

	With nocturia (%)		OR	(95 % CI)	p-value
Age			1.06	(1.05-1.08)	0.000*
Gender (reference: female)					
Women (n = 539)	193	(35.8)	1		
Men (n = 472)	192	(40.7)	1.13	(0.85-1.49)	0.399
Hypertension (n = 304)	167	(54.9)	1.58	(1.16-2.16)	0.004*
Diabetes (n = 117)	71	(60.7)	1.59	(1.03-2.45)	0.035*
Cardiovascular disease † (n = 75)	45	(60.0)	1.66	(0.98-2.79)	0.059
Stroke history (n = 21)	17	(81.0)	2.75	(0.88-8.64)	0.083
Obesity (n = 180)	86	(47.8)	1.47	(1.02-2.10)	0.038*

Multivariate logistical regression was used and adjusted for all variables mentioned above.
† Includes congestive heart failure, myocardial infarction, angina, coronary artery bypass, or angioplasty.
*Statistically significant.

chronic illnesses were significantly associated with nocturia (≥ 2 episodes/night), which implied that the nocturia may be multi-factorial in origin. Hypertension was significantly associated with nocturia, similar to the findings of some previous studies (6,9,15), but not others (10,11). Based on nocturnal polyuria, an important pathogenetic factor characterized by natriureses, McKeigue and Reynard suggested that hypertension caused by increased urolitidin secretion can depress the circadian rhythm of sodium secretion and lead to nocturnal polyuria (16). However, the mechanisms between them are not completely clear.

Diabetes mellitus was another independent predictor for nocturia. The result is congruent with numerous previous studies (6-9,15,17,18),

but a few studies have disagreed (10,11). The osmotic diuresis and global polyuria induced by hyperglycemia in diabetes patients has been demonstrated as the major cause of urinary storage symptoms (19). Thus, it is logical to hypothesize that the association between the effects of hyperglycemia may, at least in part, affect the occurrence of nocturia. In addition, the lack of arginine vasopressin (AVP) secretion and renal AVP resistance induced by diabetes may play a crucial role (20,21).

People with obesity (BMI of > 27 kg/m^2) had higher odds for suffering from nocturia, as compared to those without obesity, which was consistent with numerous epidemiological studies (5,7,8,15,17). The exact mechanism by which obesity causes nocturia is not known. There is a relationship between sleep apnea and nocturia (22-24), and both are associated with obesity. Obesity can induce both sleep apnea and hypoxia, as well as possibly increasing atrial natriuretic peptide (ANP). Thus, the mechanism of natriuresis among obeses people with sleep apnea and hypoxia should not be overlooked (25) and may account for these associations.

Heart disease had borderline significance with nocturia, which may be resulted from the relatively small number of people with heart disease (n = 75). Some earlier epidemiologic studies reported significant associations of heart disease with nocturia (7-9), while others did not (6,10,11,17). A recent study conducted by Chiu et al. demonstrated that patients with chronic heart failure suffer from three or more episodes of nocturia as compared to the controls (26). Possible reasons such as postural diuresis at night, nocturnal polyuria as a result of an increase in the atrial natriuretic peptide (ANP), or wrong timing of diuretics therapy (particularly loop diuretics) (25) may account for the association.

Only 25 subjects reported to have had stroke and there was also a borderline effects of stroke (n = 25) with nocturia. Two studies rejected the association of nocturia with stroke (6,17). However, a previous study conducted by Asplund demonstrated that stroke was significantly associated with nocturia of ≥ 3 voids (18). Also, Brittain et al. reported that nocturia was the most common urinary symptom, and that stroke survivors had a higher prevalence of nocturia than the nonstroke population (49% vs. 19%, respectively) (27). The associations of stroke with nocturia may be partially explained by the lack of the normal nocturnal rise of plasma AVP in the post-stroke patient (28) and the uninhibited detrusor contractions secondary to the neurogenic defect. However, the actual mechanisms between them are not completely clear.

The causes for nocturia were previously classified as diurnal polyuria, nocturnal polyuria, low functional bladder capacity, or a combination of these, and the plasma AVP and ANP may be crucial humoral factors (21,29). In our study, nocturia was significantly associated with several illnesses, and some plausible mechanisms or hypotheses are proposed to explain these relationships. Besides, psychogenic nocturia, sleep apnea, caffeine intake, alcohol intake, bladder outlet obstruction and detrusor overactivity have been considered as possible etologies for nocturia. Additional studies regarding these aspects in the future may also be needed.

There are some limitations in the present study. First, this analysis was based on participants' reports rather than voiding diary or the measure of functional changes of the bladder. Recently in the Krimpen study, Doorn et al. also showed that the discrepancy during assessing nocturia was significant between questionaire and voiding diary (30). Thus, the recall bias must be considered. Second, all participants were recruited from public health centers, not from hospitals. Most participants could not provide the exact drug names. Thus, the effect of medications (eg, diuretics) on nocturia could not be understood in our study. Third, our study enrolled participants from public health centers, where the patients studied tended to be either elderly or patients with chronic illnesses. Consequently, the prevalence of 38.1% for nocturia may be higher than the epidemiologic rate of nocturia (5-7). We focused here on the association between nocturia and some specific chronic illnesses, rather than the truly epidemiologic rate. Overall, despite these limitations, the findings of this study suggest relevance to clinical practice and directions for further research.

CONCLUSIONS

In our study, nocturia was significantly associated with several illnesses, and some plausible mechanisms or hypotheses are proposed to explain these relationships. When treating people with nocturia, health care providers need to be aware of the possible non-urogenital reasons for the nocturia, and provide appropriate health care.

CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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The buccal mucosa fenestrated graft for Bracka first stage urethroplasty: experimental study in rabbits

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ABSTRACT

Objective: To histologically evaluate, in an experimental study in rabbits, the integration process of the buccal mucosa fenestrated graft applied in the corpora cavernosa for Bracka first stage urethroplasty.

Materials and Methods: A urethral defect was surgically created in 16 male rabbits of the New Zealand breed through the excision of the penile urethra. The urethral defect was corrected by applying buccal mucosa fenestrated graft through two cruciform incisions in the distal portions of its longitudinal axis. The animals were sacrificed at 2, 4, 8 and 12 weeks post surgery and their genitals were subjected to clinical and histological assessment.

Results: The buccal mucosa fenestrated graft showed complete uptake in all groups, with keratinization squamous metaplasia and mucosal proliferation of the fenestrated areas. The fenestrated graft area represented an increase in length of 25% in length in relation to the original standard graft.

Conclusions: The fenestrated buccal mucosa graft presented total integration to the adjacent epithelia with re-epithelization of the incision areas of the graft (fenestrations) and no significant inflammatory or scarring reactions when compared to other mucosa transplanted areas; therefore its application is viable in cases of extensive urethral defect whenever the donating area might be insufficient.

ARTICLE INFO

Key words:
Hypospadias; Surgery; Mouth Mucosa, Transplantation; Urethra; Models, Animal

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INTRODUCTION

Urethral reconstruction surgery has been challenging the skills and creativity of surgeons since antiquity. A wide variety of flaps and grafts have been used, particularly in procedures such as complex hypospadias and urethral stenosis (1). Currently, the vast majority of techniques for hypospadias repair is based on the preservation of the urethral plate, which when intact is well vascularized, has a good nervous supply and is sustained by strong muscle and connective tissue (2,3).

However, in as many as 10% of cases, maintaining the integrity of this urethral plate is practically impossible, requiring its removal for the straightening of the penis (4,5). Once the urethral plate is removed, most authors justify that surgery should be done in two steps (two-stages). Lately we have carried out clinical and experimental protocols in order to better understand the behavior and healing of the tissue regarding these complex reconstructions, and in this context, we have conducted experimental studies in animal model (6).

In patients with previous surgery or severe hypospadias, Bracka described a two-stage repair with a free graft of buccal mucosa, reaching a success rate of approximately 87% and accounting for only 5.7% of fistulas and 7% of stenosis (7). However, when regarding major defects, it is sometimes necessary to use many segments of buccal mucosa or the combination of buccal mucosa and skin to cover the entire surface and reconstruct the urethral plate (8). In these cases, the use of fenestrated grafting simulating mesh grafting, a technique well known in correcting major defects especially by using skin grafting in burn reconstructive plastic surgery, could be an interesting option to increase the area to be covered by grafting in situations where the availability of donor tissue is limited (9). Our proposal was to evaluate the histological integration process of fenestrated buccal mucosal grafting in the corpora cavernosa as the first stage of urethral reconstruction in an experimental model in rabbits.

MATERIALS AND METHODS

After approval by the Research Ethics Committee of the Universidade Federal de São Paulo - Hospital São Paulo, the study included a total of 16 New Zealand male rabbits, aged approximately 8 weeks and weighting 2.0 to 2.5 kg. Before the surgeries, the animals were acclimated in the Department of Experimental Surgery of the Universidade Federal de São Paulo for a period of adjustment of 72 to 96 hours.

The study was performed in 4 months, including the adjustment period, 12 weeks of the longest interval between intervention and sacrifice and finally the period of histological analysis.

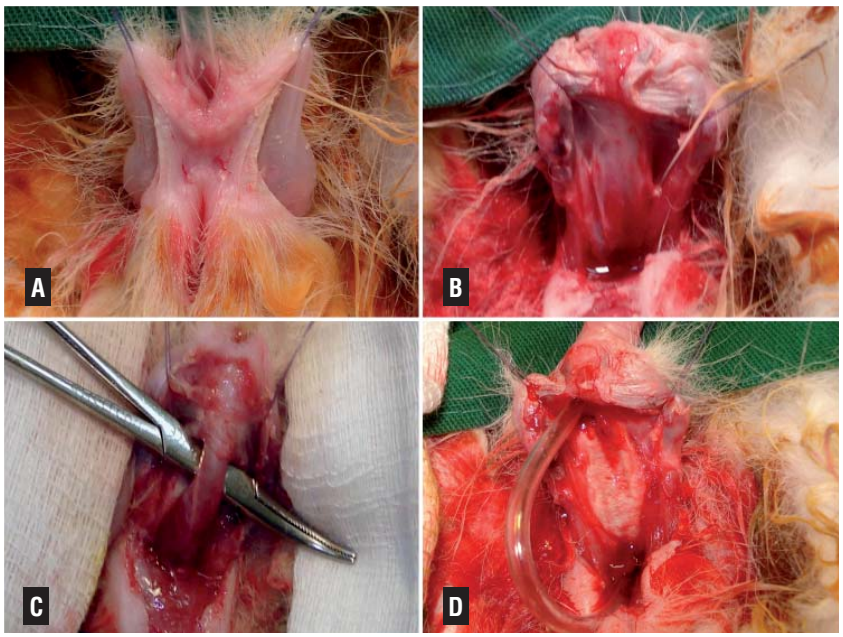
Anesthesia was initiated by the intramuscular administration of a preanesthetic agent (Midazolam - 5mg/kg) and hydration through a peripheral vein. Anesthesia was complemented with an intramuscular injection of an anesthetic solution containing ketamine hydrochloride 30 mg/kg + xylazine 5mg/kg and penile nerve block with bupivacaine 0.25% and lidocaine 1% without vasoconstriction (10,11). The surgical procedure was carried out under sterile conditions and the use of a magnifying glass of 3.5X.

First the urethra was catheterized with a urethral catheter of 8 Fr and the perineal fold between the penis and the anus of the animal was sectionalized, thus allowing access to the urethra (Figures 1A and 1B). Buck's fascia was incised at the junction of the corpus spongiosum and the corpus cavernosum on each side, thus allowing the structures to be isolated (Figure-1C). The corpus spongiosum and urethra were then exposed and completely sectioned with a scalpel, resulting in two stumps and defining a lengthy urethral defect, similar to a proximal hypospadia. Part of the distal urethral stump was excised to obtain complete exposure of the ventral surface of the corpus cavernosum (Figure-1D). To avoid a greater retraction or stenosis, the proximal urethral stump was fixed at the base of the penis and perineum with a 5.0 catgut® suture.

Next, buccal mucosa was extracted from the rabbit's jugal region. The donating site was exposed with interrupted stitches using 5.0 catgut® (Figure-2A). A centesimal solution of lidocaine at 1% with vasoconstrictor (adrenaline 1:200,000) was then injected locally into the submucosa (Figure-2B), thus facilitating the withdrawal of a buccal mucosa fragment 1.0 cm long and 0.4 cm wide (Figure-2C). After review of the hemostasis the donor region was not sutured, the wound healed by itself.

The buccal mucosa graft was harvested and fenestrated through two cruciform incisions created in distal portions of its longitudinal axis, approximately 1 mm from the superior and side edges. These incisions allowed for a macroscopical augmentation of 25% of the graft's longest strip, which went from 10 mm to 12.5 mm (Figures 2C and 2D). The fenestrated buccal mucosa graft was then brought to the urethral surgical area and applied onto the defect created in the ventral region of the penis with six interrupted stitches of polydioxanone suture (PDS® II) 6-0. The submucosa area of the graft was merged with the ventral surface of the exposed corpus cavernosum, thus constituting the neourethra plate. After the main part of urethral surgery, the penile fold was then partially rebuilt with separate stitches of 5.0 catgut® suture, while the patency of the urethrostomy was maintained without the need of a catheter.

Figure 1 – A) The rabbit's urethra catheterized with repairs exposing the fold between the phallus and the anus of the animal; B) Opening of the fold exposing the rabbit's urethra; C) Urethral spongiosum body isolated from the corpora cavernosa; D) After excision of the urethra it is observed the surface of the corpora cavernosa completely exposed.



Experimental animals were divided into 4 equal groups of 4 each and sacrificed 2, 4, 8 and 12 weeks after surgery, respectively. The genital of each animal was examined according to its ventral surface aspect. The penises were sectioned along the base, allowing the withdrawal of surgical parts that were immediately fixed in 10% formalin. These fixed segments were longitudinally sectioned, from the glans to the base of the penis, to allow for a full analysis of the two fenestrated areas in relation to the graft itself. It was compared the integration and histological changes that occurred during the different times of sacrifice. All blocks were cut to produce segments of 5 microns in thickness and stained with hematoxylin-eosin (HE) and Masson's trichrome. The animal group was not revealed to the pathologist for histological evaluation enabling an unbiased analysis.

The inflammatory response was classified as acute, when there was infiltration by polymorphonuclear cells, and chronic, with infiltration by mononuclear cells. We adopted a semi-quantitative assessment of the intensity of inflammation, graded on a 0 to 4 + scale, as follows: (0) no inflammatory infiltrate, (1+) minimal inflammation, (2+) moderate inflammation, (3+) severe inflammation and (4+) finding of aggregates of leukocytes / lymphocytes with formation of micro-abscesses. The degree of sub-epithelial fibrosis was also analyzed in a semi-quantitative scale, graded from 0 to 3+, as follows: (0) no scarring, (1+) minimal scarring, (2+) moderate scarring and (3+) severe fibrotic scar.

RESULTS

There were no deaths related to the surgical procedure. Moreover, no signs of local or systematic bacterial infection were verified. All animals were sacrificed on the pre-established dates. At the time of sacrifice and emasculation, the location of the graft was macroscopically identifiable, demonstrating its good integration and the fenestration areas were all fulfilled, being impossible to distinguish them from the buccal mucosa grafted (Figure-3).

Figure 2 – A) Exposition of the jugal mucosa with submucosal injection of anesthetic substance with vasoconstrictor; B) Fragment of mucosa that will be transformed into graft; C and D) Demonstrating the increased size of the graft after fenestration at the benchmarking of its length.

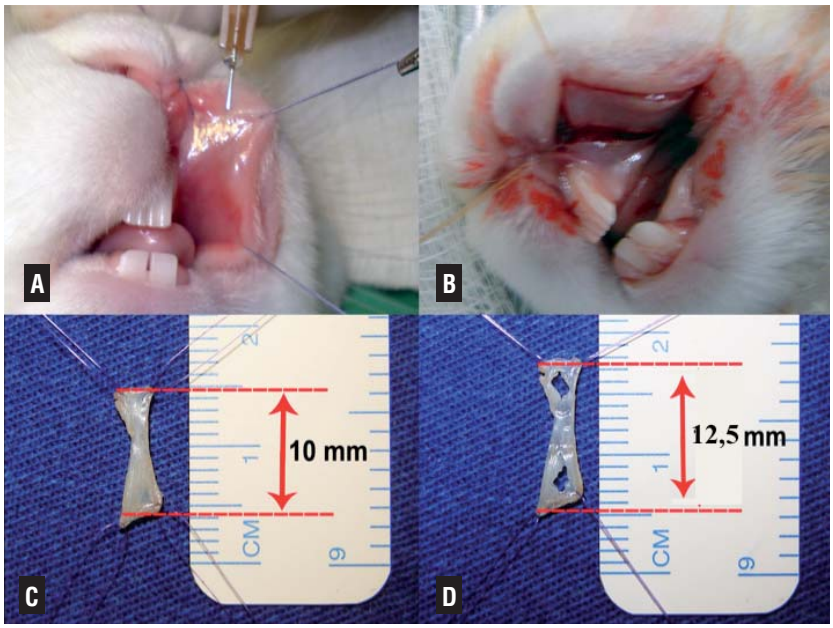
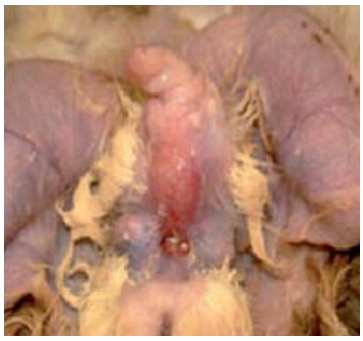


Figure 3 - Aspect of the ventral surface of the penis of the rabbit to be sacrificed after four weeks of surgical procedure. Notice the good integration of graft that is shown in red coloring.



After two weeks of surgery the histological assessment showed an intense inflammatory infiltrate with the presence of neutrophils and scarce eosinophils, featuring a recent inflammatory process. These signs of acute inflammation gradually reduced as the postoperative period increased and within 12 weeks we noticed a minimal inflammatory response with scarce lymphocytes (Figures 4A, 5A and 5B).

In relation to changes that occurred over time in the epithelial mucosa, we realized that after 2 weeks of surgery the mucosa had thickened, with keratinization of some areas, the presence of a few vessels, areas of urothelium epithelization and recent metaplasia, particularly in cuts where the fenestrated graft was found, from the incisions made in the mucosa. With time, this epithelium had become squamous keratinized and in the 8th week it was separated by small areas of squamous non-keratinized epithelium (Figure-4B). Within 12 weeks of post-operative care there were only small areas of immature squamous epithelium or in keratosis in probable areas of fenestration. It is important to stress the common histology of the buccal mucosa of rabbits, which consists of stratified epithelium with no attachments and no grainy layer, therefore, without any production of keratin.

The healing took place through the formation of a moderate sub-epithelial fibrosis, most

Figure 4 – A) Subgroup of 2 weeks. Mucosa and transition to younger epithelium. Moderate fibrosis (HE 40 x); B) Subgroup of 8 weeks. Metaplasia of the mucosa with moderate organized fibrosis (Masson 40 x).

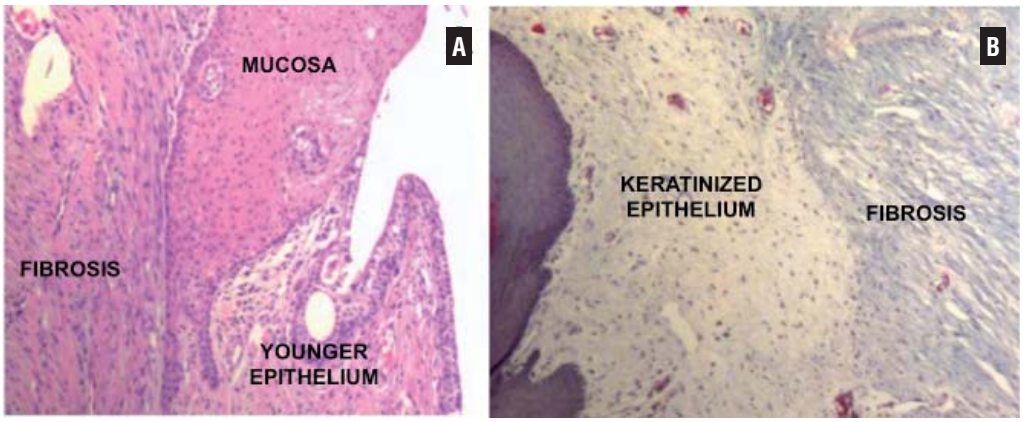
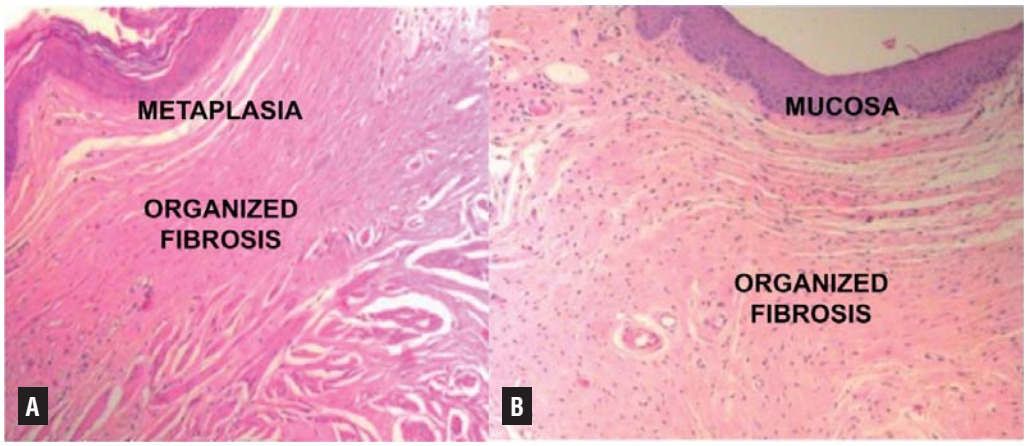


Figure 5 - A) Subgroup of 12 weeks. Squamous mucosal metaplasia with mature healing of the graft (HE 40 x); B) Subgroup of 12 weeks. Well-integrated mucosa without signs of inflammation and mature scarring of the graft (HE 40 x).



evident on the border of the grafts within two weeks of postoperative and becoming more noticeable in 4 weeks when we could perceive the emergence of young fibroblasts (rounded nuclear), and areas of mature collagen. Within 8 weeks the fibrosis remained moderate, however, with organized fibers and deposition of collagen more evident in some areas. Finally, within 12 weeks accentuated fibrosis was noticed with a disposition of collagen more evident on the borders of the grafts (Figures 5A and 5B). Resume of histological findings are shown in Table-1.

DISCUSSION

The application of grafts or autologous flaps in urethral surgery is technically more feasible with the use of certain perigenital tissues such as foreskin or tunica vaginalis. However, and especially in reoperations, these tissues may no longer be available, making the use of free grafts from other tissues necessary. In these applications, and especially in the correction of serious forms when it is necessary to excise the urethral plate, one of the most studied tissues is the buccal

Table 1 - Resume of histological findings. Inflammatory response: (0) no inflammatory infiltrate, (1+) minimal inflammation, (2+) moderate inflammation, (3+) severe inflammation and (4+) finding of aggregates leukocyte / lymphocyte with formation of microabscesses. Sub-epithelial fibrosis: (0) no scarring, (1+) minimal scarring, (2+) moderate scarring and (3+) severe fibrotic scar.

	2 Weeks	4 Weeks	8 Weeks	12 Weeks
Acuteness of the inflammatory response	2+/3+	2+	1+/2+	0 / 1+
Types of inflammatory cells	Neutrophils and rare Eosinophils	Lymphocytes	Eosinophils and Lymphocytes	Rare Lymphocytes
Level of Subepithelial Fibroses	2+/3+	2+/3+	2+	2+/3+
Modification of the area in 'Mesh'	Urotelial Epithelization and Young Metaplasia	Stratified Epithelium with Keratinization	Squamous Keratinized Epithelium and Squamous non-keratinized	Squamous Keratinized Epithelium and of the immature squamous type
Other Findings	Cysts of Inclusion with micro abscesses	Microfocos of Ulceration	Acanthosis on the margins of the graft	-

mucosa, whose usage has also provided superior results (12-14). Secrest has reviewed indications and techniques of staged urethroplasty and has made a historical survey concluding that the staged technique is the oldest form of urethral reconstruction still in use today (15). The author also credits the best current results of staged urethroplasty to the works of Venn et al and especially to the changes and results of Schreiter et al., who applied urethroplasty techniques by using mesh grafts prepared for staged procedures in great defects (16-18).

Bracka has published excellent results with the use of buccal mucosa for the staged treatment of great urethral defects, especially in reoperations (19,20). The author performed this procedure in 121 patients and obtained a success rate of almost 90% after the second period of surgery. Rosito et al. demonstrated in an experimental study that the tunica vaginalis graft is an alternative tissue for the confection of the neo urethral plate in the first surgical period of a staged urethroplasty, however, broader clinical studies and longer follow-ups are needed until the tunica vaginalis is to be considered an alternative of general use (21). Barroso et al. suggested that the buccal mucosa graft in Bracka's technique could be applied in "U" shape, thus increasing the lateral area of the graft (22-25). This shape allows a more feasible tubularization in the second period, presenting positive results in 10 children and only two complications after the second period of urethroplasty.

Based on the innovations and advantages of Schreiter's and Bracka's techniques, we decided to investigate histological changes that occur in an experimental model of staged correction of urethral defects, adding to the same procedure the innovative precepts postulated by the two authors. We also evaluated the integration of fenestrated buccal mucosa graft in the first stage of this surgical technique. The advantage of fenestrated graft is to cover a larger area of the urethral surface with the same donor area.

The rabbit was the chosen animal for being docile and easy to handle, and also for its penis size, which is compatible with that of a 12 month infant. Moreover, it is known that the embryological development of the phallus of the rabbit is similar to that of men, including the

formation of the foreskin (26). We also considered the fact that the healing process in rabbits has already been extensively studied and that this animal has often been used as a model in penile surgeries (27). No deaths occurred during the surgical procedure or the post-operative period. It is our belief that local anesthesia together with the extraction of the buccal mucosa patch decreased the need for general anesthetics, thus reducing the potential mortality inherent to the act of anesthesia.

Macroscopically, an augmentation of the buccal mucosa fenestrated graft was observed, confirming an increase in length of 25%. It was also observed that the grafts presented no important signs of macroscopic retraction in any of the sacrificed subgroups and that the buccal mucosa grafted area was flat and flawless.

Our histological results were consistent with the findings of previous studies, where the characteristically intense inflammatory reaction and infiltrated polymorphonuclears were gradually reduced into a discrete and chronic response, mainly of lymphocytes (6). These findings show a greater integration of the mucosa and are macroscopically noticeable at the moment of the animals' sacrifice. The histological response of the presence of fenestrated graft in our model was compared to the findings of Mokhless et al., who evaluated 31 patients who underwent the staged reconstruction proposed by Bracka through biopsies and histological analysis of the buccal mucosal grafts at the time of the second period of the reconstruction, six months after the grafting (28).

Mokhless' findings were similar for all patients, showing no statistical difference regarding their age or the specific area where the transplanted buccal mucosa originated. The authors found minor reaction modifications, mild acanthosis, mild epithelial hyperplasia and mild focal keratosis with a discreet lengthening of the lamina propria papillae. There was still a discreet infiltrated lymphocytic in the lamina propria papillae, and all grafts presented perfect vascularization, the same as the original buccal mucosa. The stratified squamous non-keratinized epithelium of our buccal mucosa graft presented aspects of immature squamous metaplasia, evident until the last sacrificed subgroup with 12 weeks of transplantation. The fenestrated areas did not alter the integration or vascularization of any of the grafts.

One of the major differences that occurred in our evaluation was the presence of non-keratinized tissue areas, separated by areas of keratosis and keratinized tissue, with a thinner appearance and with less edema, thus presenting a more organized tissue. This suggests that non-keratinized buccal mucosa undergoes squamous metaplasia, covering the surface of the corpus cavernosum including the fenestrated area, stimulating a non-keratinized mucosal proliferation. However, the reaction found does not seem to be definitive and can change even after the second period of a staged correction, as shown by Smith et al. (29).

Subepithelial fibrosis assessment did not show any important changes in the expected behavior of graft reaction, with the classic evolution of the scarring process and without fiber optic reactions that might occur in fenestrated areas, being this effect similar to the clinical outcome of Schreiter (30).

In summary, our findings have enabled us to define that a fenestrated graft has a behavior similar to a healthy graft, without the presence of macroscopic retraction or any evidence of greater macroscopic scarring process. Through histological examination the behavior of epithelium confirmed metaplasia and keratinization with no relevant difference between healthy and fenestrated areas.

CONCLUSIONS

Buccal mucosa fenestrated graft, dorsally applied onto the corpus cavernosum of a rabbit, has shown in this histological evaluation full integration to the adjacent epithelium with re-epithelization of the fenestrated areas of the graft, without presenting an inflammatory reaction or scarring significantly greater than in the different areas of the transplanted mucosa. The epithelium has undergone squamous metaplasia and began to present keratinization.

ABBREVIATIONS

HE = Hematoxylin-Eosin

CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Effect of Mesenchymal Stem Cells Associated to Matrixen on the Erectile Function in the Rat Model with Bilateral Cavernous Nerve Crushing Injury

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ABSTRACT

Objectives: To evaluate the effect of mesenchymal stem cells (MSCs) and MSCs mixed with Matrixen as a cell carrier on the erectile dysfunction caused by bilateral cavernous nerve crushing injury.

Materials and Methods: White male Sprague-Dawley rats were divided into 4 groups: sham-operated control group (n = 5), bilateral cavernous nerve crushing group (BCNC group, n = 10), BCNC administered with MSCs group (n = 10,1x106 in 20 µL), BCNC administered with Matrixen group (n = 10.1x106 in 20 µL), BCNC administered with MSCs/Matrixen group (n = 10.1x106 in 20 µL). After functional assessment at 4 weeks, major pelvic ganglion (MPG) and penile tissue were collected. Immunofluorescent staining of MPG was performed with PKH26 and Tuj1. Western blot analysis of endothelial nitric oxide synthase (eNOS) and neuronal nitric oxide synthase (nNOS) were done in corpus cavernosum.

Results: ICP/MAP ratios of BCNC with MSCs and MSCs/Matrixen groups were significantly increased compared with BCNC and BCNC with Matrixen group. Moreover, ICP/MAP ratios of MSCs/Matrixen group were significantly increased compared with BCNC with MSCs group. In MPG, the more implantation of MSCs and increased expression of nerve cells were observed in MSCs/Matrixen group compared with BCNC with MSCs group. Significant increase expression of eNOS and nNOS was also noted in BCNC with MSCs/Matrixen group.

Conclusion: The erectile function was more preserved in MSCs/Matrixen group compared with the administration of MSCs alone in the rats with bilateral cavernous nerve crushing injury. Therefore, we consider that the use of transplant cell carrier such as Matrixen may help the implantation of MSCs and improve the therapeutic effect of MSCs.

ARTICLE INFO

Key words:
Cavernous nerve; Erectile dysfunction; Matrixen; Mesenchymal stem cell

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INTRODUCTION

Diagnosis of prostate cancer has been elevating due to the increase in prostate-specific antigen (PSA) screening. In addition, this earlier diagnosis increases the number of patients presenting with clinically organ-confined disease and therefore, it has led to an increase of the number of candidates for radical prostatectomy (1,2). Radical prostatectomy has been associated with complications such as postoperative urinary incontinence and erectile dysfunction. Erectile dysfunction is a bothersome complaint among pa-

tients submitted to radical prostatectomy and it is an important reason for the decrease of the quality of life in these patients. Therefore, nerve-sparing procedures have been proposed to reduce short--term and long-term functional complications such as erectile dysfunction (3-5). However, some patients submitted to radical prostatectomy with nerve-sparing technique have been experiencing erectile dysfunction and need other treatments to improve their symptoms (6).

Mesenchymal derived stem cells (MSCs) from bone marrow seems to be one of the attractive treatments for tissue regeneration and engineering due to their differentiation potentials into various adult tissues, including endothelial cell, smooth muscle and nerve (7). Therefore, there have been several studies using MSCs and the treatment effect of MSCs has been studied in the animal model of erectile dysfunction caused by cavernous nerve injury. As a result, stem cell therapy may be one of the treatment options for the patients that lost their potency after radical prostatectomy. However, some difficulties appeared in stem cell therapy in spite of their therapeutic effect. For example, stem cells were easily spread-out to the adjacent tissue after stem cell injection into the target tissue according to the report by Lee et al. (8). This may cause unexpected, unfavorable events and moreover, we are not able to get anticipating results from the stem cell therapy, neither. To overcome this weakness, some investigators used polymers to promote the retention of stem cells in the targeted tissue (9-11).

Among various biomaterials, Matrixen is a collagen based biocompatible polymer and it has been used widely as scaffolds in medical field. In addition, Matrixen is easy to handle depending on the investigator's purpose due to its characteristics of thermo-dependant sol-to-gel transition. Therefore, we used Matrixen to enhance the implantation ability of MSCs and compared the effect of MSCs and MSCs mixed with Matrixen in rats with cavernous nerve crushing injury.

MATERIALS AND METHODS

Animals

White male Sprague-Dawley rats aged 8 weeks with weight distribution of 300-350 gm were purchased from Saemataco Inc. (Osan, Korea). Then, the rats were divided into 5 groups: the control group (n = 5), the bilateral cavernous nerve crushing group (BCNC group, n = 10), the administration of mesenchymal stem cells following the bilateral cavernous nerve crushing (BCNC with MSCs group, n = 10), the administration of Matrixen following the bilateral cavernous nerve crushing (BCNC with Matrixen group, n = 10), and the administration with MSCs mixed with Matrixen following the bilateral cavernous nerve crushing (BCNC with MSCs/Matrixen group, n = 10). The experimental protocol was approved by the Catholic University Animal Ethics Committee (CUMC-2009-0045-01).

Preparation of MSCs

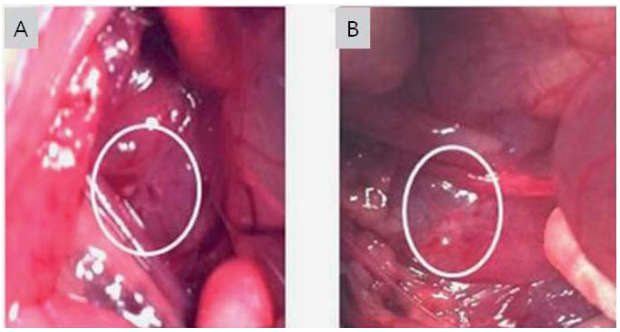
For preparation of rat bone marrow--derived MSCs (rBM-MSCs), bone marrow cells were collected from the femurs and tibias of 3-4 week-old Sprague-Dawley rats by flushing the respective tissues with Hank's balanced salt solution (HBSS, WelGENE, Korea) containing 2% fetal bovine serum (FBS; Hyclone, USA). After red blood cells were removed, bone marrow cells were filtered through a 40 μm cell strainer (BD Bioscience, San Jose, CA) and separated using Ficoll density gradient centrifugation. Isolated bone marrow cells were re-suspended and cultured in Dulbecco's Modified Eagle Medium (DMEM, 1000 mg/L glucose; WelGENE) with 20% FBS, 100 U/mL penicillin and 100 g/mL of streptomycin (Invitrogen, Carlsbad, CA) for about 10 days until colonies formed. Colonies were harvested and used for subsequent experiments as MSCs. For animal experiments, ex-vivo expanded MSCs (less than passage 5) were seeded into a 70 mm flask at an initial density of 1.4x106 cells and incubated overnight at 37 °C. Cells were then trypsinized, washed with PBS, and injected into the rats (1x106 cells/rat).

Bilateral cavernous nerve crushing and administration of MSCs or MSCs mixed with Matrixen

Tiletamine (Zoletil) 0.2 mL was injected intraperitoneally to anesthetize the animals. A lower midline incision was made and the prostate gland was exposed. After identifying the major pelvis ganglion (MPG) on the lateral side to bi-

lateral prostates, the cavernous nerves, tracking posterolaterally, were identified and isolated. In control group (sham surgery), there was no further surgical manipulation. In the remaining groups the cavernous nerves were isolated and a crush injury was induced using a hemostat clamp for 2 min. In the BCNC group, the abdomen was closed after the bilateral cavernous nerve crushing. In the BCNC with MSCs group, MSCs (1x106 in 20 µL) were administered to the MPG, Matrixen (20 µL) was administered to the MPG, and MSC (1x106 in 20 µL) mixed with Matrixen (Bioland, Cheongwon, Chunbuk, KOREA) administered to the MPG in BCNC with MSCs/Matrixen group after bilateral cavernous nerve crushing (Figure-1).

Figure 1 - The gross finding of MSCs plus collagen based cell carrier after immediate administration into MPG. A: MPG of normal rats, B: Gel formation of collagen based cell carrier contacting of MSCs after implantation, MPG: major pelvic ganglion, MSCs: mesenchymal stem cells.



Erectile functional assessment

The rats were anaesthetized with an intraperitoneal injection of 0.2 mL Tiletamine (Zoletil®). With the rat in the supine position, the penis was dissected and the corpus cavernosum and crus of the penis were exposed. A low, midline abdominal incision was made to access the pelvis, and the MPG lateral to the right prostate was exposed. For the measurement of the intracavernosal pressure (ICP), a heparinized 23G butterfly needle was inserted in the corpus cavernosum of penile proximal portion after penile skin was degloved and the corpus cavernosum identified. For the measurement of mean arterial pressure (MAP), a PE-50 tube was inserted into the carotid artery. Then a bipolar electrical stimulator was placed on the ganglion to stimulate the cavernous nerve for 50 seconds at 1.5 mA, 20 Hz, pulse width 0.2 ms. The cavernous nerve stimulation was conducted at least 3 times and the interval between stimulations was maintained for over 10 minutes. At the completion of functional analysis, the MPG and penis were excised for histopathology.

Immunofluoescent staining of MSCs in MPG

Immediately following ICP measurement, the ganglion was removed. The tissue was then snap frozen using 2-methylbutane pre-cooled in liquid nitrogen. Cryostat sections of the ganglion embedded in tissue-Tek OCT were obtained. From each ganglion, consecutive sections (5 µm) were collected on 4-5 slides, and thus each contained a similar collection of 10-15 serial sections from the same animal.

Ganglion sections were washed three times for 5 min with PBS and to avoid nonspecific antibody binding; they were then incubated for 60 min with 2% normal goat serum (Chemicon International, Temecula, CA) containing 0.1% Triton X-100. To evaluate neuronal differentiation of MSCs, the samples were incubated with anti--beta-tublin III (Tuj1) (diluted to 1:200; Abcam, Cambridge, UK) overnight at 4 °C in a humidified chamber. Sections were incubated with the secondary antibody (Alexa Fluor 488 goat anti-rabbit IgG; Invitrogen) for 2 hours in the dark in a humidified chamber at room temperature. After a washing (3 times, 10 min. each) with PBS, the nuclei were counterstained with 4,6-diamino-2--phenylindole dihydrochloride (DAPI;vector Labs, Burlingame, CA). Immunofluorescence was visualized using an Olympus BX51 fluorescence microscope.

eNOS and nNOS protein expression tests: Western blot

The corpus cavernosum was obtained from all rats and homogenized individually in a buffer solution of 0.32M sucrose, 0.2M Hepes, (pH 7.4), 1mM ethylenediaminetetraacetic acid (EDTA), 1mM dithiothreitol (DTT), 10 µg/mL leupeptin, 2 µg/mL aprotinin, 1 µg/mL pepstatin, 10 µg/mL trypsin inhibitor and 1mM phenylmethylsulfonyl

fluoride (PMSF). The homogenized buffer solution was placed on ice for 15 min and centrifuged at 4 °C for 13,000 rpm for 15 minutes. The supernatant solution was separated. The separated solution was utilized in the bovine serum albumin. 30 µg of the quantitative protein was denatured at 95 °C for 5 min. and electrophoresis was performed on a 12% discontinuous sodium dodecylsulfate (SDSPAGE)--polyacrylamide gel. The proteins were then electroblotted onto a 0.2 µm polyvinylidenedifluoride (PVDF, Amershambioscience, USA) membrane for 150 minutes at 25V. The membranes were reacted with blocking buffer (5% skim milk in TBS-T buffer) for 30 minutes at the ambient temperature. The eNOS and nNOS (BD Biosciences, USA) antibodies were added for 2 hours, and the membrane was washed 3 times using TTBS at intervals of 10 min. The secondary antibodies, anti-mouse IgG-HRP and anti-goat IgG-HRP (1:2000 dilution) (Zymed Laboratories Laboratories, USA) were added at the ambient temperature for 1 hours and the membrane was washed again with TTBS for 6 times with an interval of 5 minutes between each washing. Chemiluminescence was detected using ECL Western blotting detection reagents. Densitometric assessment of the bands on the autoradiogram was performed using Bio1D software (version 97; Vilber Lourmat, France).

Statistical analysis

All measurements were expressed as mean ± standard deviation (SD). Statistical analysis was obtained via Sigma stat for Windows. An inter--group comparison was made with the use of Neuman-Keuls multiple comparison test. The cut-off value of statistical significance was $P < 0.05$.

RESULTS

Erectile functional assessment

The erectile function was assessed by tracing the ICP under cavernous nerve electrical stimulation at 4 weeks after operation. The analysis is presented in ICP/MAP ratios (Figure- 2). The ICP/MAP ratios in the control group were 0.83 ± 0.02, which was significantly higher compared with all other groups ($P < 0.05$). BCNC group showed significantly decreased ICP/MAP ratios 0.23 ± 0.04 ($P < 0.05$). The ICP/MAP ratios (0.21 ± 0.08) of BCNC with Matrixen group was significantly decreased compared with control group ($P < 0.05$). The ICP/MAP ratios of BCNC with MSCs group (0.49 ± 0.1) and BCNC with MSCs/Matrixen group (0.65 ± 0.03) were significantly higher compared with BCNC group (0.23 ± 0.04) ($P < 0.05$). Moreover the the ICP/MAP ratios of BCNC with MSCs/Matrixen group (0.67 ± 0.09) was significantly higher compared with BCNC with MSCs group (0.49 ± 0.1) ($P < 0.05$).

Expression of MSCs in MPG using immunofluoescent staining

MPG in BCNC group showed decreased expression of neuronal cells compared with that of control group. The expression of neuronal cells was increased in BCNC with MSCs and BCNC with MSC/Matrixen groups compared with BCNC group. In the MCSs/Matrixen group, increased implantation of MSCs was observed in MPGs compared with BCNC with MSCs group (Figure-3).

Quantification of eNOS and nNOS proteins

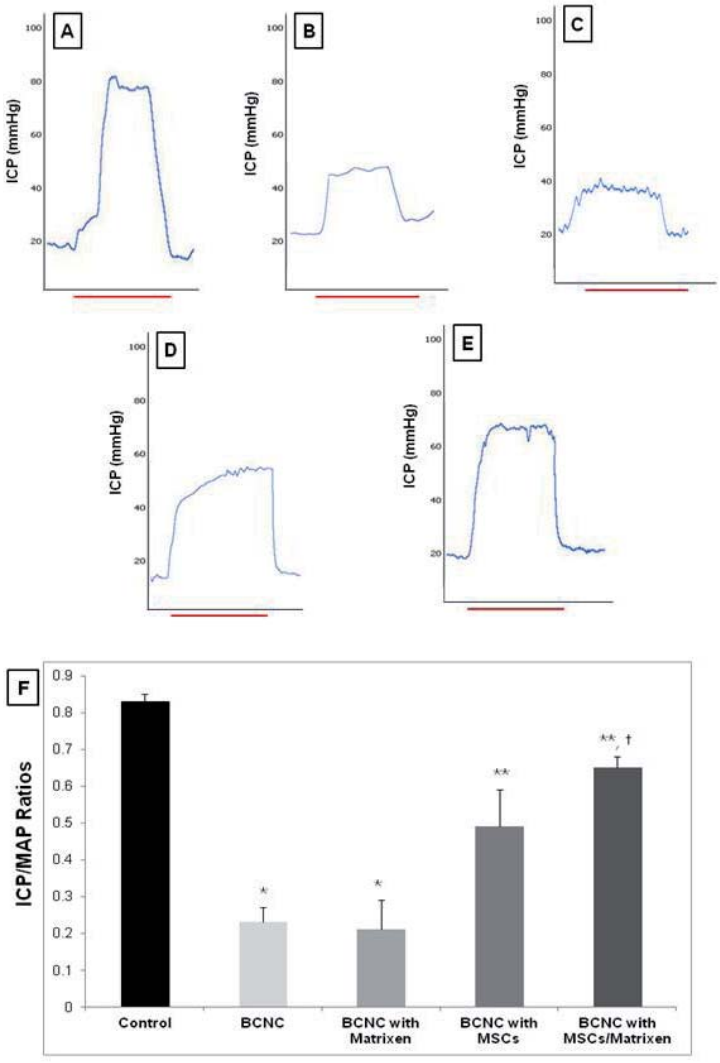
Decreased expressions of eNOS and nNOS were observed in the BCNC and BCNC with Matrixen groups than the control group ($P < 0.05$). The increase expressions of the eNOS and nNOS was observed in the BCNC with MSC and BCNC with MSCs/Matrixen groups compared with BCNC group ($P < 0.05$). The significant increase expression of the eNOS and nNOS was observed in BCNC with MSC/Matrixen group compared with BCNC with MSCs group ($P < 0.05$) (Figure-4).

DISCUSSION

We observed that the administration of MSCs into major pelvic ganglion (MPG) showed the preservation of erectile function after bilateral cavernous nerve crushing injury. Moreover, the implantation of MSCs was enhanced in the MPG with the help of collagen based cell carrier, Matrixen, and the improvements of functional and histological results were noted in this study.

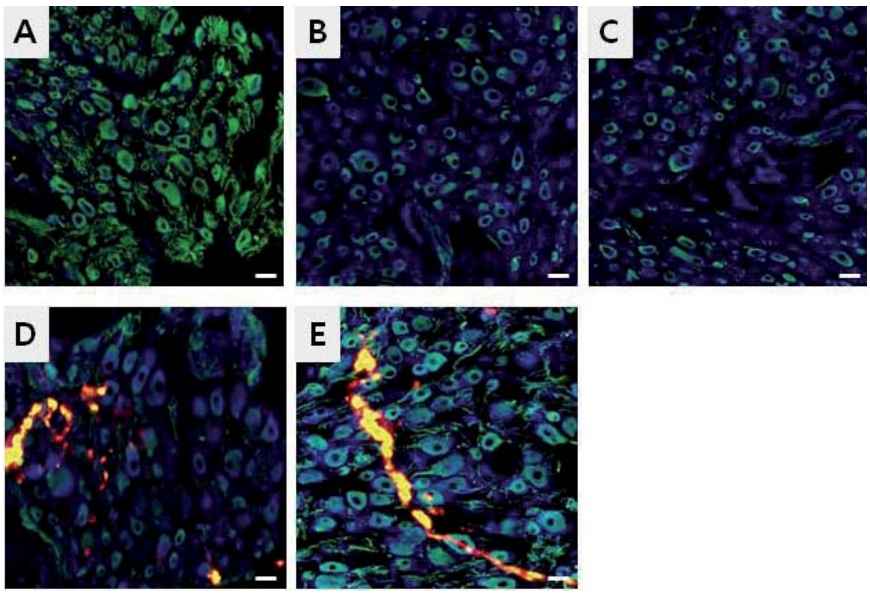
Animal model induced by bilateral cavernous nerve crushing injury has been widely used

Figure 2 - Erectile functional assessment under cavernous nerve stimulation (CNS) at week 4 in control (A); bilateral cavernous nerve (BCNC) crushing (B); BCNC with Matrixen (C); BCNC with mesenchymal stem cells (MSCs) (D); BCNC with MSCs mixed with Matrixen group (E). The voltage dependent erectile response to CNS represented by the ratios of intracavernous pressure (ICP) / mean arterial pressure (MAP) (F). *P < 0.05 compared with the control group, **P < 0.05 compared with BCNC group, †P < 0.05 compared with BCNC injected with MSCs group.



because this type of injury was designed to mimic the partial nerve damage that occurs during nerve-sparing radical prostatectomy through mechanical nerve stretch (11). And several studies were performed about the periodic changes of MPG in response to the cavernous nerve injury in the animals with erectile dysfunction induced by cavernous nerve injury (12,13). For example, Palma et al. (12) observed the sprouting of injured postganglionic axons in MPG of adult male rats at the first week after unilateral cavernous nerve transaction or crushing. And the recovery of cavernous nerve probably partly depends on sprouting of the remaining neural tissue after adverse manipulation. However, clinically, the recovery of erectile function cannot be observed in all patients who are

Figure 3 - The expression of mesenchymal derived stem cells (MSCs) in MPG at 4 weeks in control control (A); bilateral cavernous nerve (BCNC) crushing (B); BCNC with Matrixen (C); BCNC with mesenchymal stem cells (MSCs) (D); BCNC with MSCs mixed with Matrixen group (E). (x 400, blue:DAPI, green:tuj1, red: PKH26)

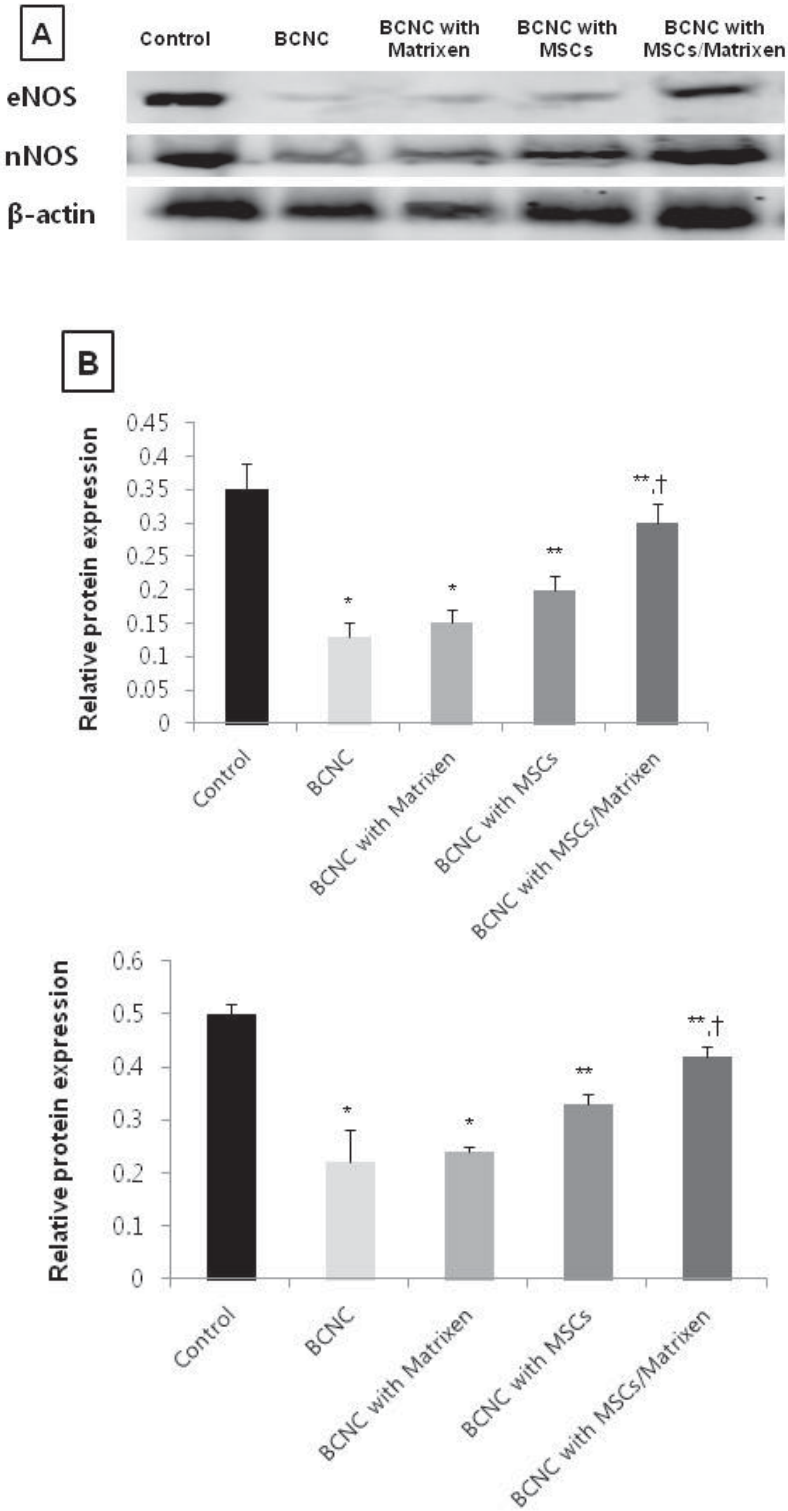


supposed to experience the partial nerve damage that occurs during nerve-sparing radical prostatectomy (6). As a result, stem cell therapy has been introduced as one of therapeutic options to help the preservation and restoration of erectile function in these patients (4). Therefore, we administered MSCs to MPG to evaluate the influence on the recovery of neurogenic erectile dysfunction by bilateral cavernous crushing injury. Moreover, we evaluated the role of Matrixen as a stem cell carrier to promote the implantation ability of MSCs in MPG by administration of MSCs mixed with Matrixen.

At 4 weeks after injury, ICP/MAP ratios in BCNC with MSCs and BCNC with MSCs mixed with Matrixen were significantly elevated compared with BCNC group. Even more elevated ICP/MAP ratio was observed in the BCNC with MSCs/Matrixen group than in the BCNC with MSCs group. This result supposed that more implantation of MSCs was possible in MPG, since Matrixen prevented the spread-out of MSCs to the adjacent, unexpected area form the MPG. And this result was identified with increased expression of stem cell in MPG in the BCNC with MSCs/Matrixen group compared with the BCNC with MSCs group from the immunofluorescent staining results.

Currently, MSCs suspended in cell culture medium are administered and therefore, there may be a limitation in the cell retention and transplant survival (15,16). There have been several studies to promote cell survival and retention, for example, the use of biomaterial scaffolds, which provide a microporous framework to improve cell survival (16,17). Xu et al. (9) reported treatment effect of the muscle derived stem cell (MDSC) mixed with fibrin glue on the restoration of urethral sphincter function resulted from the pudendal nerve transaction rat model. They observed an obvious increase of the thickness of muscle mass and of the muscle/collagen ratio, as well as a higher neovasculature density after MDSC mixed with fibrin glue injection. There is another study about the effect of MDSC mixed with polymer such as alginate (Alg)/polycaprolactone (PCL) in the same urethral sphincter dysfunction animal model (10). It also observed significant functional improvement compared with injection with MDSCs, alone and

Figure 4 - Westerm blot analysis of eNOS and nNOS in corpus cavernosum at 4 weeks control, bilateral cavernous nerve crushing (BCNC), BCNC with Matrixen, BCNC with mesenchymal stem cells (MSCs), BCNC with MSCs mixed with Matrixen group (A). Densimetric analysis to β-actin of eNOS and nNOS (B).
***P < 0.05 compared with control group. **P < 0.05 compared with BCNC group. †P < 0.05 compared with BCNC with MSCs group.**



suggested that the polymer may promote the stem cell viability and bulking effect. To be clinically useful cell transplant carriers, there are several required conditions. They need to be biocompatible without toxic side effects and biodegradable. And the ideal preparation must be liquid because liquid would be shaped and administered more easily.

We used Matrixen as stem cell carrier because it is collagen based biocompatible polymer and also shows thermosensitive character. It has easily sol-to-gel transition character that depends on the temperature. Matrixen remains as a sol state at the low temperature; therefore, it can be easily mixed with MSCs. After implantation into MPG with MSCs mixed with Matrixen, we could observe the immediate sol-to-gel transition and the gel formed three-dimensional shape at the body temperature. This means that the gel formation rate is fast. If the process of gel formation is slow Matrixen spreads to form a sheet like structure (19,20) and it may disturb the retention and localization of MSCs in MPG. Therefore, the better preservation of eNOS and nNOS levels in BCNC with MSCs/Matrixen group may be due to the stable immobilization of MSCs by Matrixen in MPG compared with BCNC with MSCs group. In addition, there was a study that suggested the necessity of a cell transplant carrier in other aspect. They observed that relatively large amount of MDSCs was needed for the functional improvement in the rats of intrinsic sphincter deficiency due to the spread-out of MDSCs (21). Practically, it is impossible to use a large number of MSCs for the regeneration of damaged tissue because the acceptable capacity may be limited according to the size of damaged tissue. However, the administration with Matrixen after BCNC did not show functional and molecular improvement compared with BCNC with MSC/Matrixen group. From this finding, we consider that Matrixen does not have a role in the restoration of damaged nerve tissue and its ability seems to be a simple cell carrier to enhance cell implantation.

The safety of MSCs has been well demonstrated (22,23), however, there is a probable chance to occur unexpected adverse events after MSCs treatment. Thus it is important to make MSCs act on the targeted damage tissue as possible and we expect the better treatment outcome of MSCs with the help of transplant cell carrier such as Matrixen. As a result, Matrixen is supposed to enhance the implantation of MSCs and differentiate to neural cells in MPG.

CONCLUSIONS

After administration with MSCs, alone, functional and histological restoration was observed in the rats with bilateral cavernous nerve crushing injury. In addition, the effect of MSCs on recovery of erectile function might be improved by using the cell carrier such as Matrixen. The reason of this result seemed that Matrixen might prevent spread-out of MSCs after administration. Therefore, we considered that the use of transplant cell carrier such as Matrixen may help the implantation of MSCs and improve the therapeutic effect of MSCs.

ACKNOWLEDGMENT

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CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Effect of ischemia preconditioning on renal ischemia/reperfusion injury in rats

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ABSTRACT

Objective: To study the effect of ischemia preconditioning (IP) on renal ischemia/reperfusion (I/R)-associated functional injury and expression of renal adhesion molecules in rats.

Materials and Methods: The ischemia preconditioning plan adopted in this experiment involved renal warm ischemia for 6 min. and blood flow for 4 min., repeated four times. The Wistar rat kidneys used for warm ischemia preconditioning were subjected to 60 min of renal warm ischemia followed by reperfusion. The rat kidneys with ischemia/reperfusion were compared with the ischemia preconditioning group to observe rat renal function and changes in the expression of renal adhesion molecules ICAM-1, P--Selectin, and E-Selectin.

Results: The expression of rat renal adhesion molecules (ICAM-1, P-Selectin, and E-Selectin) with ischemia preconditioning was significantly lower than that of the ischemia/reperfusion group. Serum creatinine was significantly lower than that in the ischemia/reperfusion group after 48 hours.

Conclusions: Ischemia preconditioning has a protective effect on renal function. Reduced expression of renal adhesion molecules is likely a mechanism involved in the observed protection.

ARTICLE INFO

Key words:
Ischemic Preconditioning; Intercellular Adhesion Molecule-1; P-Selectin; E-Selectin; Ischemia; Reperfusion Injury

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INTRODUCTION

Acute kidney injury (AKI) with high mortality and morbidity occurs frequently in critical patients. Ischemia is currently the leading cause of AKI in hospitalized patients. Kidney ischemic/reperfusion (I/R) injury is the major cause of renal injury in ischemic AKI. Avoiding IRI is very difficult in organ transplantation. The occurrence mechanism of kidney I/R injury is complex and data show that inflammatory mediators, adhesion molecules, and a variety of cytokines are involved in kidney I/R injury, but the exact pathogenesis has not been elucidated. If the condition in AKI cannot be improved in time, I/R injury may be persistent for several weeks or even years and evolves always to irreversible chronic kidney disease (CKD).

Ischemia preconditioning (IP) refers to an effective protective measure produced by tissues and organs after a brief ischemia/reperfusion, which can produce tolerance to ischemia-reperfusion injury (1-3). Research shows that preconditioning stimulation can protect the target organs or distant parts of organs and tissues (4,5). This phenomenon can be observed in many organs, such as skeletal muscle (6) and the heart (4). In the early stage, kidney tolerance can be induced by applying intermittent blood flow interruptions in the early phase of reperfusion (7). The kidney is a vital organ in which preconditioning can probably produce a protecti-

ve effect. Given the need for high energy and a complex vascular network, kidneys are extremely sensitive to ischemia/reperfusion (8,9). Kidney I/R injury is also an important cause of the delayed recovery of function after renal transplantation (10-12). Animal experiments have confirmed that local and distant preconditioning are effective in protecting the kidneys (13,14). Meta-analysis indicated that IP can reduce serum creatinine, blood urea nitrogen and histological renal damage after kidney I/R injury as compared to controls, suggesting that IPC effectively reduces renal damage after kidney I/R injury. Increased secretion of adhesion molecules after renal injury caused adhesion of inflammatory cells to endothelial cells, resulting in release of multiple cytokines. These cytokines can also enhance expression of adhesion molecules which not only may result in aggravation of renal injury but also make the rejection of transplanted organ occurs or aggravates. Nowadays, most studies are limited to the macroscopic analysis of the protective effect of renal function. The mechanism behind the renal ischemia preconditioning phenomenon is yet to be elucidated.

The inflammatory cascade caused by ischemia/reperfusion is an important factor leading to renal I/R injury. Research has shown that leukocyte activation, invasion, adhesion, and impaction evidently occur in the tissue of an AKI model. As a result, perfusion is difficult to recover, and a variety of hazardous substances (such as free radicals, lysosomal enzymes, and various cytokines) is released, causing histiocyte damage (15,16). The adhesion of leukocytes to the endothelium is mainly mediated by intercellular adhesion molecule-1. Previous studies have confirmed that the expression of adhesion molecules increases after kidney I/R injury (17-20). This paper established an ischemia/reperfusion disease model in rat and studied the expression level of adhesion molecules in IP. The relationship between adhesion molecule expression and kidney I/R injury after IP was also investigated.

MATERIALS AND METHODS

Animals

One hundred and fifty 2- to 3-month-old Wistar rats (200 g to 250 g; male or female; Third Military Medical University Animal Institute) were used in this experiment. Goat anti-rat ICAM-1 antibody and goat anti-rat P-Selectin antibody (Beijing Zhongshan Biotech Co., Ltd.,. China); Goat anti-rat E-Selectin antibody (Ancell, USA); ASBC immunohistochemistry (IHC) kit (Boster Bio-Engineering Co., Ltd. Wuhan, China); Biotinylated Goat Anti Rabbit IgG (Boster Bio-Engineering Co., Ltd. Wuhan, China); Rat Creatinine (Cr) ELISA Kit (Shanghai Xitang Biological Technology Co., Ltd., China) were also used. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal use protocol has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of The General Hospital of Shenyang Military Region.

Experimental Methods

The Wistar rats were randomly divided into three groups: sham-operated, ischemia/reperfusion (I/R), and IP groups. Each group was further sorted by time point at 1, 2, 12, 24, and 48 hours (each subgroup with 10 rats). Sodium pentobarbital (1%) was given to the rats via intraperitoneal anesthetic injection (30~40 mg/kg). The abdominal cavity was opened under aseptic conditions. The right kidney was excised, and the left renal pedicle was freed. In the sham-operated group, the incisions were closed immediately after 60 min. In the ischemia/reperfusion group, the left renal artery and veins were occluded for 60 min. using microaneurysm clamps, and then the incisions were released and closed. In the IP group, the renal artery and veins were occluded for 6 min using microaneurysm clamps and the blood was allowed to flow again for 4 min. The whole procedure was repeated four times. After these procedures were conducted the renal artery and veins were occluded for 60 min. After the warm ischemia, the microaneurysm clamps were released, and the incisions were closed.

Rats in each group were euthanized by cervical vertebra luxation while still under anesthesia and samples were collected from their blood, tissues at 1, 2, 12, 24, and 48 hours after the incisions were closed. The left renal biopsy specimens

were taken and fixed in mercuric chloride (4.5 mL) + formaldehyde (0.5 mL) solution for 5 min., and then stored at 4 °C in picric acid (5 mL) until pathological examination. Venous blood was collected to determine the level of serum creatinine in the vena cava at the time of sacrifice.

Immunohistochemical staining

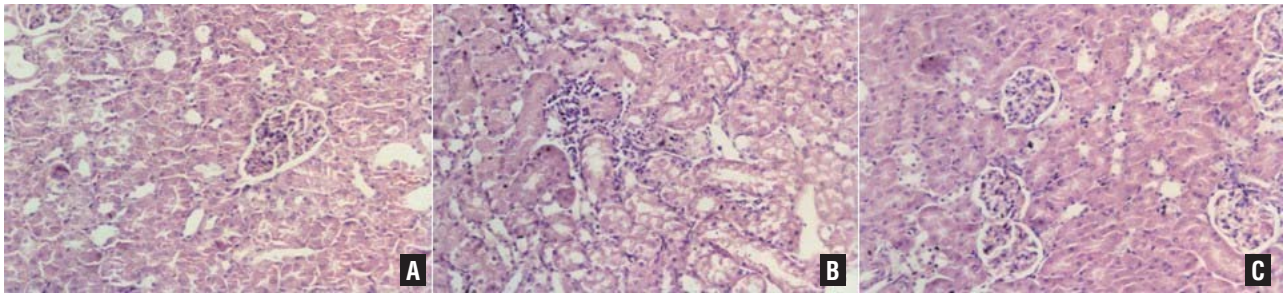
All the renal tissue specimens were dehydrated by automatic tissue dehydration machine, paraffin embedded, cut into 5 µm sections and mounted onto glass slides. Two percent (v/v) APES in acetone was used as adhesive for fixed tissue. At least six pieces of sections were cut in each specimen: one of the slides was stained with HE and the rest of specimens were placed at 70 °C overnight. Immunohistochemical staining was performed according to the product manual. Each kidney specimen was stained with ICAM-1, P-Selectin, and E-Selectin antibodies for 10 sections, with three sections extracted randomly thereafter. The concentrations of the primary and secondary antibodies were 1:100 dilution. PBS was used as negative control. The specimens were stained with SABC method. Briefly, the specific steps are as follows (ICAM-1 as an example). (1) slice conventional dewaxing to water progressively: Xylene (I), 5 min.; Xylene (II), 5 min.; 100% ethanol, 95% ethanol, 90% ethanol, 85% ethanol, 75% ethanol, 5 min. each; rinse in distilled water, 5 min. (2). 1% hydrogen peroxide was used to treat slices at RT for 15 min. to eliminate endogenous peroxidase activity (3). Wash with distilled water and soak in PBS 3 times for 5 min. each (4). Antigen retrieval in microwave for 11 min. Gradually cooled to RT and soak in PBS for 5 min. (5). Non-immune serum of normal animal block at RT for 15 min. to reduce non-specific staining (6). Dumping of serum and dropping goat anti-rat ICAM-1 antibody (1:100 dilution). Incubate at 37 °C for 1 hour and overnight at 4 °C (7). Wash with 0.01M PBS 3 times for 5 min. each (8). Dropping Biotinylated Goat Anti Rabbit IgG and incubation at 37 °C for 1 hour (9). Wash with 0.01M PBS 3 times for 5 min. each (10). Add avidin and incubate at 37 °C for 1 hour (11). Wash with 0.01M PBS 3 times for 5 min. each (12). DAB color developed for 5 min. Terminate the chromogenic reation until the yellow particles were observed in the cells under microscope (13). The nuclei were stained with hematoxylin (14). Wash with water for 5 min. Dehydrate with graded alcohol series (70% ethanol, 80% ethanol, 90% ethanol, 95% ethanol, 100% ethanol, 5 min each). Clarify with Xylene for 15 min. Mounting with neutral resin.

The extent of renal injury was evaluated by counting the number of neutrophils infiltration in renal tissue. Three sections were randomly selected for counting the number of neutrophils in five adjacent high-power fields. The average was defined as the number of positive cells (neutrophils) for each detection indicator (Figure-1).

Statistical analysis

SPSS was used to analyze all data. The results were expressed as mean ± SD. Statistical significance of differences among groups (defined as $P < 0.05$) was evaluated using one-way analysis of variance (ANOVA) with Bonferoni correction and Student's t-test.

Figure 1 - The results for the histological examination. A) Control; B) IR pathology; C) IP.



RESULTS

Changes in ICAM-1 expression after renal IP

A few positive cells were observed in the sham-operated group. Immunohistochemistry showed that renal ICAM-1 expression was enhanced after ischemia/reperfusion (Figure-2). The number of ICAM-1-positive cells increased at 2 hours after ischemia/reperfusion and peaked at 12 hours. A downward trend was observed at 24 hours. The expression of ICAM-1 was mainly observed in the glomerular endothelial cells, peritubular capillaries, and renal proximal tubule epithelial cells. After IP, the number of ICAM-1--positive cells decreased correspondingly. The expression level of ICAM-1 in the IP group was less than that in the I/R group at each time point ($p < 0.05$; Table-1 and Figure-3).

Changes in P-Selectin expression after renal IP

The number of positive cells in the sham--operated group was extremely few (Figure-4). One hour after ischemia/reperfusion, P-Selectin was widely expressed in the kidney, particularly in the glomerular mesangial, capillary loop, tubular, and renal interstitial sections. The expression was most significant in the renal tubular epithelial cells. It peaked 12 hours later and then declined. P-Selectin expression decreased significantly after IP ($p < 0.05$; Table-2 and Figure-5).

Changes in E-Selectin expression after renal IP

E-Selectin was rarely expressed in the sham-operated group (Figure-6). Two hours after ischemia/reperfusion, the cells expressing E-Selectin increased, specifically those in the peritubular capillaries. The expression peaked at

Figure 2 - Histology figures of ICAM-1 expression in the three groups. A) Control; B) IR; C) IP.

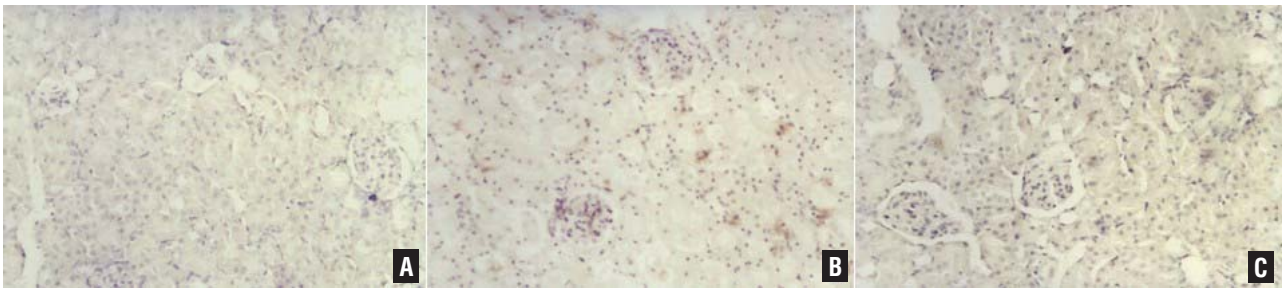


Table 1 - Effects of ischemic preconditioning on the expression of ICAM-1 in rat kidney (positive cell number/HP).

	1h	2h	12h	24h	48h
Sham-operated	0.6 ± 0.70	1.0 ± 0.67	1.2 ± 0.92	1.1 ± 0.74	1.2 ± 0.63
I/R	2.1 ± 0.99	7.6 ± 1.43▲	12.9 ± 2.33▲	10.6 ± 2.59▲	7.1 ± 1.37▲
IP	1.1 ± 0.74	5.0 ± 1.63▲*	10.4 ± 1.43▲*	8.3 ± 1.49▲*	5.2 ± 1.31▲*
F	4.3366	32.1898	68.4419	38.8833	34.0986
P	0.0383	0.0000	0.0000	0.0000	0.0000

▲ $P < 0.01$ vs. sham-operated; * $P < 0.05$ vs. I/R.

Figure 3 - Effects of ischemic preconditioning on the expression of ICAM-1 in rat kindney.

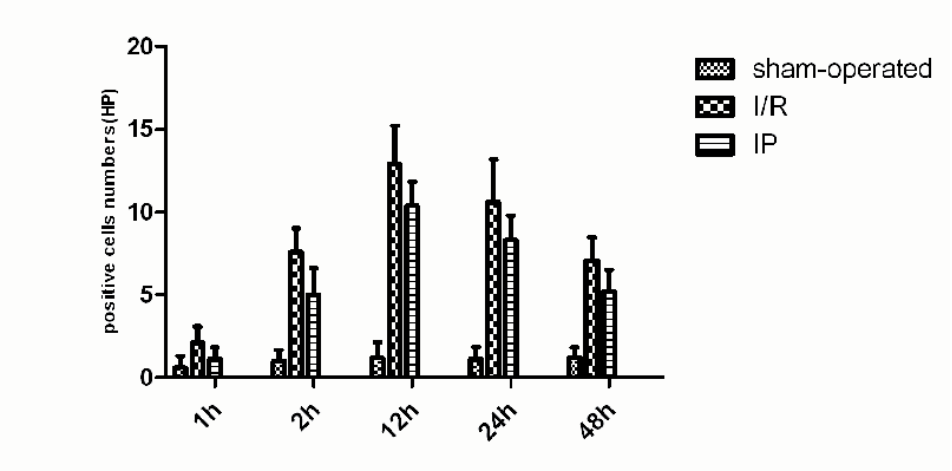


Figure 4 - Histology figures of P-Selectin expression in the three groups. A) Control; B) IR; C) IP.

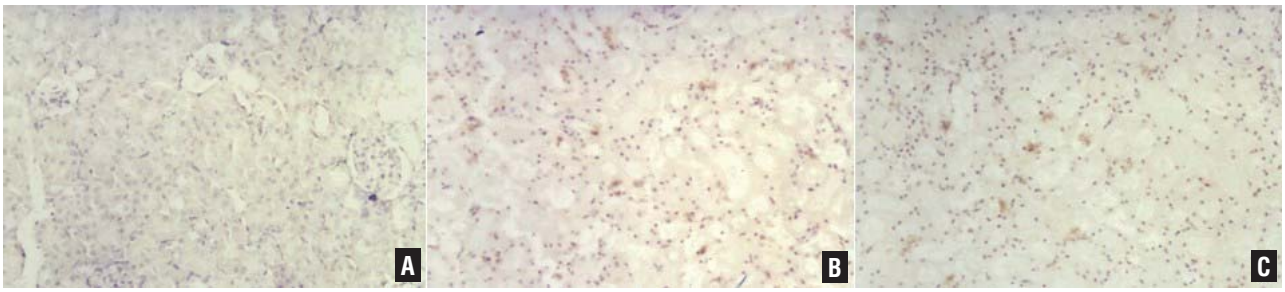


Table 2 - Effects of ischemic preconditioning on the expression of P-Selectin in rat kidney (positive cell number/HP).

	1h	2h	12h	24h	48h
Sham-operated	1.5 ± 0.85	1.5 ± 0.85	1.3 ± 0.67	1.2 ± 0.63	1.5 ± 0.85
I/R	6.0 ± 1.15▲	9.9 ± 1.52▲	13.0 ± 2.53▲	4.7 ± 1.16▲	3.89 ± 0.94
IP	5.4 ± 1.26▲	6.4 ± 1.65▲*	10.8 ± 1.81▲*	4.4 ± 1.65▲	3.78 ± 1.33
F	24.6518	46.3999	57.2739	12.6428	8.0908
P	0.0001	0.0000	0.0000	0.0001	0.0060

P < 0.01 vs. sham-operated; * P < 0.01 vs. I/R.

Figure 5 - Effects of ischemic preconditioning on the expression of P-Selectin in rat kidney.

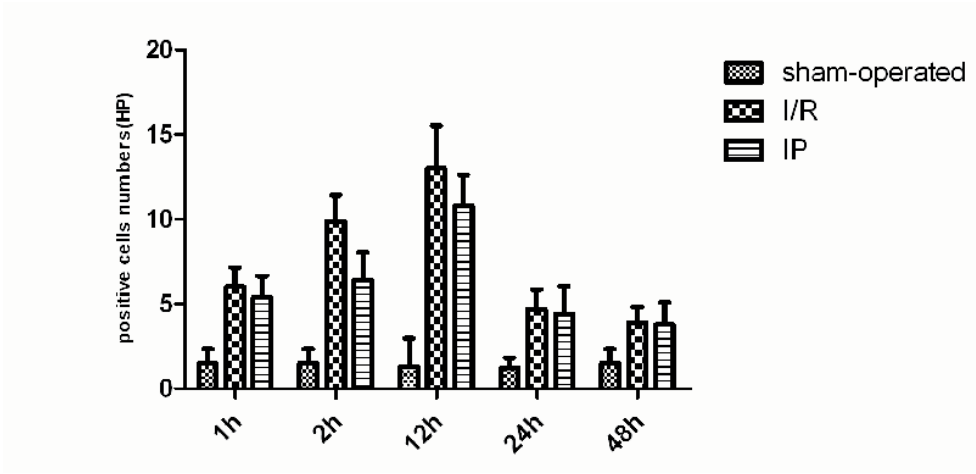
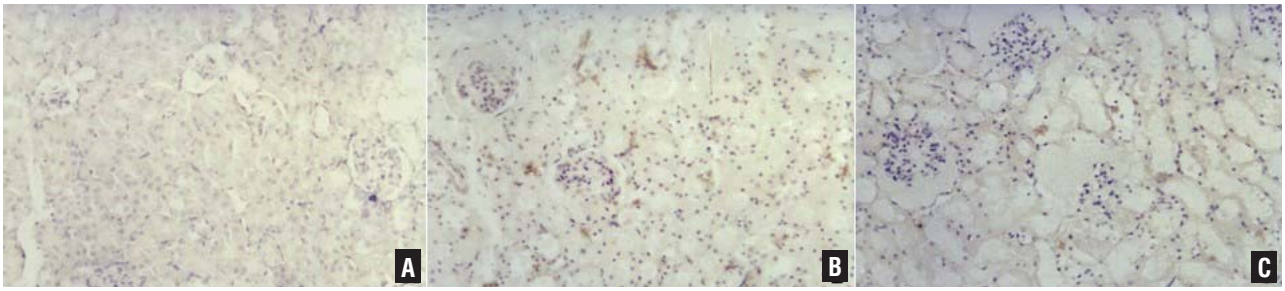


Figure 6 - Histology figures of E-Selectin expression in the three groups. A) Control; B) IR; C) IP.



24 hours and then declined gradually. The number of positive cells in the IP group was significantly reduced ($p < 0.05$; Table-3 and Figure-7).

Changes of leukocyte infiltration in renal tissues

One hour after ischemia/reperfusion, the number of leukocyte infiltration increased obviously. The number peaked at 24 hours and was significantly reduced at 48 hours. The infiltration occured mainly in vascular, interstitial and tubular areas of the kidney. Compared with those in IR group, the number of leukocyte infiltration was significantly reduced, especially at 24 hours ($p < 0.05$; Table-4 and Figure-8).

Renal ischemia/reperfusion and changes in renal function after IP

Serum creatinine levels increased after renal ischemia/reperfusion. In the first 24 hours, serum creatinine level was significantly higher than at 1, 2, 6, and 12 hours. ARF (acute renal failure) occurred when the creatinine level reached 140 μmoL/L at the first 48 hours. By contrast, the creatinine level in the IP group also increased but at a slower rate. Compared with each corresponding time point of IP, no significant difference was observed at the first 1, 2, 12, and 24 hours. Compared with the IP group at the first 48 hours, the difference was significant ($p < 0.05$;Table-5 and Figure-9).

DISCUSSION

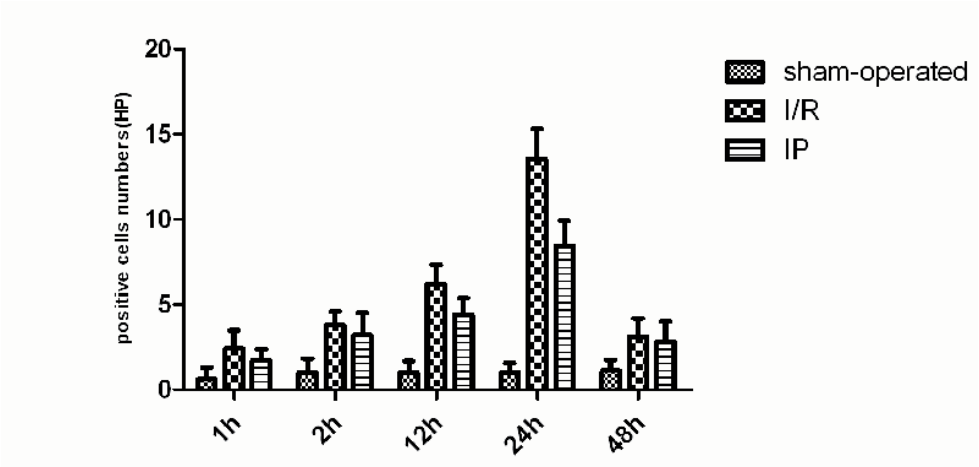
Preconditioning is a tolerance or adaptability to a secondary stimulus exhibited by organisms and organs after primary exposure to a stimulus. This phenomenon known as IP is common in biology. Organs can stand a longer time of

Table 3 - Effects of ischemic preconditioning on the expression of E-Selectin in rat kidney (positive cell number/HP).

	1h	2h	12h	24h	48h
Sham-operated	0.6 ± 0.70	1.0 ± 0.82	1.0 ± 0.67	1.0 ± 0.57	1.11 ± 0.63
I/R	2.4 ± 1.07	3.8 ± 0.79▲	6.2 ± 1.14▲	13.56 ± 1.75▲	3.1 ± 1.1▲
IP	1.7 ± 0.67	3.2 ± 1.32▲	4.4 ± 0.97▲*	8.44 ± 1.49▲**	2.8 ± 1.23▲
F	5.9267	10.7276	38.8934	106.6971	5.5342
P	0.0162	0.0021	0.0000	0.0000	0.0198

▲ P < 0.01 vs. control; * P < 0.05, ** P < 0.01 vs. I/R.

Figure 7 - Effects of ischemic preconditioning on the expression of E-Selectin in rat kidney.



warm ischemia after brief ischemia/reperfusion. In 1986, Murry first observed the IP phenomenon in the heart (4). In later studies, many organs such as skeletal muscle have been demonstrated to exhibit IP (6).

In relation to renal preconditioning in a rat model, since the new concept of IP was proposed by Murry and protective effect of IP has been confirmed in cardiac ischemia-reperfusion injury, many scholars have established the heart IP model and explored its mechanism. If different preconditioning program was used in the renal IP model, the conclusions could not have been the same. Preconditioning involved the sequential clamping of the left renal artery for 4 min. and its release for 11 min., a total of four times. The results of the above preconditioning program showed that no protection to kidney I/R injury was observed (21). However, Toosy et al. reported that an IP regimen applied only 5 min. before exposure to a 40 min. period of RI in the rat significantly protected it from the functional impairment associated with kidney I/R (22). Furthermore, the results of an IP regimen (three 2 min. periods of ischemia separated by 5 min. of reflow prior to 45 min. of bilateral renal ischemia followed by

Table 4 - Changes of leucocyte Infiltrated into renal parenchyma after ischemic preconditioning.

	1h	2h	12h	24h	48h
Sham-operated	1.4 ± 0.55	1.8 ± 0.84	2.0 ± 0.71	1.6 ± 0.89	1.8 ± 0.83
I/R	6.2 ± 0.84▲	11.4 ± 2.30▲	22.8 ± 4.98▲	32.6 ± 4.40▲	16.0 ± 1.58▲
IP	5.2 ± 1.30▲	7.8 ± 1.30▲*	13.6 ± 2.07▲**	23.4 ± 2.41▲**	8.8 ± 1.82▲**
F	35.6547	45.9040	55.0738	146.4627	116.3796
P	0.0000	0.0000	0.0000	0.0000	0.0000

▲ P < 0.01 vs. control; * P < 0.05<** P < 0.01 vs. I/R

Figure 8 - Changes of leucocyte Infiltrated into renal parenchyma after ischemic preconditioning.

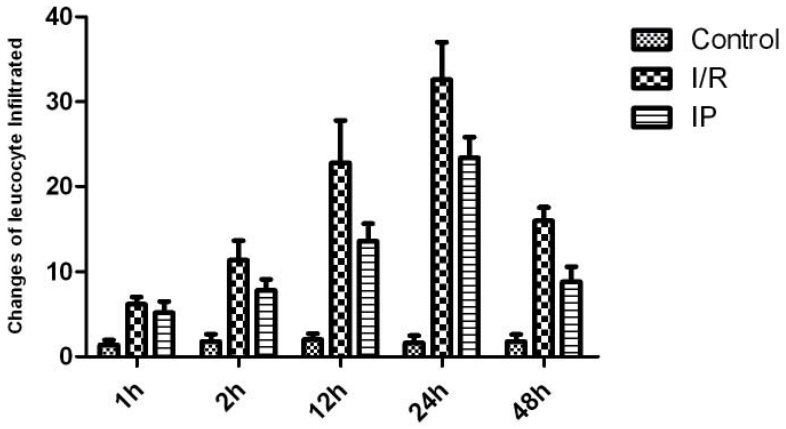
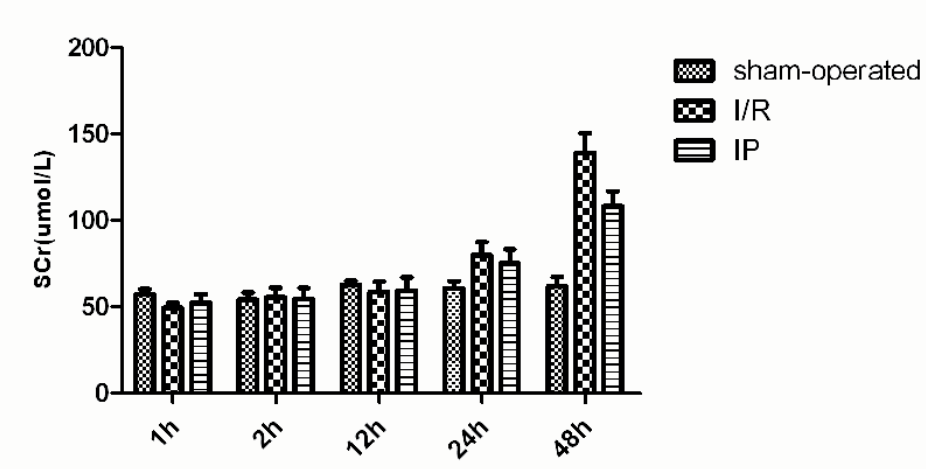


Table 5 - Changes of serum creatinine after renal ischemic preconditioning (umol/L).

	1h	2h	12h	24h	48h
Sham-operated	57.0 ± 3.16	54 ± 4.18	62.4 ± 2.65	60.6 ± 4.22	61.8 ± 5.67
I/R	49.4 ± 2.79	55.4 ± 5.59	58.6 ± 5.98	79.8 ± 7.6	139.0 ± 11.55▲
IP	52.2 ± 5.07	54.6 ± 6.10	59.2 ± 7.85	75.2 ± 7.99	108.0 ± 8.86▲*
F	5.0972	0.0861	0.5996	10.8128	92.7601
P	0.0250	0.9180	0.5647	0.0021	0.0000

▲ P < 0.01 vs. sham-operated; * P < 0.05 vs. I/R.

Figure 9 - Changes of serum creatinine after renal ischemic preconditioning.



24 hours of reperfusion) applied by Cochrane et al. found that IP would ameliorate ischemic renal injury (23). The preliminary experiment of preconditioning method in our study found that the protective effect of prolongation of reperfusion time on renal I/R injury was not that obvious. The results of our preliminary experiment suggested that if the time and frequency of ischemia were fixed in a repeated cycle of short-time IR the protective effect on kidney would decline with the prolongation of the reperfusion interval. When a certain critical point is exceeded, such as fixed four 6 min. periods of ischemia separated by more than 10 min. of reperfusion, the role of IP protection would be lost. IP regimen with multiple cycles of short-period ischemia followed by short-period reperfusion had a significant protective effect on kidney I/R injury. At the same time, according to our previous experimental experience with I/R injury, an IP regimen applied 6 min. period of ischemia followed by 48 hours period of reperfusion was the most appropriate method to observe the renal function. Therefore, based on the previous study of the kidney IP model, the following modified IP regimen was used in the present study: the sequential clamping of the renal artery for 6 min. and its release for 4 min., a total of four times; 60 min. of renal ischemia followed by 48 hours of reperfusion. The results of our study showed that this IP regimen may significantly reduce the incidence of renal I/R injury, suggesting that the experimental animal model was successfully established and may provide a reliable preadaptation program for further studies. According the observations of a preliminary study, changes of experimental data at 1, 2, 12, 24 and 48 hours after IP were obvious. Therefore, these time points were chosen as the observation point. Because one of the aims was to provide experimental data of IP protection for the kidney transplant, no prolonged observation time and the protective effect of later IP were further investigated in the present study.

Most studies have been limited to the macroscopic study of the protective effect of renal function in terms of the renal IP phenomenon. Wever conducted a meta-analysis of animal experimental studies on renal IP (24). Creatinine, urea nitrogen, and histological changes were used as indicators of renal IP. Meta-analysis showed that compared with the control group, serum creatinine and blood urea nitrogen levels decreased significantly and changes in the renal tissue was less important in the IP group. No difference was observed between local and distant preconditioning on the protective effect of renal ischemia/reperfusion. IP significantly improved kidney I/R injury, especially in the advanced window period (≥ 24 hours). Nevertheless, significant differences were found between studies in terms of the

results of the best protection window. The effect of IP in each animal species varies. Indisputably, renal IP has protective effects to subsequent ischemic/reperfusion injury. However, the mechanism remains unclear. Mahfoudh-Boussaid (25) used Wistar rats to study IP and found that renal IP reduces cell lysis and lipid peroxidation and improves renal function. Compared with the ischemia/reperfusion group, the IP group showed elevated levels of endothelial nitric oxide synthase, nitrite, and hypoxia inducible transcription factor-1α. Early preconditioning through reduction of oxidative and endoplasmic reticulum stress has been believed to protect against kidney I/R injury. Kim (26) believed that the effect of IP on the renal protective effect is related to the increase in isocitrate dehydrogenase (IDH1) activity. IDH1 enhanced NADPH levels, elevated the GSH-to-total glutathione ratio, and reduced oxidative stress. Chen (3) believed that NF-kappaB is the key medium to reperfusion injury. The activation of NF-kappaB depended on the phosphorylation of its inhibitor. The inhibitor was IkappaB, which had a specific NF-kappaB kinase subunit. Animal experiments confirmed that renal IP reduces renal acute injury by inhibiting IKKbeta activity. The IP protocol adopted in this experiment involved four repetitions of warm ischemia for 6 min. and blood flow for 4 min. Under the condition of 1 hour warm ischemia and 48 hours perfusion, IP significantly reduced the expressions of renal adhesion molecules ICAM-1, P-Selectin, and E-Selectin. At the same time, granulocyte infiltration in renal tissue after IP was significantly lower than that in the I/R group. Adhesion molecules are the major molecules which mediate adhesion of polymorphonuclear leukocytes to endothelial cells. Because of reduced expression of adhesion molecules after IR, adhesion of granulocytes to endothelial cells decreased leading to reduction of granulocyte infiltration in renal tissue. The serum creatinine level of the rats was significantly lower than that of the I/R group. This result proves the explicit protective effect of IP. The mechanism of kidney protection was closely related to the reduction of adhesion molecule expression caused by IP.

We believe that the reduction of adhesion molecule expression is one of the main mechanisms of IP in preventing ischemic/reperfusion injury. ICAM-1 distributes in a variety of cells. The degrees of ICAM-1 expression in different cells are not the same: the strongest is in vascular endothelium followed by peripheral blood leukocytes, interstitial macrophages and fibroblasts. Expression of ICAM-1 which is low in normal cell is enhanced by cytokines such as interleukin-1 (IL-1), tumor necrosis factor (TNF). Expression of ICAM-1 increased by the role of cytokines in the injured endothelial cells, resulting in promoting the adhesion of leukocytes to vascular endothelium and migration from intravascular to the extravascular matrix. E-selectin was expressed on the surface of the stimulated endothelial cells. There was no E-selectin in the endothelial cells. E-selectin was generated by transcription 4-6 hours after stimulation. Its function is to mediate the adhesion of neutrophils and memory T cells to the infected endothelial cells. P-selectin expressed in the stimulated endothelial cells or the activated platelets is stored separately in the Weibel-Palade bodies of endothelial cells and α-granules of platelets. P-selectin can move to the cell surface within minutes after stimulation of inflammatory mediators. The function of P-selectin is to mediate endothelial cells adhesion to inflammatory cells and activated platelet adhesion to monocyte and neutrophil. Many studies confirmed that the expression of adhesion molecules increases after renal ischemic/reperfusion. In a research involving cultured human umbilical vein endothelial cells and human microvascular endothelial cell line in vitro, a lack of oxygen could induce a large number of Weibel-Palade corpuscles in endothelial cells to be released, thereby increasing P-Selectin expression. E-Selectin expression in endothelial cells also increases after hypoxia reoxygenation (27). The kidneys can be protected from ischemic/reperfusion injury by blocking the expression of P-Selectin and E-Selectin (19,28). Klein et al. found that the expression of ICAM-1 does not change significantly during the hypoxic process but increases after hypoxia reoxygenation. Rats with ICAM-1 gene knockout and ICAM-1 monoclonal antibody were used in the studies, which confirmed that ICAM-1 was the key mediator of ischemic AKI and has important role on the kidney I/R injury. Furthermore,

when ischemia occurred, expression of adhesion molecules increased with the increasing secretion of TNF-α (29) and IL-1 in macrophages and monocytes (30). Increased expression of ICAM-1 induced by IL-1β and TNF-α may lead to an increase of leukocyte infiltration in kidney and synergistically aggravating kidney I/R injury. Zhou (31) used rats to study renal ischemic/reperfusion. He discovered that after ischemia/reperfusion, ICAM-1 expression in renal vascular endothelial cells and P-Selectin expression in renal tubular epithelial cells increase. If anti-P-Selectin antibody was given, kidney I/R injury could be reduced, confirming the important role of ICAM-1 and P--Selectin in kidney I/R injury. The expression of adhesion molecules could be significantly reduced through IP. The results confirmed that preconditioning had a protective effect on reperfusion kidneys. The data in another study confirmed that ischemic preconditioning could prevent postischemic P-selectin expression in the rat small intestine (32). The expression of ICAM-1 of pig lung tissue obviously decreased in group IP than that in control group (33). The study on protective mechanism of ischemic preconditioning in cultured rat endothelial cells found that changes of post--ischemic expression of adhesion molecules such as ICAM-1 may be related to activation of protein kinase C and production of nitric oxide and free radicals. And this is associated with a lesser adhesion of neutrophils to endothelial cells. Such prevention of neutrophil adhesion may contribute to the protective effect of preconditioning against reperfusion-induced endothelial injury (34). Similar to other methods of blocking the expression of adhesion molecules, we reconfirmed that the increase in adhesion molecule expression plays an important role in kidney I/R injury. Remote ischemic preconditioning can significantly reduce the risk of AKI in patients undergoing cardiopulmonary bypass-assisted cardiac surgery (35). However, the data in some studies indicated that the effect of ischemic preconditioning had limitations and uncertainty. Young et al. reported that 96 adults undergoing high-risk cardiac surgery were randomized to group of remote ischemic preconditioning or control. Main endpoints were plasma high-sensitivity troponin T (hsTNT) levels at 6 and 12 hours, worst post-operative acute kidney injury (AKI). The results showed that there were no significant differences between the experimental and control group, suggesting that IP does not reduce hsTNT, AKI in high-risk cardiac surgery (36).

Compared with other methods that block a certain type of adhesion molecule alone, IP is a much more comprehensive method that can reduce a variety of adhesion molecules with higher precision and reliability. The protective effect of whole kidney can be achieved by ischemia/reperfusion, even the protective effect of other organs can be achieved by controlling inflammatory cytokines. It can also reduce the incidence of multiple organ dysfunction syndrome, which can help meet the needs of clinical and practical use. Unfortunately, some meaningful means of detection can not be performed because of our limited laboratory conditions, making the experimental results in the present study have certain limitations. There also has a certain gap with the complex situation in the actual clinical environment of human body and such findings acquired in the ideal conditions in rats need to further validation at the appropriate internal environment of the human body.

CONFLICT OF INTEREST

None declared.

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RADIOLOGY PAGE

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MRI findings in Marion's disease: the bulb and the doughnut signs

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A 77-year-old female presented to our facility with a five-year history of progressive urinary bladder retention and voiding difficulties. She underwent a pelvic magnetic resonance imaging (MRI) examination as a part of her lower urinary tract symptoms evaluation, and for treatment planning.

MRI with a torso phased-array superficial coil was performed in a 1.5T magnetic field equipment. We obtained multiplanar T1- and T2-weighted pre-contrast sequences, with and without fat suppression techniques, axial diffusion weighted images, axial apparent diffusion coefficient (ADC) map, and multiplanar T1-weighted fat suppressed images after the administration of intravenous gadolinium.

MRI revealed an exuberant concentric hypertrophy of the entire urethra. This characteristic imaging finding resembled a bulb of a sphygmomanometer on the sagittal and coronal planes, and a doughnut on the axial ones (Figure-1). The affected musculature was isointense on T1- and slightly hyperintense on T2-weighted images compared with unaffected muscles. The diffusion sequence showed restriction to free water molecules movements, making the hypertrophied muscles hyperintense on this sequence and hypointense on the ADC map (Figure-2). Additionally this patient also exhibited a marked concentric hypertrophy of the internal anal sphincter muscle, probably related to the same bladder neck neuropathological mechanism. This latter finding was previously misdiagnosed in another facility as a rectal tumor. After the intravenous contrast administration, there was slight enhancement of the hypertrophied muscle. The combination of the MRI findings together with the patient´s symptoms and urodynamic testing was consistent with the diagnosis of primary bladder neck obstruction (PBNO) due to severe muscular hypertrophy.

DISCUSSION

PBNO was first described in men by Marion (1), a condition wherein the bladder neck fails to open adequately during voiding, resulting in increased striated sphincter activity or obstruction of urinary flow in the absence of another anatomic obstruction, such as that caused by benign prostatic enlargement in men or genitourinary prolapse in women (2,3).

Men typically present with symptoms for years before a correct diagnosis of PBNO is made. PBNO can present with a variety of symptoms including voiding or obstructive symptoms (decreased force of stream, hesitancy, intermittent stream, incomplete emptying), storage or irritative symptoms (frequency, urgency, urge incontinence, nocturia), or a combination of

both. Occasionally, the initial presentation may be urinary retentions (3). Presenting symptoms appear to be similar in men and women, with a combination of voiding and storage symptoms being common (4). Currently PBNO is diagnosed primarily by videourodynamics and cystourethroscopy, but the increasing use of MRI can introduce new imaging findings (5).

ABBREVIATIONS

ADC: apparent diffusion coefficient
MRI: magnetic resonance imaging
PBNO: primary bladder neck obstruction

Figure 1 – Coronal (a), sagittal (b), and axial (c), T2-weighted MRI images showing exuberant concentric hypertrophy of the muscular planes surrounding the urethra along its complete extension (arrows). This characteristic finding resembled the bulb of a sphygmomanometer in "a" and "b", and a doughnut in "c".

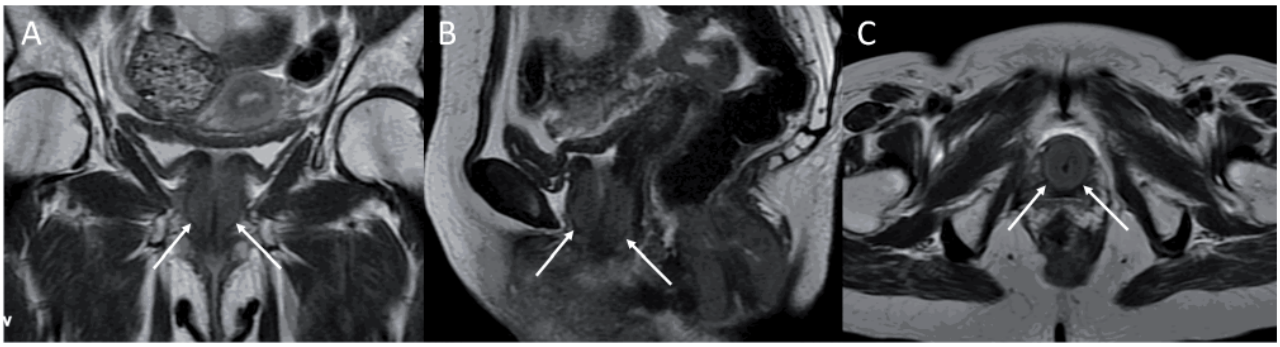
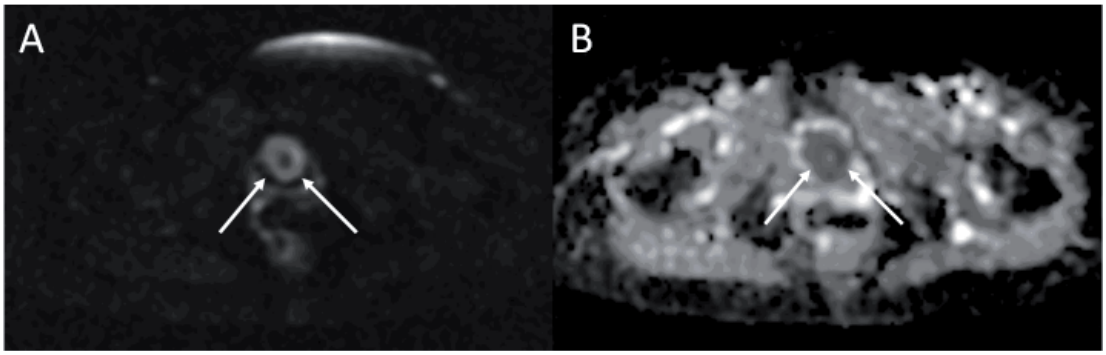


Figure 2 – (a) Axial diffusion sequence showed a ring-like hyperintensity of the peri-urethral hypertrophied muscles. (b) The axial ADC map demonstrated hypointensity in the affected muscles. In both images the doughnut sign is clearly depicted (arrows).



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A case of septic pulmonary embolism caused by urinary tract infection

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A 56-year-old woman with diabetes mellitus (DM) presented at our department with intermittent low-grade fever, right-side back pain and general fatigue, having taken antibiotics (cefotiam) for the preceding two weeks as prescribed at another healthcare facility. Despite the absence of any cough or sputum production, chest X-ray revealed multiple nodular lesions in the peripheral lung fields (Figure-1). These nodules were not observed at the time of initial treatment at the referring hospital. Chest CT indicated multiple nodules with necrotic centers in the peripheral bilateral lung fields (Figure-2). Abdominal CT showed a swollen right kidney and diffuse abscesses (Figure-3). A diagnosis of septic pulmonary embolism accompanied by pyelonephrosis was made. Accordingly, an alternative antibiotic (cefotaxime) was administered against sepsis. The multiple nodules in the lung and the diffuse renal abscesses simultaneously disappeared within two months after this course of highly potent anti-bacterial therapy. DM control was also accomplished by insulin therapy.

Septic pulmonary embolism has been defined as a lung embolism caused by a blood clot infected with any of several bacterial or fungal species. Typically, patients present with high-grade fever, productive cough, general malaise and hemoptysis. The presence of DM has an unfavorable impact on disease progression and prognosis. The most common radiographic findings are bilateral peripheral nodules with feeding vessels and cavity formations less than 3 cm in diameter (1). Septic pulmonary emboli are classified according to their source, i.e.

Figure 1 - A chest roentgenogram showed poorly marginated nodules in the bilateral peripheral lung fields.

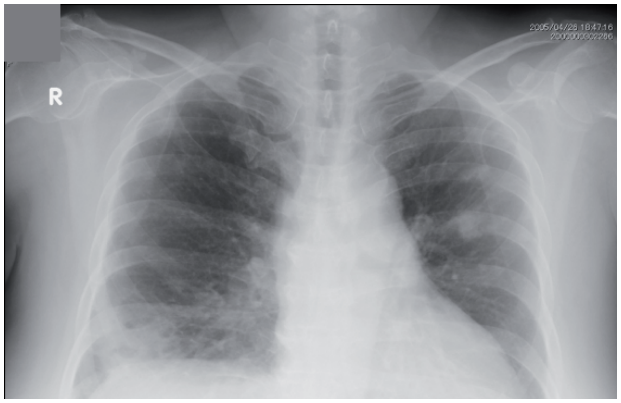


Figure 2 - Chest computed tomography (CT) showed multiple peripheral nodules with cavity formation in the bilateral lung fields.

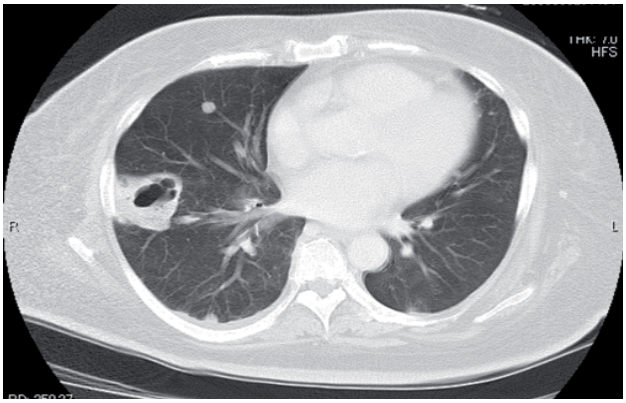
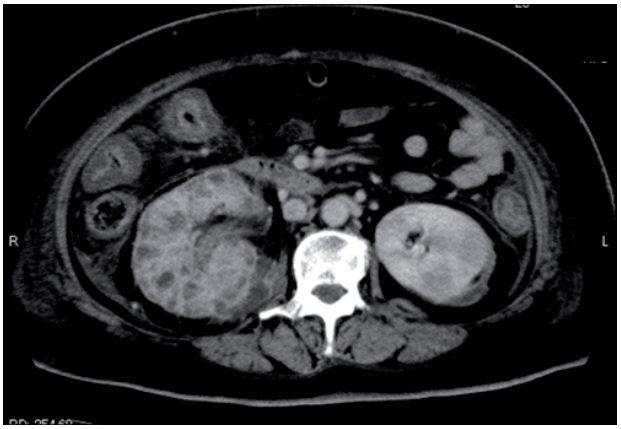


Figure 3 - Abdominal CT showed a swollen right kidney and multiple abscesses.



endocarditis, peripheral endogenous origin, or exogenous origin. Endogenous origins include peripheral abscesses without cardiac lesions (2). While septic pulmonary embolism occurring secondary to UTI appears to be rare (3,4), it should be considered a possibility in DM patients with abnormal multiple nodules revealed in radiographic examinations. Although the mechanism underlying this disease is still unknown, it is well known that renal cell carcinoma can form tumor thrombi and metastasize to the lungs via the venous vessels rather than through lymphatic spread. Thus, the septic pulmonary embolism in our case was thought to have developed from an infected thrombus originating in a renal abscess derived from the patient's UTI.

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VIDEO SECTION

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LESS Sacrocolpopexy: step by step of a simplified knotless technique

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ABSTRACT

Introduction: Pelvic organ prolapse is an ordinary disease with around 200.000 surgeries performed annually in the US to treat this condition. The surgical treatment for complete vaginal vault prolapse after hysterectomy involves abdominal or vaginal sacrocolpopexy. The purpose of this video is to demonstrate the steps of a laparoendoscopic single-site surgery (LESS) sacrocolpopexy performed by a simplified knotless technique.

Materials and Methods: A 52 year-old female submitted a total hysterectomy five years ago due to miomatosis who developed vault prolapse and urinary incontinence after surgery. She was treated by transumbilical LESS cutaneous retractor and a surgical glove attached to three trocars through a 3.5 cm umbilical incision. Patient was positioned in lithotomy, the Y-shape polypropylene mesh was passed through the trocar. Only conventional laparoscopic instruments were used for intrabdominal dissection of vagina and peritoneum. The mesh was fixed to the vaginal fornix using 3 continuous sutures held in extremities by polymeric clips. The last helical suture was fixed by polymeric clips to the sacral periosteum from the promontory to achieve good vaginal positioning without tension. The posterior peritoneum was closed over the mesh.

Results: The operative time was 150 minutes, blood loss of approximately 100 mL and the patient was discharged after 18 hours with no immediate complications and a 3 months follow-up free of vault prolapse and urinary incontinence until now.

Conclusions: LESS sacrocolpopexy performed with conventional instruments is feasible and a safe procedure reproducing surgical steps of conventional laparoscopic or robotic surgery.

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EDITORIAL COMMENT

The authors from the Department of Urology, ABC Medical School report on a case of a 52 year old female who developed vault prolapse and urinary incontinence subsequently after undergoing Total Hysterectomy. Her pathology was formally diagnosed with Pelvic Organ Prolapse Quantification (POP-Q) stage 3. The authors then highlight the steps involved in performing a Transumbilical LESS sacrocolpopexy. There are good graphic representations of the steps involved in this repair. Mesh and suture material were accurately described. Specific surgical parameters of the case were also disclosed (surgery time, estimated blood loss). Finally, post-operative report was given in support of this procedure. Limitations were acknowledged with this relatively new technique.

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