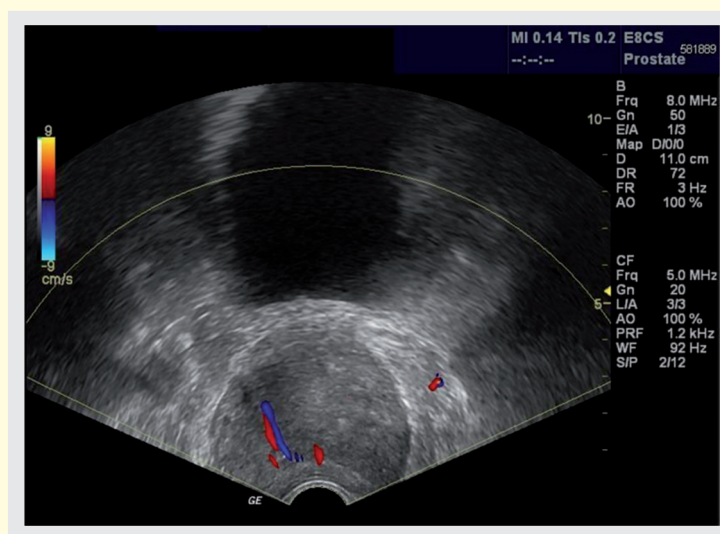




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PDUS examination of the prostatic vasculature showing urethral arteries. (Page 246)



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Editorial for the Challenging Clinical Cases Section

Why and what to write as “Challenging Clinical Cases” in the evidence based era.

Astute observations can add to our understanding of genesis, natural history, pathophysiology and treatment of diseases. Clinical cases generate new hypotheses, providing the bases for future researches that will lead to evidence-based behavior; they exercise shrewd observation skills and a curious analytical mind to understand unexplained features of a given circumstance.

Publication of case reports provides a rich opportunity for students, residents, and fellows in training who have an interest in pursuing a career in academic medicine.

Especially, case reports are appropriate to describe a new disease entity, alert others to unexpected treatment responses, and inform about an groundbreaking treatment for a rare condition, none of which circumstances is fitting for a full clinical trial.

While evidence-based medicine is utterly concerned with finding the best evidence for clinical judgments (i.e. should we apply a specific treatment or diagnostic test for an individual patient?), a case report invariably is hypothesis generating and brings a clear learning point, occasionally of an otherwise known field, an additional compelling point. “The first snap, the initial glimpse....”, that must undergo the hierarchy of evidence with a randomized trial on top.

In other words case reports are involved in the “invention phase” of the expansion of knowledge in medicine (not in its quantifiable validation); they underscore observations, the first step in the scientific method and a necessary supplement to the ambitions of evidence-based medicine. At the end, virtuous case reports always promote further research. The subsequent hypotheses generated from the report are the tentative explanations for the observation and deserve further investigations.

Nevertheless, under this critical and realistic rational it is not easy to get published on the modality, mainly considering the current vast and rapidly growing literature and the brutal competition for a space in the prominent journals. In this context, to mine good observations and ideas is fundamental and at the end undeniable essential and poignant questions must be answered: “What has been learned? Does the case truly contribute to the literature? Is there a new idea encouraging additional research?”

On the other hand, while conducting a thorough review of the literature in preparation of the report is essential, merging “a case report and a literature review” usually yields a lengthy and poorly performed hybrid. Contemporary literature reviews require evidence-based designs and have precise purpose and style. As a rule, case reports and literature reviews are separate study designs and the last is not able to retrieve or hide poor case reports.



Although it is clear that the observation of a single patient can add to our understanding of etiology, pathogenesis, natural history, treatment of particularly rare diseases, and the training of potential junior investigators; recently, the notion that case reports represent a lower quality of evidence in the design hierarchy of studies, and the increasing application of the impact factor (IF) in medical scientific publications as a metric, have frequently relegated case report to the lowest step of the hierarchy of study design and consequently under scrutiny and disfavor among some in the medical scientific publication community.

In the current evaluation system of academic journals, publication of many case reports may negatively affect the rating of an academic journal, driving many journal editors to discontinue or decrease the number of case reports to improve their journals' ratings (i.e., impact factor).

However, despite the potential effects on impact factor, we have the challenging duty of rescuing the documentation of perspicacious clinical observations aiming to revisit the important value of case reports. Many essential facts have been published initially as case reports. In addition, there may be a room for reports with educational value.

In the mission of taking the best advantage of case reports, carefully consider the information for authors in the <http://www.brazjurol.com.br/>. It is our hope that case reports still have a vital place in medical education and in the practice of medicine as the first line of sustenance in documenting clinical phenomena in the peer-reviewed literature; indisputably not in the measurable validation of previous postulations but ultimately supporting the fundamental move of offering new ideas and endorsing further research.

PROF. DR. LEONARDO OLIVEIRA REIS

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The role of radiotherapy in urinary bladder cancer: current status

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ABSTRACT

The role of radiotherapy (RT) in the treatment of urinary bladder cancer has undergone several modifications along the last decades. In the beginning, definitive RT was used as treatment in an attempt to preserve the urinary bladder; however, the results were poor compared to those of radical surgery. Recently, many protocols have been developed supporting the use of multi-modality therapy, and the concept of organ preservation began to be reconsidered. Although phase III randomized clinical studies comparing radical cystectomy with bladder preservation therapies do not exist, the conservative treatment may present low toxicity and high indexes of complete response for selected patients. The aim of this study was to review the literature on the subject in order to situate RT in the current treatment of urinary bladder cancer.

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radiotherapy; urinary bladder; neoplasms; review

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INTRODUCTION

An estimated 386,300 new cases and 150,200 deaths from bladder cancer occurred in 2008 worldwide (1).

The TNM (2) staging system is the most important independent prognostic variable in invasive urinary bladder cancer. This system is used for urothelial carcinoma, squamous cell carcinoma, undifferentiated carcinoma and urinary bladder adenocarcinoma. The spectrum of urinary cancer includes non-muscle invasive

(superficial) tumors, muscle invasive tumors and metastatic disease. Each one of these tumors presents a specific clinical behavior, prognostic and treatment. Regardless of the tumoral stage, the standard treatment for urinary bladder cancer is surgery. From endoscopic resections to radical cystectomies, surgery still offers the best locoregional control (3). However, radical cystectomy may be associated with a decrease in the patients' quality of life, especially the functional quality of the neo-bladder, even taking into account the advances of the reconstruction techniques used

for this organ (4-6). Occurrences such as urinary incontinency and erectile dysfunction (in cases in which radical prostatectomy was performed during the surgery) are not rare and can be the tiebreakers for choosing a conservative option of organ preservation.

Historically, radical radiotherapy (RT) with curative intent was offered only to patients with severe clinical comorbidities, or in an advanced stage of the disease, or older age, at the moment of the diagnostic. With the advances of the therapeutic modalities, combined multidisciplinary treatment is, at present, the one that offers the best possibilities of cure with preservation of the bladder (7).

Up to this date, there are no phase III randomized studies that compare radical surgery versus more conservative multimodality approaches. Only informations from retrospective studies or non randomized prospective series are available. Besides, RT also plays an important role in symptoms palliation in curable or incurable cases and presents different indications in the treatment of urinary bladder cancer.

This study presents a literature review, in order to situate RT in the current curative treatment of urinary bladder cancer.

Radiotherapy for superficial cancer

Information on the use of RT in the treatment of superficial urinary bladder cancer dates from before the success obtained with endoscopic treatments. Nowadays, there is no support in the literature that justifies its routine use in this group of patients (8-10).

In most reports, RT is used in patients with progressive disease or in recurrences, after many transurethral resections of bladder tumors (TURB) combined or not with intravesical therapy.

Despite of the relatively high rate of complete responses in stages Tis and Ta, it is known that approximately 50% of the patients will present recurrence in the first year after RT; in five years, this rate will be 90% (11).

There is no evidence that RT offers a higher probability of urinary bladder preservation than other treatments (TURB and intravesical therapy). However, Weiss et al. (12) suggested

that TURB followed by radiochemotherapy could be an alternative treatment for high-risk tumors (T1, G3). In view of the satisfactory results obtained with TURB and BCG, the use of RT in this context is rarely justified and therefore, should be considered only in an individual basis.

Radiotherapy for Invasive Cancer

Preoperative Radiotherapy

Preoperative RT emerged in an attempt to minimize the possible dissemination of the disease during surgery, as well as to eradicate microscopic tumor focuses that might had been situated beyond the resection margins.

At the beginning, many retrospective studies confirmed a real benefit in the use of preoperative RT compared to cystectomy alone (13-15). A review published by the University of Florida (16) in the 80's with stage T3 patients concluded that preoperative RT would give a 5-year survival benefit of 5% (15% versus 20%) when compared to surgery alone.

Conflicting results were found in studies that compared preoperative RT followed by immediate radical surgery versus radical treatment with RT and salvage surgery at recurrence (17-21). It is important to note that the RT scheme used was different in each of these studies. And, most of the times, a small number of patients were included which limited the statistical power of these analyses. Except for the MD Anderson experience (Miller) (21) that showed statistically significant benefit favoring bimodal therapy, the other studies did not find any difference in terms of patient's survival in the evaluated groups. However, the study of Miller also must be interpreted with caution considering the small number of patients analyzed and the fact that only patients with large T3 tumors were considered.

Moreover, a meta-analysis involving five randomized studies concluded that the available data from those clinical studies do not support the routine use of preoperative RT (22).

Therefore, although it has been considered an option in the past, preoperative RT is not recommended in the treatment of invasive urinary bladder cancer.

Postoperative Radiotherapy

After radical cystectomy, RT is rarely administered in the postoperative setting of patients with urinary bladder cancer. This is mainly due to the presence of high rates of side effects related to irradiation in the postsurgical period, after the abdominal manipulation and consequent fixation of both large and small bowel to the pelvis, as the neo-bladder itself. Complication rates vary around 20%. Nevertheless, the pelvic control of the disease is in general satisfactory with surgery only (13),

Positive margins or lymph node involvement are adverse risk factors for recurrence and patient survival (23,24).

Postoperative RT should be considered only in individualized situations such as recurrences of pelvic disease.

Definitive Radiotherapy

Until the development of modern surgical techniques for the performance of cystectomy, RT was widely used as a radical and exclusive treatment modality. Nowadays, RT alone should be used only in patients who present a high risk of complications during surgery or with advanced disease.

RT effectiveness in the treatment of urinary bladder cancer was repeatedly demonstrated in retrospective studies in Europe and Canada. However, these studies, most of the time, demonstrated lower local control and shorter patient survival when compared to cystectomy.

The results of a Scottish retrospective study revealed that 45.9% out of 963 patients, treated with RT alone, presented local tumoral regression (25). An English retrospective study showed 40% five-year survival with 41% of local control in 182 patients with T2 and T3 urinary bladder cancer treated with RT (26).

A Norwegian retrospective series (27) focused on patient survival after radical treatment of transitional cell carcinoma of the bladder. Forty five patients underwent cystectomy, and ninety patients underwent RT. After surgery, the ten-year overall survival for superficial and muscle-invasive tumors was, respectively, 67% and 26% and for patients treated with RT, 26% and 5%, respectively.

Fossa et al. (28) studied patients with T2 to T4 bladder cancer in whom total cystectomy could not be performed due to medical contraindications and were treated with curative RT alone. Five-year survival was 22%.

A study performed at Princess Margaret Hospital (29) in Toronto reported results after a long follow-up period of patients with invasive bladder cancer (T1 to T4) treated with radical RT. Of 340 patients evaluated, 247 received RT alone. Complete response rate was 63.5% for the whole group. Overall and cause-specific survival and local control rate in ten years was 19%. In 131 patients with disease limited to the bladder wall (T2N0M0), cause-specific survival and local control rate in ten years were 68% and 60% ($p = 0.02$) with the absence of carcinoma in situ and 47% and 28% ($p = 0.03$) with the presence of carcinoma in situ, respectively. In the multivariate analysis, younger age, lower T stage, and absence of carcinoma in situ were associated to a significant increase in local control and survival ($p \leq 0.01$). The study concluded that RT-based treatment is a good alternative to radical cystectomy, regarding local control and survival and may be used in selected patients.

There is a sub-group of patients who may benefit from RT alone: T2 tumors, patients with no urethral obstruction, complete transurethral resection, solitary tumor and absence of T4 disease.

A study conducted by Shipley et al. at the Massachusetts General Hospital (30) revised the results of 55 patients treated with RT alone in order to identify factors associated with tumor radioresponsiveness and patient cure. Sixty seven percent of the patients presented T2 or T3 clinical stage and 33% were T4. Overall 5-year survival was 28% for the whole group. When comparing T2/T3 with T4 patients, survival was 45% versus 9%, respectively ($p = 0.009$). Within T2/T3 group the most important prognostic factor was the histological finding of tumor on the papillary surface of the bladder, with 63% local control rate and 62% 5-year survival versus 20% and 0% in the presence of solid or bulky tumors. Other important prognostic factors in five-year survival in this group were the extent of complete transurethral resection (54% complete versus 17% incom-

plete, $p = 0.009$) and urethral obstruction in the intravenous pyelogram (47% without obstruction versus 14% with obstruction, $p = 0.01$). The study concluded that RT may be used in the treatment of muscle-invasive tumors, with a better probability of success in patients with less advanced clinical stages, in the presence of tumors on the papillary surface, in the absence of urethral obstruction, and with the possibility of complete transurethral resection.

Similar findings were published by Mameghan et al. (31) in which bad prognostic factors for vesical recurrence were tumor multiplicity, the presence of urethral obstruction and larger tumor size.

Recently, a German series (32) reported the results of 75 patients with localized muscle-invasive bladder carcinoma (T2, $n = 34$; T3, $n = 32$; T4, $n = 9$). Patients were considered not suitable for radical surgery due to advanced age, comorbidity or inoperability. Definitive RT was delivered with tridimensional conformal technique without concomitant chemotherapy. Complete response was obtained in 2/3 of the patients and the 3-year overall survival rate was 56.9%. Although the short follow-up, they concluded that RT is an effective treatment option in terms of local control and survival even in elderly patients with locally advanced bladder cancer not suitable for cystectomy.

Multimodal therapy

The rational of a multidisciplinary approach of invasive bladder cancer is based on the promising results of combined therapeutic modalities when compared to radical surgery.

TURB is used to reduce the tumor volume to be irradiated. Moreover, it is known that transitional cell carcinoma of the bladder is highly chemoresponsible. Chemotherapy attempts to eradicate local and systemic disease and to increase RT effect on locoregional control. This multimodality approach was pioneered by Housset et al. (33).

The National Cancer Institute of Canada Clinical Trials Group (34) in a prospective randomized study showed the benefits of adding chemotherapy to RT in patients with muscle invasive bladder cancer. Patients with T2 to T4 tumors

were randomized to receive or not concomitant chemotherapy with RT. A lowest percentage of pelvic recurrence was observed in the arm that received the combined therapy (59% versus 40% $p = 0.038$). Overall survival after three years was higher in the group that received cisplatin (47% versus 33%), however, with no statistical significance ($p = 0.34$).

Recently, a phase III study (BC 2001 CRUK/01/04) (35) demonstrated that radiochemotherapy increases the locoregional control of tumors invading the muscular layer, when compared to RT alone, with preservation of the vesical function and without increase in acute or late toxicity. Patients were randomized to receive 5-fluorouracil (5-FU) with mytomicin C, concomitant to RT and a 2-year locoregional control rate of 67% was achieved, compared to 54% for patients submitted to RT only.

Until now, there are no level I evidence studies that compared radiochemotherapy with radical cystectomy. Data obtained from prospective and retrospective series point out the real benefits of the combination. The largest experiences are from the groups of the Massachusetts General Hospital, USA, of the University of Erlangen, Germany, and of the University of Paris, France. Combined treatment protocols (TURB followed by radiochemotherapy) come from these institutions.

The German study, conducted by Rödel et al. (36), analyzed the combined treatment with bladder preservation and tried to identify factors that could predict treatment response, risk of recurrence and survival. They evaluated 415 patients (89 high risk T1; 326 T2 to T4) who were treated with RT (126) or radiochemotherapy (289) after TURB of the tumor. Six weeks after treatment, the response was evaluated by re-staging TURB. In case of a complete response, the patients were kept under observation in regular intervals. In case of tumor persistence or invasive tumor recurrence, salvage cystectomy was recommended. Seventy two percent of the patients had complete response with maintenance of local control and 64% of the patients did not present recurrence, after 10 years of follow-up. Distant metastases were diagnosed in 98 patients with a 35% rate

in 10 years. Ten-year disease-free survival was 42% and more than 80% of the surviving patients had their urinary bladder preserved. Radiochemotherapy was more effective than RT alone regarding local control and survival. The initial stage of the tumor and a complete TURB were the most important predictive factors of both local control and survival.

Weiss et al. (12) included 112 patients with muscle invasive urinary bladder cancer or high risk T1 (grade 3, associated Tis, multifocal, diameter > 5 cm). The protocol consisted of TURB followed by cisplatin and 5-FU administered from days 1 to 5 and 29 to 33 of RT. Treatment response was evaluated by re-staging TURB 4 to 6 weeks after radiochemotherapy. In case of residual invasive tumor or recurrence, salvage cystectomy was recommended. Complete response was observed in 88% of the patients, and 72% did not present local or distant recurrence. Overall and cause-specific survival rates for all patients were 74% and 85% in 5 years, respectively. Of all surviving patients, 82% remained with their own bladder and of these, 79% were satisfied with their urinary condition.

Patients with high risk T1 tumors (G3, associated Tis, multifocal, or recurrent) should be evaluated with caution. In many of them, TURB followed by intravesical therapy (BCG or chemotherapy, for example) is used. However, a considerable percentage of these patients (15 to 40%) present local recurrence, jeopardizing the possibilities of initial cure (37). This is the reason why some authors indicate radical cystectomy as the first line standard treatment for these patients. As a consequence of these uncertainties, many patients are undertreated (TURB followed by intravesical therapy) or overtreated (radical cystectomy as initial treatment). Moreover, many patients submitted to staging with TURB may be understaged, presenting more invasive tumors in up to one third of the cases (38,39). Considering this, RT would play a role in the treatment of tumors invading deeper layers and in eventually involved lymph nodes. The results presented in Weiss's study (12) seem to be superior when compared to TURB studies followed by intravesical BCG (40,41) and are relatively equivalent to radi-

cal cystectomy (42). This suggests that TURB followed by radiochemotherapy may be an adequate treatment for patients with high risk T1 stage.

A more recent study by Caffo et al. (43) supports these findings. They studied 26 patients who underwent transurethral tumor resection followed by a radical dose of external radiotherapy with concomitant cisplatin and weekly gemcitabine therapy. With a median follow-up of 74 months, 5-year overall survival rate was 70.1%, with 5-year disease-specific survival and bladder-intact survival rates of 78.9% and 73.8%, respectively.

So, in general, we can consider that RT alone is inferior to the combined scheme with chemotherapy. Also, it is important to note that the complete pathological response rates observed in re-evaluation cystectomy or at the end of the combined treatment varies from 50% to 90%, being consistent in approximately 70% of the patients. Five-year overall survival is approximately 60% and about at least half of the patients maintains a functional urinary bladder after 5 years of follow-up. However, much of the evidence of the multimodality approach including endoscopic resection and radiotherapy combined with chemotherapy is retrospective and involves treating locally advanced poor-risk patients (44). Prospective evaluation of these treatments for truly localized (T1/2) bladder cancer should be encouraged.

Induction chemotherapy followed by radiotherapy/radiochemotherapy

For a long time, many institutions established two cycles of induction chemotherapy before radiochemotherapy for the treatment of tumors in an advanced stage (45). However, more recently, many authors have questioned the success of urinary bladder preservation based on the treatment with induction chemotherapy (46,47).

Arias et al. (48) in a prospective study included 50 patients in good clinical conditions who had operable urinary bladder invasive cancer (T2 to T4). The treatment protocol was as follows: 1) TURB; 2) two cycles of chemotherapy (methotrexate, vinblastine, adriamycin and cisplatin - MVAC); 3) 45 Gy RT with concomitant chemotherapy (cisplatin); 4) cystoscopic evaluation: in the presence of a complete response, RT was complet-

ed until 65 Gy; if the response was incomplete, cystectomy was performed. Sixty eight percent of the patients had a complete response. Five-year local control and overall survival were 48% and 47%, respectively. For patients who presented a complete response, 65% and 70%, respectively.

The role of induction chemotherapy was tested in a phase III study conducted by the Radiation Therapy Oncology Group (RTOG) (49). The objective of this study was to test the efficacy of neoadjuvant methotrexate, cisplatin and vinblastine (CMV) in patients with muscle-invasive urinary bladder cancer treated with an organ preservation approach. One hundred and twenty three patients (clinical stage T2 to T4aNXM0) were randomized to receive pelvic irradiation (39.6 Gy) with concomitant cisplatin with (arm 1, 61 patients) or without (arm 2, 62 patients) two cycles of neoadjuvant CMV. Patients that presented complete response received additional RT (25.2 Gy) with one more dose of cisplatin. Patients whose responses were not complete were submitted to cystectomy. Seventy four percent of the patients completed the protocol (67% arm 1 and 81% arm 2). Five-year overall survival was 49% (48% arm 1 and 49% arm 2). Thirty five percent of the patients presented evidence of distant metastases in 5 years (33% arm 1; 39% arm 2). Survival rate in 5 years with functioning bladder was 38% (36% arm 1; 40% arm 2). These differences were not statistically significant.

Additionally, Perdonà et al. (50) reported a promising result with 2 cycles of neoadjuvant chemotherapy followed by radiotherapy (n = 43 patients) or radiochemotherapy (n = 78 patients). Complete response was observed in 102 out of 119 evaluable patients. With a median follow-up of 66 months, the 5-year tumor-specific, overall, and bladder-intact survival rates were 73.5%, 67.7%, and 51.2%, respectively.

The larger randomized study of neoadjuvant chemotherapy using the CMV regimen (3 cycles), followed either by surgery or RT or both, was conducted by the Medical Research Council / European Organization for Research and Treatment of Cancer (MRC/EORTC) with 976 patients (51). At first, a non-significant increase of overall survival in the group that received neoadjuvant

therapy was observed (HR = 0.85; 95% CI: 0.71 - 1.02; p = 0.075). The results of this study were recently updated and the survival advantage of neoadjuvant CMV became statistically significant with 16% reduction in the risk of death (hazard ratio, 0.84; 95% CI, 0.72 to 0.99; p = 0.037, corresponding to an increase in 10-year survival from 30% to 36%) after CMV (52).

Concluding, for deeply invasive bladder cancer, induction chemotherapy with CMV followed by definitive local therapy presents a clinically relevant survival benefit and may be considered as first-line adjunctive treatment and should be viewed as the state of the art, as compared with cystectomy or radiotherapy alone (52).

Alterations in Radiotherapy Fractionation

Usually, RT is fractionated in 1.8 to 2 Gy per day, 5 days a week. A total dose of 45 to 50 Gy is delivered to the pelvis and 55 to 70 Gy to the bladder tumor bed, achieving favorable rates of local control (53).

A Dutch review (54) emphasized the importance of the treatment dose in which 10 Gy increments in the final dose of RT yielded an increase of about 50% in local control rate in three years.

Some groups have used RT schemes with altered fractionation (hyperfractionated or accelerated fractionation) in an attempt to improve the results of irradiation. Hyperfractionation consists in increasing the number of fractions per day, with a lower dose per fraction, but with an increase in the total daily dose, ending the therapy in a period similar to conventional RT, with an absolute higher final dose. Accelerated RT implies in the administration of the same number of fractions in a shorter period of time, in other words, an equivalent higher total dose (55).

A pilot study conducted by the Oxford group (56) treated 24 patients with invasive urinary bladder cancer with RT with 54 to 64 Gy, using 1.8 to 2 Gy fractionation, twice a day with a six hour interval between the fractions. Grade 4 or 5 acute urinary toxicity was not registered. Expected survival and local control in two years were 35% and 56%, respectively.

Näslund et al. (57) in a randomized study with 168 patients compared two schemes of RT: hyperfractionation with 1 Gy, three times/day and 84 Gy total dose versus 2 Gy, once/day with 64 Gy total dose (conventional fractionation). After a ten year follow-up, a higher local control rate and patient survival was observed in patients submitted to hyperfractionated RT.

A meta-analysis (58) comparing several schemes of hyperfractionated RT used in different kinds of cancer also pointed out a gain in favor of altered fractionation.

A prospective randomized study elaborated at the Royal Marsden Hospital (59) evaluated the effectiveness and the toxicity of accelerated fractionation in the treatment of invasive urinary bladder cancer. Two hundred and twenty nine patients (T2 or T3, N0 or N1, M0) were randomized in two groups: accelerated fractionation - 60.8 Gy in 32 fractions in 26 days; conventional fractionation - 64 Gy in 32 fractions in 45 days. Acute toxicity was observed in 121 patients of the accelerated fractionation and in 96 patients of the conventional fractionation group. There was no statistically significant difference in local control and disease-free and overall survivals. Accelerated RT was not more effective than the conventional fractionation for T2 and T3 tumors and was associated to higher acute toxicity.

Hypofractionated RT which consists on the administration of larger daily fractions (2.5 Gy to 6 Gy, in general) was also studied. Only one phase III randomized study compared conventional RT (1.5 Gy/day with a total dose of 60 Gy) with hypofractionated RT (3 Gy/day fractions totalizing 30 Gy, followed by a four week rest and a second course with 30 Gy, 1.5 Gy/day). The arm that received hypofractionated RT presented a lower 5-year survival (39% versus 52%) (60).

In conclusion, total RT dose is important for locoregional control. Hyperfractionated RT schemes, most probably due to the higher total dose of the treatment, present a real benefit in relation to conventional RT. Accelerated and hypofractionated RT schemes are not recommended, and may present higher toxicity.

Perspectives

RT current techniques using tridimensional planning systems, intensity modulated RT (IMRT) and image-guidance allow a greater dose-escalation to the treatment targets, with lower doses to the normal adjacent tissues and, consequently, less treatment related toxicity. However, regarding urinary bladder cancer, due to the anatomic alterations inherent to the organ filling that occur inter and intrafractions, the use of high precision techniques may be more limited, both for target definition and protection of organs at risk. Besides that, such techniques are promising and provide the tools for dose-escalation and improvement in local control and, eventually, in survival.

Comprehensive summary

- RT for superficial tumors is not indicated.
- Pre or postoperative RT is not indicated.
- Radiochemotherapy is an option in the conservative treatment of selected cases of invasive urinary bladder tumors: solitary tumor (T2 to T4) less than 5 cm, absence of hydronephrosis, complete transurethral resection and normal bladder function.
- RT alone should be indicated with palliative intention, or for unresectable tumors, or in inoperable patients, when combination with chemotherapy is not possible.

CONFLICT OF INTEREST

None declared.

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EDITORIAL COMMENT

The authors reviewed the current status of radiation therapy (RT) in the management of bladder cancer, which become more attractive to the patients due to the possibility to retain the urinary bladder and having better quality of life. Beside that, this approach will be an alternative for patients who could not underwent operation due to their medical condition. Based on the recent studies the authors concluded that radiochemotherapy is an alternative for selected muscle invasive bladder cancer patients instead of radical operation.

Several studies reported that trimodality treatment: complete TUR-BT, chemotherapy, and RT could have similar results compared to radical cystectomy. Five-year overall survival reaching 70% and about 80% of the surviving patients could preserve their bladder (Ref. 12, 36, and 43 in the article). The limitations are delivery of ad-

equate dose of chemotherapeutic agents into the tumor site and its systemic adverse events. To overcome this problem, some investigators developed novel approach such as balloon-occluded intra-arterial infusion of gemcitabine and cisplatin with concomitant hemodialysis and concurrent RT (1). In the same time, more understanding of the tumor biology and incorporating molecular markers as predictive factor for successful treatment lead toward better patient selection; development of new chemotherapeutic, including targeted therapy drugs, in combination with RT, will improve the effectiveness of this treatment strategy for tumor control (2).

Important thing to be remembered when choosing this treatment modality is the need for strict follow-up by periodic cystoscopy and also the patients should be aware of the possibility for cystectomy that needs to be done in case of disease progression.

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Comparison of the Epidemiologic Features and Patterns of Initial Care for Prostate Cancer between Public and Private Institutions: A Survey by the Brazilian Society of Urology

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ABSTRACT

Objective: To describe the epidemiological features and patterns of initial care for prostate cancer at public and private institutions in the State of Sao Paulo, Brazil.

Materials and Methods: A total of 1,082 physicians affiliated to the Sao Paulo Section of the Brazilian Society of Urology were invited to participate in this cross-sectional, web-based survey. Between September 2004 and September 2005, participating urologists entered data on demographic, clinical and pathological characteristics of patients diagnosed with prostate cancer in their practice. Data on patients attended at public institutions were analyzed and compared with those patients attended at private practice.

Results: One hundred and ten society members contributed with data from 1915 patients, 1026 (53.6%) of whom from public institutions. When compared with patients attended at private institutions, those attended at public institutions were older and more likely to be black, had higher serum prostate specific antigen (PSA) levels, had a higher probability of being diagnosed with metastatic disease, but were less likely to undergo prostatectomy (all $P < 0.001$). In multivariate analysis, age, biopsy Gleason score, and being attended at a public institution were independently associated with metastatic disease upon diagnosis. The significant predictors of nonsurgical treatment were age, black race, and higher serum levels of PSA.

Conclusions: A statewide registry provides valuable information regarding patient demographics, clinical features, and patterns of care. The results of this study suggest that significant disparities exist for patients with prostate cancer attended at different health-care systems. The relative contribution of biological versus socioeconomic features remains uncertain.

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Cross-sectional studies; epidemiology; prostatic neoplasms; insurance; health

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INTRODUCTION

Prostate cancer is one of the leading neoplasms in the male population worldwide (1); it is currently the most frequent tumor type in

Brazil, where an estimated 52,000 new cases are diagnosed each year (2). With nearly 21% of the country population (3), the State of Sao Paulo contributes with an estimated 13.160 annual cases of prostate cancer, or nearly 25% of all new cases

of this disease in Brazil (2). In the State of Sao Paulo, approximately 38% of the population had access to private health insurance in 2003, and 40% in 2008 (4). Accordingly, an estimated 37% of patients with prostate cancer in the State of Sao Paulo received medical care under coverage by private insurance companies (5). The vast majority of the remaining individuals not covered by private insurance receive medical care through the public health-care system, whose hierarchical structure dictates patient referrals to more complex health organizations in geographically defined areas.

Since prostate cancer accounts for a sizeable proportion of the workload of many urologists, many of whom base their practice in publicly-funded organizations, the Brazilian Society of Urology (Sociedade Brasileira de Urologia, SBU) committed to undertake a statewide survey, whose overall results have been published (5). The aim of that survey was to describe the demographic, clinical, and pathological characteristics, as well as the patterns of initial care, for patients with prostate cancer diagnosed and treated by members of SBU in the State of Sao Paulo. In the current analysis, the aim was to compare in detail such features among patients treated at public and private institutions in the State of Sao Paulo during the study period.

MATERIALS AND METHODS

Overall study design

In this cross-sectional study, a web-based survey was conducted with 1,082 SBU members from the State of Sao Paulo, who were invited to participate in the study through mailed announcements and advertising of the study on the SBU website (www.sbu.org.br). The questionnaire used in the survey was available at the website for the duration of the study, and could also be sent by regular mail or fax upon request. In addition, supplementary information and reminders were regularly sent by regular mail and e-mail to all SBU members in the State of Sao Paulo. The study protocol was approved by three academic institutional review boards (IRBs) and by the State Health Secretariat, and all participating patients

signed an informed consent document. Protocol submission to local IRBs was left to the discretion of investigators according to local policies.

Data collection

Participating physicians were asked to collect data from patients with prostate cancer seeking medical attention at public or private practices between September 2004 and September 2005. The questionnaire contained 23 questions related to characteristics of the participating physician, demographic and socioeconomic patient features, and clinical and pathological aspects of prostate cancer. Special attention was given to the flow of patient care, and places of residence, diagnosis and care were ascertained from each case. For staging purposes, the 2002 International Union Against Cancer/Tumor Node Metastasis (TNM) classification system was used (6). All filled questionnaires were kept for final analysis in a central database that was maintained under the supervision of SBU, whose scientific committee vouched for the data presented herein.

Statistical analysis

Descriptive statistics were used for the demographic, clinical, and pathological variables. Exploratory analyses were conducted for comparisons between groups of patients according to medical or socioeconomic features of interest. The chi-square test was used to compare the frequency of categorical variables between groups, and the Mann-Whitney test was used to compare continuous variables with non-normal distribution. Logistic regression models were used to explore the associations between selected potential risk factors and categorical outcomes. All P values were two sided, and $P < 0.05$ was considered significant. All data analysis was conducted using the MedCalc software, version 11.0.0.0 (MedCalc, Mariakerke, Belgium).

RESULTS

Physician and patient characteristics

One hundred and ten of the 1,082 (10.2%) registered SBU members contributed with data from a total of 1915 patients, an average of 17.4

patients per urologist over the 1-year period. Participating urologists had an average of 44 years of age (range, 26 to 74). Among the patients included in the study, 1,026 (53.6%) had been attended at public institutions. Table-1 shows the chief demo-

when black patients were compared with those of other races (15.4% versus 12.8%, respectively; $P = 0.394$). However, black patients had higher serum levels of PSA than white and Asian patients (median of 12.5 versus 9.6 ng/mL; $P = 0.001$).

Table 1 - Demographic characteristics of patients attended at public institutions.

Characteristic	Number	Percent
Age, years		
Mean (± SD), range	68 (± 8), 36 to 95	
Race		
White	828	80.7
Black	188	18.3
Asian	10	1.0
Family history of prostate cancer		
No	737	71.8
One case	86	8.4
Two or more cases	15	1.5
Unknown	188	18.3

SD: Standard Deviation.

graphic characteristics of these patients. Their mean age was 68 years (range, 36 to 95), nearly 80% of patients were reportedly white, and approximately 70% had no family history of prostate cancer.

Disease characteristics

The clinical characteristics of patients attended at public institutions are shown in Table-2. The serum level of prostate specific antigen (PSA) upon diagnosis was available for 976 patients (95.1%); the median value was 10.0 ng/mL, ranging from 0.1 to 2890.0 ng/mL. The biopsy Gleason score was not known for nine patients (0.9%). In the remaining cases, the most frequent scores were 5 or 6 (51.8%), followed by 7 (32.1%); only 13.3% of patients had a Gleason score of 8 to 10. There was no significant difference in the percentage of patients with a Gleason score of 8 to 10,

Regarding the anatomic disease stage, slightly over two-thirds of patients had localized disease, 20.2% had locally advanced disease, and 10.4% had metastatic disease (Table-2). Among patients with metastatic disease, the most frequent primary sites of metastases were the bones (65.4%).

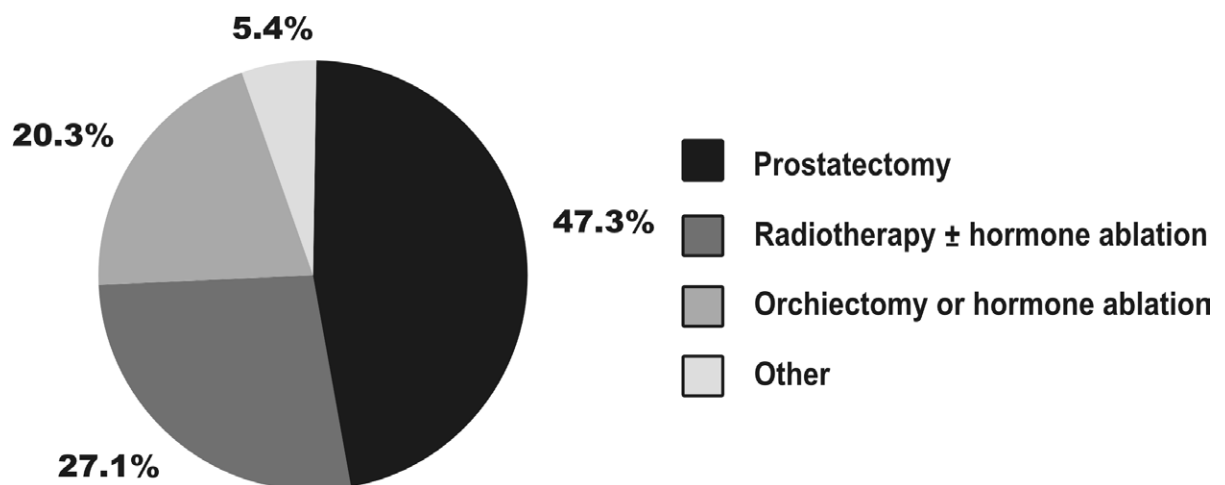
Patterns of initial care

Figure-1 displays the treatment modalities recommended for patients attended at public institutions. Surgery was the most frequent modality and was recommended for 485 patients (47.3%). Of these patients, 321 (66.2%) were recommended to undergo retropubic prostatectomy, 161 (33.2%) perineal prostatectomy, and only three were referred to laparoscopic surgery. Radiotherapy with or without hormone therapy was the recommendation in 278 cases (27.1%), orchiectomy or oth-

Table 2 - Clinical characteristics of patients attended at public institutions.

Characteristic	Number	Percent
PSA, ng/mL		
Median (IQR), range	10.0 (6.4 to 23.0), 0.1 to 2890.0	
Mean (± SD)	36.7 (± 143.3)	
Gleason score		
2 to 4	21	2.0
5 or 6	531	51.8
7	329	32.1
8 to 10	136	13.3
Unknown	9	0.9
Disease stage		
Localized	712	69.4
Locally advanced	207	20.2
Metastatic	107	10.4

IQR: Interquartile Range; **PSA:** Prostate Specific Antigen.

Figure 1 - Initial treatment modality recommended for patients attended at public institutions (N = 1026).

er types of hormone ablation alone in 208 cases (20.2%), and palliative care or other modalities were recommended for only 55 cases (5.4%).

Comparisons between public and private institutions

When compared with patients attended at private institutions (N = 889), those attended at public institutions were slightly older (median ages of 67 versus 69 years; $P < 0.001$) and more likely to be black (7.0% versus 18.3%; $P < 0.001$). Patients attended at public institutions had a higher median value of PSA (10.0 versus 6.8 ng/mL; $P < 0.001$) and a higher probability of being diagnosed with metastatic disease (10.4% versus 4.2%; $P < 0.001$) than patients attended at private institutions. As shown in Table-3, adjustment for age, black race and Gleason score disclosed that being attended at a public institution was independently associated with metastatic disease upon

diagnosis when the total sample of 1915 patients was considered. Other independent variables for the presence of metastatic disease upon diagnosis were age and biopsy Gleason score. In addition, patients attended at public institutions were significantly less likely to undergo prostatectomy than patients attended at private institutions (47.3% versus 61.3%; $P < 0.001$). However, after adjusting for age, race, serum PSA upon diagnosis, and Gleason score, being attended at a public institution was not associated with treatment modalities other than prostatectomy. The only significant predictors of such modalities (i.e., no prostatectomy) were age, black race and higher serum levels of PSA considered as a continuous variable (Table-4). Similar results were observed when the analysis was restricted to patients with no metastatic disease at diagnosis (data not shown). Finally, patients attended at public and private institutions were compared regarding the

Table 3 - Multivariate analysis of factors associated with metastatic disease upon diagnosis in the total sample of 1915 patients.

Variable	Multivariate OR (95% CI)	P value
Age	1.03 (1.01 to 1.06)	0.002
Black race	1.09 (0.67 to 1.78)	0.718
Gleason score	1.19 (1.15 to 1.25)	< 0.001
Diagnosis at public institution	2.38 (1.59 to 3.56)	< 0.001

CI: confidence interval; OR: odds ratio.

Table 4 - Multivariate analysis of factors associated with prostatectomy as initial treatment in the total sample of 1915 patients.

Variable	Multivariate OR (95% CI)	P value
Age	0.88 (0.86 to 0.89)	< 0.001
Black race	0.57 (0.40 to 0.81)	0.002
Serum PSA	0.95 (0.94 to 0.96)	< 0.001
Gleason score	0.99 (0.96 to 1.02)	0.773
Diagnosis at public institution	0.84 (0.67 to 1.05)	0.127

CI: confidence interval; OR: odds ratio; PSA: Prostate Specific Antigen

need of displacement from the city of residence to another city to be diagnosed or receive care. For this analysis, patients attended at private institutions were divided into two groups based on whether they have used or not a health insurance; nine patients attended at private institutions had no available information on the use of a health insurance and were excluded from this specific analysis. As shown in Figure-2, patients attended at private institutions with no health insurance were more likely to dislocate from the city of residence (126/172; 73.3%), followed by patients attended at public institutions (705/1026; 68.7%) and patients attended at private institutions using a health insurance (266/708; 37.6%). There was a significant difference between the three groups regarding the need of displacement ($P < 0.0001$), with patients using a health insurance being significantly less likely to have to dislocate.

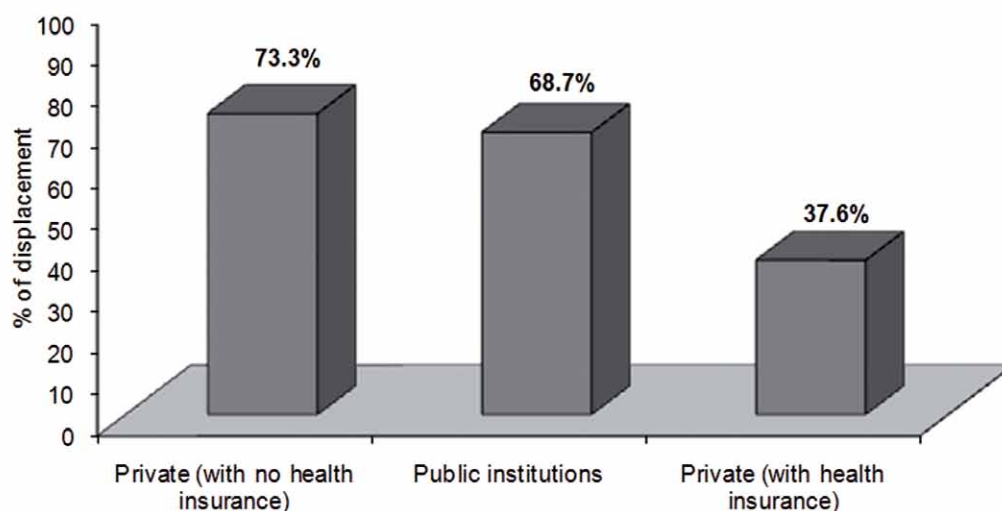
DISCUSSION

The current study represents an effort to characterize the demographic and clinical features, as well as the patterns of care for patients with prostate cancer attended at public and private institutions in the State of Sao Paulo, Brazil.

During the study period, data were collected on 1,915 patients, a figure that corresponds to 16% of all cases estimated by the Brazilian National Cancer Institute for that State in 2005 (7). Of those cases, 53.6% were attended at public institutions, a percentage that conforms to the proportion of the population in the State of Sao Paulo with no coverage by private insurance companies (4). Therefore, it is likely that the data presented herein are representative of the whole population of patients with prostate cancer in the State of Sao Paulo, Brazil. On the other hand, it is possible that the method of data collection and the profile of the urologists that decided to participate in the study may have introduced unknown biases. However, there is not a priori reason to believe that such biases are present in the study. Moreover, to our knowledge, this study represents the one with the highest number of participating urologists contributing with data in the state of Sao Paulo, Brazil.

Overall, the data presented herein support the notion that patients attended at public institutions are slightly older and more likely to be black, and have higher levels of PSA and a higher probability of being diagnosed with metastatic disease than patients attended at private institutions. In addition, patients attended at public institutions

Figure 2 - Need of displacement from the city of residence, according to the health-care system.



appear to be significantly more likely to undergo treatment modalities other than prostatectomy, possibly as a result of demographic and clinical characteristics, such as the older age and the higher serum levels of PSA in public institutions, when compared with private institutions.

The biological differences in prostate cancer between black and non-black patients have been highlighted in the literature for several decades (8). The incidence and mortality rates for prostate cancer are higher in blacks and African-Americans than in other ethnic groups (9). When adjusted for other biological disease features, PSA levels are higher in blacks and African-Americans than in other groups, regardless of socioeconomic factors (10-13). In the United States, black patients were more likely to receive nonsurgical treatment for prostate cancer (14,15). In agreement with these findings, in the current study, black individuals had higher serum levels of PSA than whites and Asians, and black race was associated with the use of treatment modalities other than prostatectomy. On the other hand, no association was found between race and the percentage of patients with biopsy Gleason scores of 8 to 10, and black race was not identified as an independent variable for the presence of metastatic disease upon diagnosis. Of note, contrasting findings among studies conducted in different countries may reflect distinct ancestors and, thus, genetic differences between Brazilian and North-American black population. In this regard, it is known that the Bantu haplotype predominates in Brazil, as the Brazilian black population is mostly originated from Angola, Congo and Mozambique, while other haplotypes predominate in the North American black population (16,17). In addition, countries differ regarding the degree of miscegenation. As recently published by the Instituto Brasileiro de Geografia Estatística (IBGE), in Brazil, black race is associated with lower annual and familiar income, lower educational level as well as access to health assistance, when compared with other races (18), factors that probably contribute with the unfavorable kind of disease presentation in this group.

Since patients attended at public institutions were more likely to be older and present higher PSA levels, these factors may have contrib-

uted to the lower frequency of prostatectomy observed for patients attended at these institutions. Furthermore, patients treated at reference public hospitals, most of the time, are referred from different areas and in many times the treatment is postponed after the diagnosis. In this regard, we found that displacement from the city of residence to another city in order to seek for diagnosis and care was more frequently required among patients attended at public institutions or at private institutions with no health insurance than in the group of patients attended at private institutions, but with health insurance. Although similar rates of dislocation were observed for patients attended at public institutions and those attended at private institutions with no health insurance, reasons for dislocation probably differed between these two groups, although not accessed in this study. Patients attended at public institution seem to dislocate to a different city as part of the patient flow that is established at the public health-care system, while patients attended at private institutions choose where they want to be treated at. Contrasting with these two groups, patients with health insurance were less likely to dislocate, what may be related to the fact that health insurance companies generally provide and obligate the complete treatment in a city not allowing patients to choose where they want to be treated. The follow up schedule (need to travel) and the perception level of patients and families regarding disease severity are other factors that may influence the decision on screening an asymptomatic patient and choose the treatment modality.

Screening can be defined as the application of diagnostic tests or procedures to asymptomatic people for the purpose of dividing them into two groups: those who have a condition that would benefit from early intervention and those who do not, other issues of equal importance include using scarce resources efficiently and rationally. It is important to recognize that the ultimate purpose of screening is to reduce morbidity and mortality. If improved outcomes cannot be demonstrated, the rationale for screening is lost. Although screening is unquestionably important, other issues of equal importance include using scarce resources efficiently and rationally. In private offices the physi-

cian-patient relationship is crucial and can be influenced by economic factors, resources available and patient's characteristics; on the other hand, in public institutions screening policies are different from the one adopted at private institutions and not infrequently the treatment is postponed due the disparity between the number of patients requiring treatment and the scarce resources.

The debate on the association between a lower socioeconomic status and variables such as stage at diagnosis, use of less aggressive treatment, and mortality, has been ongoing in the United States and other countries for several years (19-21). Socioeconomic factors are clearly associated with the patterns of care for various tumor types, including prostate cancer (14,19).

Brazil is a country with larger inequalities in socioeconomic status and more uneven access to medical care between socioeconomic groups. Three recent studies (22-24) two from the United States and one from Brazil found that prostate cancers were more commonly advanced at diagnosis and the prostate cancer mortality was higher among men with lower socioeconomic status than among those with higher socioeconomic status. Therefore, it is possible that differences in patterns of care between public institutions and private institutions in Sao Paulo State are due to a set of factors such as discrepant local screening policies, tumor biological behavior, socioeconomic status and geographic differences, among patients attended at these two types of health-care system (25,26).

In the United States, regional differences have been found in how physicians and patients select treatment options (15,27). The results of the current study should not be extrapolated to Brazil as a whole, because cultural differences, screening policies, public and private health systems and treatment resources availability may vary across the country.

CONCLUSIONS

The results presented herein confirm that statewide registry of prostate cancer is feasible and provides valuable information regarding patient demographics, clinical features, and patterns

of care, analogously to national studies previously conducted by SBU in other disease settings (28,29). The results of this study suggest that significant disparities exist for patients with prostate cancer attended at public and private institutions, but the relative contribution of biological versus socioeconomic features remains uncertain at present. Further studies with longer follow-up are necessary to assess the impact in quality of life and prostate cancer mortality in patients attended at public and private institutions.

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CONFLICT OF INTEREST

None declared.

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EDITORIAL COMMENT

Limiting the rationale of its handling, the prostate cancer (PCa) natural history is varied, often long, and not consistently predictable and in a life period in which competing causes of mortality are high and erratic.

In this complex scenario, although it is known that the only valid comparisons are in the context of randomized clinical trials, head-to-head comparisons are frequent because of the paucity of adequate trials. Add to this the fact, the population-based studies in Brazil are even scarcer and very limited. Even including about

only less than 10% of exposed urologists-patients, the study of Nardi et al. is reasonably balanced and seems to represent a reliable random sample of the target population.

Considering the routine use of serum PSA testing and screening programs, resulting in a significant stage migration and improvement in outcome for patients with PCa (while some men depending on the public health system tend to have their treatment delayed), those under private practice tend to be over-tested and over-diagnosed due to a myriad of circumstances (including

medical income), supporting the classic aphorism: “Too late or too soon?”

In the highlighted study the possibility of selection and lead-time biases should be emphasized - once careful assessment of every diagnostic and therapeutic step taken in the study patients is mandatory to ensure that no stage or grade migrations have occurred. Those in the public system may have limited access to diagnostic tests and screening. In this regard, many uncontrolled variables, especially pre-diagnosis exposure to PSA and DRE and the impact of university-level education of institutions, should ideally be considered and compared between groups, mainly in future studies.

While this provocative study should be read with caution due to the complexity of interpreting prostate cancer outcome data (making comparisons among different groups of patients receiving different treatments at different institutions and over different conditions fraught with very serious errors and fundamentally unreliable), it stimulates important reflections.

“What would be the equilibrium between over and under diagnosis in the private-public dichotomy of the Brazilian medical system?” “Were all the biopsies necessary and all cancers required treatment? Especially in the private system?”

In our risk-averse society, clinically insignificant cancers are indeed found with pros-

tate biopsy and over-treated. Survival and quality of life endpoints would make future studies stronger, pursuing better evidence to support and guide population-based preventive actions and health system improvement. “Are the patients in the compared groups different?” (reinforcing the hypothesis of selection bias). Or are they the same patients in different moments of the time-line and/or under different conditions?” (supporting the lead-time bias).

The Will Rogers phenomenon is a more obscure bias that is easily overlooked and that cannot be excluded in the development of the presented scenario represented by an apparent improvement in outcome for groups of patients with no actual improvement for any individual patient.

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EDITORIAL COMMENT

Understanding how socioeconomic status affects early diagnosis, treatment plan and outcomes are pivotal to elaborate any long term public and private health care plan. Comprehensive data regarding epidemiology is extremely valuable.

Prostate cancer is the most common non-cutaneous human malignancy and its epidemiology has been well reported in some parts of the World. Here at the United States, several publications and databases have been performed to better understanding this prevalent disease. From our institution, Byers et al. (1) demonstrated that low socio economic (SES) status is a risk factor for all-

cause mortality after a diagnosis of cancer, largely because of a later stage at diagnosis and different treatment options availability. CAPSURE (Cancer of the Prostate Strategic Urologic Research Endeavor), an American observational disease registry, was created in 1995 and since that several studies have been published on Prostate cancer epidemiology (2,3).

Brazil has just become the world's sixth largest economy and only the state of Sao Paulo represents more than 1/3 of Brazil's gross domestic product. Unfortunately social and health improvements haven't matched economy's improvement yet, and lack of disease epidemiologi-

cal data remains an issue even at the richest state of the country. To the best of my knowledge, this is the first publication describing epidemiological feature and patterns of prostate cancer patient's care both in public and private institutions. The authors performed a cross sectional web based survey study to determine features and patterns of how prostate cancer has been treated and its implication in terms of diagnosis, treatment and outcomes. All SBU (Brazilian Urological Society) members from Sao Paulo state were invited to participate on the survey. Although only 10% of the members contributed with some data, almost 2000 patient's data were gathered and analyzed. Very interesting conclusions were made from the data such as patients attended at public institutions were more likely to have late stage disease.

I would like to congratulate the authors and the SBU-Sao Paulo for this project. It was a tremendous effort and so much important information was already obtained. The merits of this study are not only based on its conclusion, but mostly on the descriptive data collected that will be extremely useful to compare Sao Paulo state with other geographical areas and also to assess improvement in the health system within the state

overtime. I encourage all the urologists to participate in upcoming surveys and make sure that SBU can count on every member's contribution and leads the research to better understand common urological conditions such as prostate cancer.

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Underexpression of MMP-2 and its Regulators, TIMP2, MT1-MMP and IL-8, is Associated with Prostate Cancer

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ABSTRACT

Objective: Extracellular matrix homeostasis is strictly maintained by a coordinated balance between the expression of metalloproteinases (MMPs) and their regulators. The purpose of this study was to investigate whether MMP-2 and its specific regulators, TIMP-2, MT1-MMP and IL-8, are expressed in a reproducible, specific pattern and if the profiles are related to prognosis and clinical outcome of prostate cancer (PCa).

Materials and Methods: MMP-2, TIMP-2, MT1-MMP and IL-8 expression levels were analyzed by quantitative real-time polymerase chain reaction (qRT-PCR) in freshly frozen malignant and benign tissue specimens collected from 79 patients with clinically localized PCa who underwent radical prostatectomies. The control group consisted of 11 patients with benign prostate hyperplasia (BPH). The expression profile of the MMP-2 and its regulators were compared using Gleason scores, pathological stage, pre-operative PSA levels and the final outcome of the PCa.

Results: The analysis of 79 specimens of PCa revealed that MMP-2, TIMP-2, MT1-MMP and IL-8 were underexpressed at 60.0%, 72.2%, 62.0% and 65.8%, respectively, in malignant prostatic tissue in relation to BPH samples. Considering the prognostic parameters, we demonstrated that high Gleason score tumors (≥ 7) overexpressed MMP-2 ($p = 0.048$) and TIMP-2 ($p = 0.021$), compared to low Gleason score tumors (< 7).

Conclusion: We have demonstrated that MMP-2 and its regulators are underexpressed in PCa. Alternatively, overexpression of MMP-2 and TIMP-2 was related to higher Gleason score tumors. We postulate that alterations in metalloproteinase expression may be important in the control of tissue homeostasis related to prostate carcinogenesis and tumor behavior.

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INTRODUCTION

Prostate cancer (PCa) is a common disease with a multifactorial and complex etiology. It is

the most common malignancy in the male and the second leading cause of death in many countries, including Brazil. Estimates for 2011 indicate that 903,500 new cases and 258,400 deaths relat-

ed to PCa will occur worldwide (1). Tumor staging, Gleason scores and PSA serum values are the most important prognostic factors, but even when used together, they cannot be used to perfectly diagnose patients at risk for progression (2). Therefore, research has been aimed to the identification of molecular markers that can contribute to diagnosis and predict PCa progression.

Degradation of basal membranes and extracellular matrix (ECM) is essential for tumor invasion and development of metastases, and matrix metalloproteinases (MMPs) are potent proteolytic enzymes that are known to play a key role in these processes. Within the MMP family, MMP2 (gelatinase A, 72 kDa) cleaves type IV collagen and gelatin, which are the main structural components of the basal membrane (3). Expression of MMP2 has been implicated in the development and progression of many neoplasias, such as bladder (4) and colon cancer (5).

MMPs are transcriptionally regulated. MMP2 is mainly regulated by its zymogen inhibitor, tissue inhibitor of metalloproteinase 2 (TIMP2), and by its major activator, membrane type-1 MMP (MT1-MMP), also known as MMP14. MT1-MMP specifically activates the pro-gelatinase, MMP2, on the tumor cell surface *in vitro* through the formation of a complex with TIMP2 (6). IL8 upregulates MMP2 in tumor cells, which is thought to be responsible for its angiogenic activity (7).

Neoplasia, an imbalance between MMPs and their regulators, leads to excess degradation, which may be linked to the invasive character of tumor cells (8-10). The aim of the present study was to investigate the expression profile of MMP2 and its specific regulators, TIMP2, MT1-MMP and IL8 in prostate cancer, associating them with the three most important prognostic parameters: Gleason score, pathological stage and pre-operative PSA serum levels. Also, we aimed to relate these profiles to the outcome of patients with localized PCa who underwent radical prostatectomies.

MATERIALS AND METHODS

Patients

The study was conducted using surgical specimens from 79 patients with clinically local-

ized PCa who underwent radical prostatectomy in our institution between 1993 and 2007. These cases were randomly selected from our database. Patients who had undergone other adjuvant and neoadjuvant treatments for PCa were excluded from the study. All subjects provided informed consent to participate in the study and to allow their biological samples to be genetically analyzed. The Institutional Board of Ethics approved this study (#0453/08).

We first analyzed MMP-2, TIMP-2, MT1-MMP and IL-8 expression levels in fresh tumor specimens from the 79 PCa patients using quantitative real-time polymerase chain reaction (qRT-PCR). The clinical and pathological characteristics of these specimens are listed in Table-1. The control group consisted of tissue specimens from 11 patients with benign prostatic hyperplasia (BPH), who had lower urinary tract symptoms and had undergone retro-pubic prostatectomy (mean age 64 ± 6.0 years).

We then correlated the expression levels of the genes with Gleason scores, pathological stage (TNM 2010) and pre-operative serum PSA levels (ng/mL). For analytical purposes, the pathological stages were described as organ-confined (pT2) or non-organ-confined (pT3) diseases; Gleason scores were classified as low grade (Gleason score < 7) or high grade (Gleason score ≥ 7). Preoperative PSA levels were also used to identify patients at high risk (≥ 10 ng/mL) and low risk (< 10 ng/mL) for disease recurrence. Additionally, we related the gene expression with the biochemical recurrence free survival rate in a mean follow-up time of 60 months, considering PSA levels > 0.4 ng/mL.

RNA Isolation and cDNA Synthesis

All tumor samples were obtained from surgical specimens and immediately frozen at -170°C in liquid nitrogen. A slide with a mirror of the frozen fragment was stained with hematoxylin and eosin to verify that the tumor represented at least 75% of the fragment in patients with cancer and to demonstrate the absence of tumor in those with BPH.

Total RNA was isolated with an RNAaqueous Kit (Applied Biosystems, CA, USA) according to the manufacturer's instructions. RNA concentration was determined by measuring absorbance at

Table 1 - Demographic characteristics of 79 men who underwent a radical prostatectomy treatment for prostate cancer.

Age (years)	
Mean	63
Min - Max	41 – 79
PSA (ng/mL)	
Mean	10.8
Min - Max	2.0 – 37.0
<10 n (%)	47 (59.5)
≥ 10 n (%)	32 (40.5)
Stage	
pT2 n (%)	38 (48)
pT3 n (%)	41 (52)
Gleason Score	
< 7 n (%)	33 (41.7)
≥ 7 n (%)	46 (58.3)

260/280 nM using a Nanodrop ND-1000 spectrophotometer (Thermo Scientific). cDNA was generated using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, CA, USA). The reactions were incubated at 25° C for 10 min., followed by 37° C for 120 min. and 85° C for 5 min. The cDNA was stored at -20° C until further use.

Quantitative Real-Time PCR and Gene Expression

Expression levels of the four genes were analyzed by qRT-PCR using an ABI 7500 Fast Real-Time PCR System (Applied Biosystems). Target sequences were amplified in a 10 µL reaction containing 5 µL of TaqMan Universal PCR Master Mix, 0.5 µL of TaqMan Gene Expression Assays (primers and probes, see Table-2), 1 µL of cDNA and 3.5 µL of DNase-free water. The PCR cycling conditions were 2 minutes at 50° C, 10 minutes at 95° C, and then 40 cycles of 15 seconds at 95° C and 1 min at 60° C. A TaqMan B2M assay was used as the endogenous control (Table-2).

We used the $\Delta\Delta CT$ method to calculate the relative expression of the three target genes using

the formula $\Delta\Delta CT = (CT \text{ target gene, PCa sample} - CT \text{ endogenous control, PCa sample}) - (CT \text{ target gene, BPH sample} - CT \text{ endogenous control, BPH sample})$. The fold change in gene expression was calculated as $2^{-\Delta\Delta CT}$.

Statistical Analysis

Quantitative variables were expressed as median values, interquartile range (Q1-Q3), and minimum and maximum values. Qualitative variables were expressed as numbers and percentages. To compare the clinical characteristics of patients with PCa, we used the Mann-Whitney, chi-squared and Fisher exact tests. For descriptive analysis of MMP-2, TIMP-2, MT1-MMP and IL-8 expressions according to pathological stage, Gleason scores and PSA levels, we constructed a box plot, and for comparison between categories, we used the Mann-Whitney test. Statistical analysis was performed using SPSS 15.0 for Windows, and significance was set at $p \leq 0.05$.

RESULTS

As shown in Figure 1, the analysis of 79 specimens of PCa revealed that MMP-2, TIMP-2, MT1-MMP and IL-8 were underexpressed in malignant prostatic tissue in relation to BPH samples. MMP-2, TIMP-2, MT1-MMP and IL-8 were underexpressed at levels of 60.0%, 72.2%, 62.0%

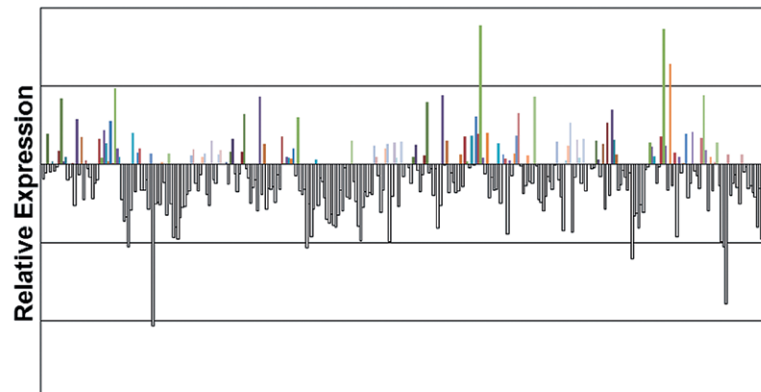
= 0.021). Only a marginal statistical difference for MT1-MMP was observed between the two tumor groups: the median was 0.89 for high-grade vs. 0.65 for low-grade PCa ($p = 0.08$). There were no differences in the expression levels of IL-8 when considering Gleason scores (Table-3).

There were no differences in the expression levels of the four genes according to the

Table 2 - Primers used in this study.

Gene symbol	Assays ID
MMP2	Hs00234422_m1
TIMP2	Hs00234278_m1
MMP14	Hs00237119_m1
IL-8	Hs99999034_m1
B2M	Hs99999907_m1

Figure 1 - Expression levels of the four genes in PCa tissue compared to BPH. Fold change in expression was calculated using the $2^{-\Delta\Delta CT}$ method.



and 65.8%, respectively, in prostate cancer tissue, compared to benign prostate tissue.

We found differences in MMP-2 and TIMP-2 expression between high-grade PCa (Gleason ≥ 7) versus low-grade PCa (Gleason < 7). The median expression of MMP-2 was 0.84 for high-grade PCa vs. 0.52 for low-grade tumors ($p = 0.048$). The median expression of TIMP-2 was 0.59 vs. 0.39 for high-grade and low-grade PCa, respectively (p

pathological stage, PSA pre-operative serum levels or biochemical recurrence.

DISCUSSION

In the present study we demonstrated that MMP-2 and its regulators are underexpressed in PCa compared to BPH tissue. Others, studying different types of cancer, have found overexpression

Table 3 - Relative expression of the four genes in malignant prostatic tissue according to Gleason scores, pathological stages, PSA values and biochemical recurrence. Fold changes in gene expression were calculated using the $\Delta\Delta CT$ method ($Q_{Rel} = 2^{-\Delta\Delta CT}$).

	MMP2	TIMP2	MMP14	IL8
Gleason Score				
< 7 (n = 32)	0.520 (0.3-1.0)	0.391 (0.3-0.7)	0.657 (0.4-1.2)	0.839 (0.5-1.3)
≥ 7 (n = 36)	0.844 (0.4-1.4)	0.598 (0.4-1.2)	0.890 (0.5-1.7)	0.689 (0.4-1.3)
p-value	0.048	0.021	0.081	0.274
Pathological Stage				
pT2 (n = 37)	0.737 (0.3-1.3)	0.493 (0.2-1.2)	0.894 (0.5-1.7)	0.767 (0.4-1.3)
pT3 (n = 40)	0.630 (0.3-1.3)	0.474 (0.3-0.7)	0.690 (0.4-1.3)	0.629 (0.4-1.3)
p-value	0.510	0.955	0.332	0.537
PSA value				
< 10 (n = 45)	0.655 (0.3-1.3)	0.479 (0.3-0.9)	0.780 (0.4-1.3)	0.577 (0.3-1.2)
≥ 10 (n = 33)	0.689 (0.3-1.3)	0.482 (0.3-1.2)	0.886 (0.4-2.0)	0.767 (0.5-1.5)
p-value	0.831	0.738	0.616	0.195
Biochemical Recurrence				
W/n (n = 29)	0.868 (0.4-1.1)	0.522 (0.3-0.8)	0.882 (0.5-1.2)	0.528 (0.3-1.0)
With (n = 28)	0.992 (0.4-1.5)	0.587 (0.3-1.6)	1.037 (0.5-2.2)	0.912 (0.5-1.3)
p-value	0.482	0.518	0.538	0.148

of these genes (11,12). However, there are reports showing that the absence of some MMP can be related to the promotion of tumors. For instance, Balbin et al., has described an increased susceptibility to skin cancer in mice that didn't express MMP-8 (13). Our results are similar to those published by Lichtinghagen et al. (2002) (14), who showed MMP-2 underexpression in prostate can-

cer tissue using RT-PCR. Conversely, they observed higher expression of MMP-2 at the protein level using immunohistochemistry, a result later confirmed by Brehmer et al. (15), indicating that there is a discrepancy between the levels of MMP-2 mRNA and protein in prostate cancer.

Interestingly, we found an association between elevated expression of MMP-2 and TIMP-2

and Gleason scores ≥ 7 , while MT1-MMP, which was also expressed at a higher level in Gleason scores ≥ 7 , only reached a level of marginal statistical significance ($p = 0.08$). The Gleason score is the main prognostic parameter for PCa, and it dictates the therapeutics in the majority of cases, although there are many pitfalls to use it as a determining factor. Because treatment options range from watchful waiting to palliative androgen deprivation, the correct characterization of histological differentiation is especially valuable for the correct treatment of the neoplasia. We have previously shown that it is possible to downgrade and upgrade PCa Gleason scores in radical prostatectomies to values of 14% and 29%, respectively, showing the weakness of using it as a prognostic factor, even when utilized by an experienced uropathologist (16). This confirms the necessity for additional biological information regarding PCa behavior. This is the first time that a study has shown a correlation between Gleason scores and MMP-2/TIMP-2 mRNA expression in PCa.

Extensive studies have revealed that malignant invasion and metastasis require ECM degradation, mainly by MMPs (17). Excessive or inappropriate expression of MMPs may contribute to the pathogenesis of cancer in a wide variety of cases by facilitating tissue degradation. Currently, there are more than 20 identified MMPs that can be categorized by substrate specificity. Despite the clinical significance of the pathogenic impact of MMPs in human cancer, including PCa, only a few studies of MMPs are available in the literature, and those mainly analyze protein expression (18).

Among the identified MMPs, MMP2 cleaves type IV collagen and gelatin, which are the main structural components of the basal membrane in the ECM, and the ECM is the first vital barrier on the course of tumor metastasis. Based on these findings, MMP-2 is thought to contribute to matrix breakdown. Today it is clear that MMP-2 participates in many deregulated signaling pathways that are used by the tumor to promote cancer cell growth and angiogenesis. The fact that we are already aware of these attributes makes MMP-2 a promising prognostic marker (19).

Interestingly, we found that MMP2 is underexpressed in prostate cancer, indicating that this MMP is not related to the promotion of PCA. However, after the establishment of the tumor, an increase in expression of MMP2 may be involved in tumor differentiation, which explains the association of elevated MMP2 expression in high-grade tumors. Miyake et al. (2010) (20), using immunohistochemistry showed that in univariate analysis MMP2 expression was significantly associated with biochemical recurrence, demonstrating the importance of this gene in the progression of PCa.

At the post-translational level, MMPs are under the control of specific tissue inhibitors (TIMPs) that bind proximal to the catalytic domain of the MMPs, preventing substrate binding. TIMPs are not simply regulators of MMP activity; they also have multifunctional roles that include cell growth promotion (8) and inhibition of angiogenesis (21). At the time of their discovery, TIMPs were considered to be tumor suppressor proteins. Increased TIMP expression is currently associated with decreased tumor growth, invasiveness and metastasis in prostate cancer and other cancer cell lines (22). However, the results of the current study demonstrate a poor prognostic significance of increased TIMP-2 expression, which is contrary to the original tumor-suppressor role that TIMPs were hypothesized to play. These results, however, are in agreement with recent evidence that supports a multifunctional and complex role for TIMPs. Nemeth et al. (23) described the growth-promoting abilities of TIMP-2 in several human cell types, including fibroblasts, keratocytes, lymphocytes and stem cells. Increased TIMP1 and TIMP2 mRNA levels have been correlated with higher tumor stages, lymph node metastasis and shortened survival in patients with carcinoma of the colon (24), breast (25), or bladder (26).

Although the paradoxical positive effects of TIMPs on tumor progression are not completely understood, the tumor promoting activity may be due either to proteolytic degradation of ECM or a direct influence on cell survival and growth. Stimulation of cell growth by TIMPs is thought to be mediated by cAMP-dependent activation of protein Kinase A and increased tyrosine phosphorylation (26).

TIMP-2 is reported to regulate matrix degradation, acting through a membrane type MMP (MT1-MMP). MT1-MMP is a key enzyme in tumor angiogenesis and metastasis: it hydrolyzes a variety of ECM components, and is a physiological activator of pro-MMP-2 (27). TIMP-2 forms a complex with MT1-MMP on the cell surface promoting hydrolysis of pro-MMP2 to its active form resulting in degradation of ECM.

We have shown that even with decreased MMP-2, TIMP-2 and MT1-MMP gene expression in cancer tissue, there was an increased expression in patients with Gleason scores ≥ 7 , an important prognostic factor of this disease. But we were not able to show a correlation between MMP-2, TIMP-2, MT1-MMP and IL-8, and the outcome of the disease following a 60-month period of follow-up. This lack of supporting evidence in this regard can be attributed to the small number of cases in each group.

We can postulate that the low expression of TIMP-2, MT1-MMP and IL-8 may be responsible for the decreased MMP-2 expression in PCa tissue, and this profile might be a characteristic of prostate cancer that can be utilized for tumor diagnosis in the future. Also, the fact that MMP-2 and TIMP-2 expression levels increase with tumor grade suggests that these genes might be involved in the differentiation of prostate cancer.

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CONFLICT OF INTEREST

None declared.

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Influence of Focal and Diffuse Extraprostatic Extension and Positive Surgical Margins on Biochemical Progression Following Radical Prostatectomy

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ABSTRACT

Purpose: The amount of extraprostatic extension and positive surgical margin correlates in most studies with biochemical recurrence following radical prostatectomy. We studied the influence of focal and diffuse extraprostatic extension and positive surgical margins on biochemical progression using a simple method for quantification.

Materials and Methods: A total of 360 prostates were step-sectioned and totally processed from 175 patients with stage T1c and 185 patients with clinical stage T2 submitted to radical retropubic prostatectomy. Extraprostatic extension was stratified into 2 groups: present up to 1 quadrant and/or section from the bladder neck or apex (Group 1, focal) and in more than 1 quadrant or section (Group 2, diffuse); and, positive surgical margin present up to 2 quadrants and/or sections (Group 1, focal) and in more than 2 quadrants or sections (Group 2, diffuse). The Kaplan-Meier product-limit analysis was used for the time to biochemical recurrence, and an univariate and multivariate Cox stepwise logistic regression model to identify significant predictors.

Results: Extraprostatic extension was found in 129/360 (35.8%) patients, 39/129 (30.2%) in Group 1 and 90/129 (69.8%) in Group 2. In univariate analysis but not in multivariate analysis, patients showing diffuse extraprostatic extension (Group 2) had a significant higher risk to develop biochemical recurrence in a shorter time. Positive surgical margin was present in 160/360 (44.4%) patients, 81/160 (50.6%) patients in Group 1 and 79/160 (49.4%) patients in Group 2. Patients with diffuse positive surgical margins (Group 2) had a significant higher risk in both univariate and multivariate analyses. Diffuse positive surgical margin was the strongest predictor on both analyses and an independent predictor on multivariate analysis.

Conclusion: Diffuse extraprostatic extension in univariate analysis and positive surgical margins on both univariate and multivariate analyses are significant predictors of shorter time to biochemical progression following radical prostatectomy.

ARTICLE INFO

Key words:

Prostatic neoplasms;
extraprostatic extension;
positive surgical margins;
radical prostatectomy;
treatment outcome

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INTRODUCTION

The amount of extraprostatic extension (EPE) and positive surgical margins (PSM) has prognostic importance in most studies (1-10).

However, during the International Society of Urological Pathology (ISUP) consensus conference on handling and staging of radical prostatectomy specimens held in Boston during the 98th meeting of the United States and Canadian Academy

of Pathology (USCAP), no consensus could be reached as to how evaluate extent of EPE and PSM (11,12). In this study we evaluated the influence of focal and diffuse extraprostatic extension and positive surgical margins on biochemical progression of patients submitted to radical prostatectomy using a simple method for EPE and PSM extent quantification that can be used in the daily practice of all pathologists who step-section and totally process the surgical specimen.

MATERIALS AND METHODS

This retrospective study was based on 360 consecutive patients with clinical stage T1c (175 patients) or T2 (185 patients) submitted to radical retropubic prostatectomy (RP) by one surgeon (UF). The clinicopathological variables studied included age at surgery, preoperative prostate-specific antigen (PSA) level, RP Gleason score, tumor extent at RP, EPE, PSM, seminal vesicle invasion and biochemical recurrence (BCR) following surgery.

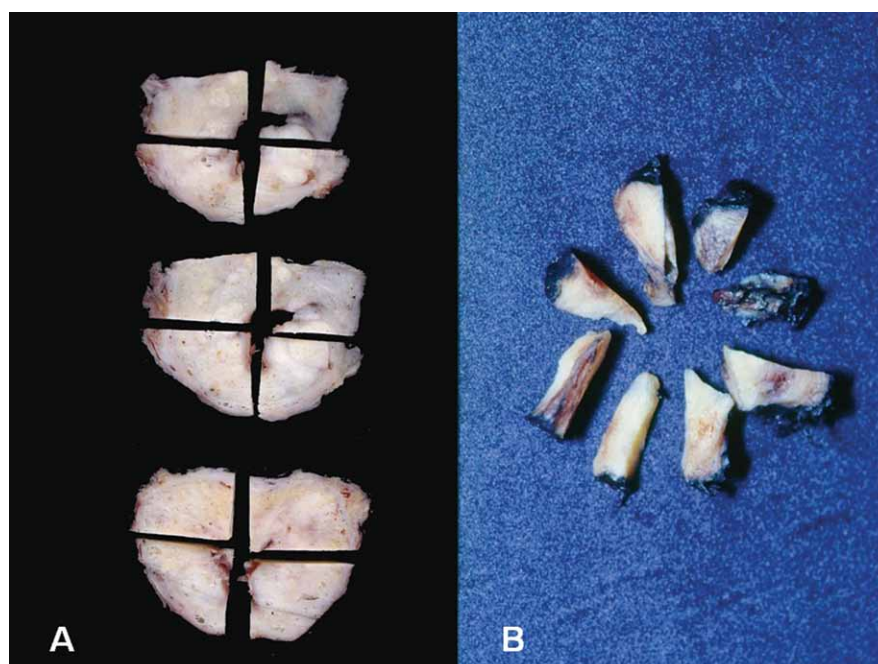
After RP, serum PSA was drawn every 3 months during the first year, every 6 months dur-

ing the second year, and annually thereafter. No patient of this series had radiotherapy or androgen manipulation before or after surgery. Only those with undetectable PSA after RP were included.

Total serum PSA was measured utilizing previous validated Immulite® PSA kit. BCR following surgery was considered as PSA > 0.2ng/mL according to recommendation of the American Urological Association (13). Patients without evidence of BCR were censored at last follow-up. The present study was approved by the Institutional Committee of Ethics of our Institution.

The surgical specimens were step-sectioned at 3 to 5mm intervals and totally embedded in paraffin. A mean of 32 paraffin blocks were processed and 6 μ m sections from each block were stained with hematoxylin and eosin. Each transversal section of the prostate was subdivided into 2 anterolateral and 2 posterolateral quadrants (Figure-1A). Using the cone method, 8 sections from the bladder neck and 8 sections from the apex were obtained (Figure-1B). The number of sections obtained from the bladder neck and the apex were constant (8 from each

Figure 1. (A) Three transversal sections of the prostate specimen subdivided into 2 anterolateral and 2 posterolateral quadrants. (B) Eight sections from the bladder neck using the cone method.



location), but the number of transversal sections varied according to the weight of the prostate. The median (range) of the prostate weight was 35g (11g-190g).

PSM was defined as cancer cells in contact with the inked specimen surface. EPE was diagnosed whenever cancer was observed in adipose tissue and, in case of desmoplastic response, whenever a protuberance corresponding to extension of tumor into periprostatic tissue was seen. Seminal vesicle invasion occurred whenever there was involvement of the muscular coat. Tumor extent at RP was evaluated by a semiquantitative point-count method previously described (14). Briefly, drawn on a sheet of paper, each quadrant of the transversal sections contained 8 equidistant points. During the microscopic examination of the slides, the tumor area was drawn on the correspondent quadrant observed on the paper. At the end of the examination the amount of positive points represented an estimate of the tumor extent. All cases were reviewed by a senior pathologist (AB).

EPE was stratified into 2 groups: present up to 1 quadrant of the transversal sections and/or sections from the bladder neck or apex (Group 1, focal EPE) and in more than 1 quadrant or sections (Group 2, diffuse EPE); PSM was stratified into 2 groups: present up to 2 quadrants and/or sections from the bladder neck or apex (Group 1, focal PSM) and in more than 2 quadrants or sections (Group 2, diffuse PSM).

Statistical analysis

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

RESULTS

PATHOLOGICAL FINDINGS

Extraprostatic extension. EPE was found in 129/360 (35.8%) patients, 39/129 (30.2%) in Group 1 and 90/129 (69.8%) in Group 2. Comparing the clinicopathological features between the groups (Table-1) there was no significant difference related to age of the patients ($p = 0.31$). In Group 2, preoperative serum PSA ($p = 0.02$) and RP Gleason score ($p < 0.01$) were significantly higher, tumors were more extensive at RP ($p = 0.04$), and more tumors presented seminal vesicle invasion ($p < 0.01$).

Positive surgical margin. PSM was present in 160/360 (44.4%) patients. The single most frequently site involved was circumferential (quadrant of a transversal section). The second most frequently involvement was the concomitance of a quadrant and the apex. Only 3 specimens showed PSM concomitantly in the bladder neck, apex and circumferential. Considering extent of PSM, 81/160 (50.6%) patients were in Group 1 and 79/160 (49.4%) patients in Group 2. Comparing the clinicopathological features between the groups (Table-2), there was no significant difference related to age of the patients ($p = 0.75$), preoperative serum PSA ($p = 0.27$), and seminal vesicle invasion ($p = 0.11$). Group 2 tumors were more extensive at RP ($p < 0.01$) and with higher RP Gleason score ($p = 0.04$).

TIME TO BIOCHEMICAL RECURRENCE

From the total of 360 men following radical prostatectomy, 115 (31.9%) patients had BCR at a mean, median and range follow-up of 18, 9, and 3-129 months; 229 (62.1%) censored men remained at risk at a mean, median and range follow-up of 46, 39, and 3-138 months, respectively; and, 16 (4.4%) men had no serum PSA data.

Extraprostatic extension. At 5 years of follow-up, 69% of patients without EPE vs 74% of patients with EPE in Group 1 were free of BCR (log-rank, $p = 0.98$); and, 69% of patients without EPE vs 38% of patients with EPE in Group 2 (log-rank, $p < 0.01$) (Figure-2). The weight of the prostates did not influence the results. Considering the median value of the weight, for prostates weighting

Table 1 - Clinicopathological features comparing 39 patients with focal EPE (Group 1) vs. 90 patients with diffuse EPE (Group 2).

Characteristic	Focal EPE (Group 1)	Diffuse EPE (Group 2)	p Value
Age (years)			
mean ± SD	63.44 ± 6.30	63.30 ± 6.38	0.31*
median	65	63.50	
range	45-75	47-74	
Preoperative PSA			
mean ± SD	9.43 ± 4.72	13.17 ± 8.34	0.02*
median	8.2	12.0	
range	2.3-21	0.60-51	
RP Gleason score			
mean ± SD	6.79 ± 0.41	7.39 ± 0.74	< 0.01*
median	7	7	
range	6-7	7-9	
Tumor extension (positive points)			
mean ± SD	50.94 ± 37.94	71.30 ± 50.21	0.04*
median	36	50	
range	7-192	9-225	
Seminal vesicle invasion			
absent	30 (88.2%)	37 (58.7%)	< 0.01**
present	4 (11.8%)	26 (41.3%)	

* Mann-Whitney test; ** Fisher's exact test

up to 35g, at 5 years of follow-up 67% of patients without EPE vs 83% of patients with EPE in Group 1 were free of BCR (log-rank, $p = 0.15$); and, 67% of patients without EPE vs 43% of patients with EPE in Group 2 (log-rank, $p = 0.03$). For prostates weighting > 35g, at 5 years of follow-up 71% of patients without EPE vs 67% of patients with EPE in Group 1 were free of BCR (log-rank, $p = 0.12$); and, 71% of patients without EPE vs 30% of patients with EPE in Group 2 (log-rank, $p < 0.01$).

Positive surgical margin. At 5 years of follow-up, 70% of patients with negative margins vs 62% of patients with PSM in Group 1 were free of

BCR ($p = 0.12$); and, 70% of patients with negative margins vs 39% of patients with PSM in Group 2 (log-rank, $p < 0.01$) (Figure-3). This latter difference was also significant considering only specimens with pathologic stage T2 ($p < 0.01$) as well as specimens pT2 and Gleason score ≤ 6 ($p < 0.01$). No influence was found according to the location of PSM as well as weight of the prostates. For prostates weighting up to 35g, after 5 years of follow-up 69% of patients without PSM vs 65% of patients with PSM in Group 1 were free of BCR (log-rank, $p = 0.26$); and, 69% of patients without PSM vs 47% of patients with PSM in Group 2 (log-rank, p

Table 2 - Clinicopathological features comparing 81 patients with focal PSM (Group 1) vs. 79 patients with diffuse PSM (Group 2).

Characteristic	Focal PSM (Group 1)	Diffuse PSM (Group 2)	p Value
Age (years)			
mean ± SD	63.03 ± 6.63	63.53 ± 7.15	0.75*
median	64	64	
range	47-74	43-75	
Preoperative PSA			
mean ± SD	9.91 ± 6.49	11.00 ± 7.07	0.27*
median	8.74	7.07	
range	0.90-35	1.22-51	
RP Gleason score			
mean ± SD	6.81 ± 0.56	7.05 ± 0.76	0.04*
median	7	7	
range	5-9	5-9	
Tumor extension (positive points)			
mean ± SD	38.86 ± 31.72	60.61 ± 47.26	0.01*
median	27.50	45	
range	1-147	4-225	
Seminal vesicle invasion			
absent	52 (64.2)	40 (50.6)	0.11**
present	29 (35.8)	39 (49.4)	

* Mann-Whitney test; ** Fisher's exact test

< 0.01). For prostates weighting > 35g, at 5 years of follow-up 78% of patients without PSM vs 59% of patients with PSM in Group 1 were free of BCR (log-rank, $p = 0.21$); and, 78% of patients without PSM vs 28% of patients with PSM in Group 2 (log-rank, $p < 0.01$).

RISK FOR SHORTER TIME TO BIOCHEMICAL RECURRENCE

In univariate Cox regression analysis, age ($p = 0.64$), EPE Group 1 ($p = 0.98$), and PSM Group 1 ($p = 0.12$) were not significantly predictive of shorter TBCR. EPE Group 2 ($p < 0.01$), PSM Group 2 ($p <$

0.01), preoperative PSA ($p < 0.01$), RP Gleason score ($p < 0.01$), RP tumor extent ($p < 0.01$), and seminal vesicle invasion ($p < 0.01$) were significantly predictive of shorter TBCR (Table-3). PSM Group 2 was the strongest predictor. In multivariate analysis, the best model predictive of shorter TBCR was PSM Group 2 ($p < 0.01$) + preoperative PSA ($p < 0.01$) + seminal vesicle invasion ($p = 0.04$).

DISCUSSION

Several studies have shown that the amount of EPE has prognostic importance. Our

Figure 2 - Kaplan-Meier biochemical (PSA) progression-free survival curve comparing 219 surgical specimens without EPE vs 37 surgical specimens with focal EPE (Group 1), and 88 surgical specimens with diffuse EPE (Group 2).

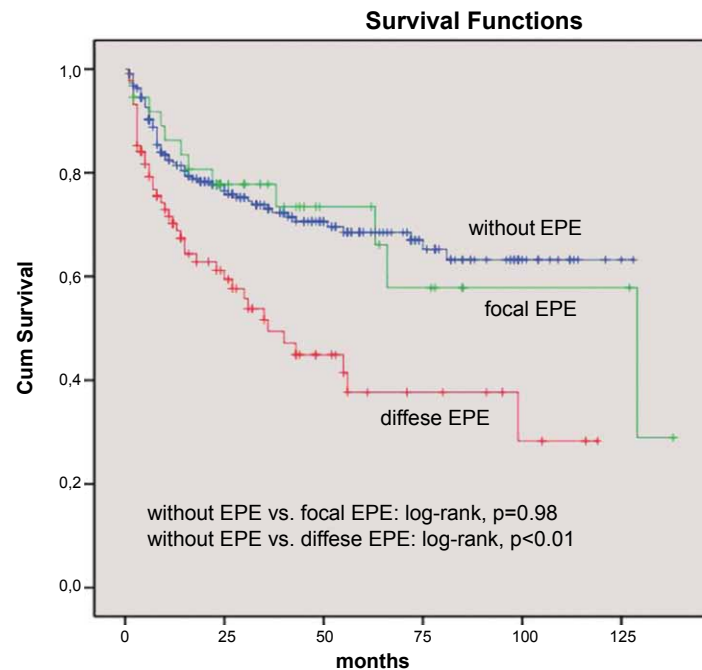


Figure 3 - Kaplan-Meier biochemical (PSA) progression-free survival curve comparing 183 surgical specimens without PSM vs 81 surgical specimens with focal PSM (Group 1), and 78 surgical specimens with diffuse PSM (Group 2).

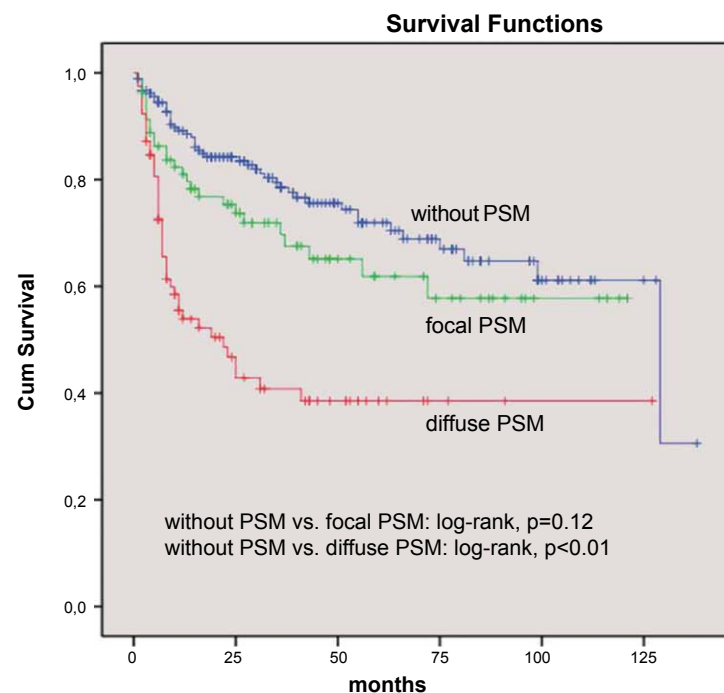


Table 3 - Cox proportional hazard analysis of several clinicopathological factors predicting time to biochemical recurrence following radical prostatectomy.

Predictors	Hazard ratio (95% CI)	Wald test	P
Univariate analysis			
Age	0.99 (0.97-1.02)	0.22	0.64
Focal EEP (Group 1)	1.01 (0.53-1.92)	< 0.01	0.98
Focal PSM (Group 1)	1.47 (0.91-2.38)	2.41	0.12
Diffuse EEP (Group 2)	1.48 (1.22-1.80)	15.17	< 0.01
Diffuse PSM (Group 2)	3.45 (2.25-5.30)	32.18	< 0.01
Preoperative PSA	1.05 (1.03-1.07)	27.15	< 0.01
Seminal vesicle invasion	2.74 (1.72-4.38)	17.81	< 0.01
RP Gleason score	1.44 (1.14-1.81)	9.28	< 0.01
RP tumor extent	1.01 (1.00-1.01)	8.09	< 0.01
Multivariate analysis			
Diffuse PSM (Group 2)	1.71 (1.37-2.13)	22.48	< 0.01
Preoperative PSA	1.04 (1.02-1.06)	11.64	< 0.01
Seminal vesicle invasion	1.79 (1.02-3.14)	4.10	0.04

study is in accordance with a more favorable biochemical progression following radical prostatectomy for patients with focal EPE than patients with extensive EPE (1,4,6). In the 7th edition of the TNM Classification of Malignant Tumours (15), the bladder neck involvement is considered pT3a and not pT4. Therefore, involvement of the bladder neck was considered EPE in this study. The incidence of EPE in the literature ranges from 28% to 48% (16). In our study the frequency was 35.8%.

Epstein et al. (1) subdivided extraprostatic extension into focal and established. Focal extraprostatic extension was defined as a few glands immediately exterior to the prostate in 1 to 2 sections. Cancers with established EPE had a higher risk of progression than those with focal EPE. Wheeler et al. (4) defined focal EPE as tumor outside the prostate to a depth of less than one

high-power field on no more than two separate sections and established EPE any amount of extraprostatic tumor more than focal. Extensive EPE was associated with progressively increased risk of seminal vesicle invasion and positive lymph nodes and lower progression free probabilities. Sung et al. (6) measured EPE considering the radial distance defined as the distance that the tumor protrudes perpendicular beyond the outer margin of the prostatic stroma. In univariate analysis, the radial distance of extraprostatic tumor measured by ocular micrometer was associated with PSA recurrence. In the multivariate analysis, radial distance remained an independent predictor of PSA recurrence.

Surgical margin status is a major predictor of biochemical recurrence following radical prostatectomy. Coupled with other variables might serve to further discriminate those patients

most likely to benefit from immediate adjuvant radiotherapy after RP. Our results are in accordance with most studies that correlate extent of margin positivity in radical prostatectomies with biochemical recurrence (2,3,5,7-10,16-18). Diffuse PSM was significantly associated to shorter time of biochemical progression and was predictive of time to TBCR in both univariate and multivariate analyses. The incidence of PSM in the literature ranges from 16% to 50% (16). In our study the incidence was 44.4%. One of the largest potential sources of discrepancy for incidence of PSM is the different methods used to process the radical prostatectomy specimens (2).

Epstein et al. (2) defined focal margin positivity as margins in which the tumor reached the inked transected exterior surface, and the involved site(s) were limited and present in only one or two areas. Cases with extensive positive margins were those in which multiple positive margins were present at different sites in the prostate. Forty-one percent of the prostate specimens studied had positive margins, 37% had focally positive margins, and 4% had extensive margins. The difference in biochemical progression in patients with focally positive versus extensively positive margins was significant. Weldon et al. (3) defined focal positive margins those present on a single step-section and 3mm long or less. Any larger positive margins were designated as extensive. Forty-four percent of the specimens had positive surgical margins, 23% focal and 21% extensive. The difference in biochemical progression between patients with focal and extensive positive margins was significant. Babaian et al. (5) stratified PSM into focal and diffuse using a cutoff of 3mm linear extent adding the length of all areas of tumor in contact with ink. The biochemical failure rate was 10% when the positive margin length was ≤ 3 mm and 77.8% when the positive margin length was > 3 mm. The authors concluded that the result stratify the probability of failure and suggested that adjuvant local therapy may be beneficial if the positive margin exceeds 3mm.

During a consensus conference held by the ISUP, no consensus could be reached as to how evaluate extent of EPE and PSM (11,12). The conference recommended that until such a consensus is reached, EPE and PSM should be reported as millimeters of linear involvement. However, mea-

surement is tedious, time consuming and not used in the daily practice of pathologists. Our proposal of EPE and PSM quantitation does not need any time consuming measurement. It implies only to consider the number of quadrants and/or sections from the bladder neck or apex showing EPE and/or PSM and can be used in the daily practice of all pathologists who step-section and totally process the surgical specimen.

The present proposal of diffuse PSM was significantly predictive of TBCR following surgery. Only patients with diffuse PSM showed significantly shorter time to BCR. This difference was also significant considering only specimens with pathologic stage T2 as well as specimens pT2 and Gleason score ≤ 6 . No influence was found according to the location of PSM as well as weight of the prostate which influences the number of transversal sections. In the present cohort of patients, diffuse positive surgical margins were the strongest predictors of TBCR in univariate and multivariate analyses. It is interesting to point out why patients with positive margins may not inevitably progress to BCR. The main reason may be local tissue destruction as a result of the effects of disrupted vasculature, cautery, and inflammation related to surgery (17).

A limitation of the present study is the relatively short mean follow-up time. Johansson et al. (19) reported that local tumor progression and distant metastases developed even after 15 years of follow-up. However, it is recognized that over 90% of patients submitted to radical prostatectomy will have a relapse within 5 years after surgery (19,20). Therefore, it is likely that the majority of cases with biochemical progression have already been identified in this study. It is also worth mention that Gleason grade in multivariate analysis was not a significant predictor of TBCR due to the small number of patients with score 8-10 ($n = 21$). If Gleason grade was significantly predictive, then maybe the significance of some of the multivariate models tested would have been different.

CONCLUSIONS

In this study we showed a statistically significant predictive value for shorter time to

biochemical progression following radical prostatectomy in patients with diffuse EPE or PSM using a simple method for EPE and PSM extent quantitation that can be used in the daily practice of all pathologists who step-section and totally process the surgical specimen. In univariate analysis but not in multivariate analysis, patients with EPE in more than 1 quadrant and/or section from the bladder neck or apex have a significant higher risk to develop biochemical recurrence in a shorter time. Patients with PSM in more than 2 quadrants and/or sections from the bladder neck or apex have a significant higher risk to develop biochemical recurrence in a shorter time in univariate and multivariate analyses. PSM was the strongest predictive finding for shorter time of recurrence in both analyses. We advocate reporting the extent of EPE and PSM as part of the surgical pathology report.

ABBREVIATIONS:

PSA: prostate specific antigen;
SD: standard deviation;
CI: confidence interval;
RP: radical prostatectomy;
BCR: biochemical recurrence;
TBCR: time to biochemical recurrence;
EPE: extraprostatic extension;
PSM: positive surgical margin;
ISUP: International Society of Urological Pathology;
USCAP: United States and Canadian Academy of Pathology.

CONFLICT OF INTEREST

None declared.

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Prognostic factors in renal cell carcinoma: analysis of 227 patients treated at the Brazilian National Cancer Institute

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ABSTRACT

Purpose: We evaluated the role of several prognostic factors in predicting death and/or progressive disease in patients with renal cell carcinoma.

Materials and Methods: Between 2004 and 2010, 227 consecutive patients with renal cell carcinoma underwent radical nephrectomy at our Institute. All histological specimens were examined by the same pathologist. We considered certain histological parameters, including histological subtype, conventional Fuhrman grade, presence of sarcomatoid features, adrenal gland infiltration, invasion of the perinephric fat, vascular embolization, collecting system invasion, presence or absence of tumour necrosis (0%, 1% to 49%, or 50% or greater) and regional lymph node metastasis.

Results: Variables significantly associated with death and/or progressive disease on univariate analysis were histological subtype ($p = 0.006$), Fuhrman grade ($p < 0.0001$), tumor necrosis ($p = 0.009$), perinephric fat invasion ($p = 0.002$), vascular embolization ($p = 0.0002$), presence of lymph node involvement ($p < 0.002$), tumor size ($p = 0.0006$), TNM stage ($p < 0.00001$) and presence of metastasis ($p < 0.00001$). In the multivariable model histological subtype, tumor necrosis, lymph node involvement and presence of metastasis were independent risk factors for disease-free survival ($p = 0.011$, 0.042 , 0.025 and $p < 0.0001$, respectively).

Conclusion: Histological subtype, tumor necrosis, lymph node involvement and presence of metastasis proved to be independent prognostic factors for disease-free survival. Therefore, the presence and rate of tumor necrosis should always be informed by the pathologist and lymphadenectomy should be performed in all patients.

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INTRODUCTION

In the United States, renal cancer represents respectively the 7th and 9th most common malignancy in men and in women, accounting for about 3% of cancer deaths. In this country, the National Cancer for Health Statistics Insti-

tute (NCHSI) (1) estimated that 57,760 new cases would be diagnosed and 12,980 deaths would occur in 2009. In 2002, the global estimate was 20,800 new cases and 10,200 deaths, accounting for about 1.9% of tumors in general. The highest incidences occurred in North America, Australia/New Zealand and Europe, with lower rates in

Africa, Asia and the Pacific (2). We have in our national literature a lack of data concerning the general characteristics of patients with renal malignancy, since the disease is not among the 10 most frequent tumor types annually reported by the Brazilian National Cancer (3). The Brazilian Society of Urology, through the ENCARE study, initiated a project aimed to carry out an epidemiological study of renal cancer in our country. Recently complete data from this study was published (4). The current work aims to show, from a survey of factors relevant to disease, the profile of patients with this type of pathology at the National Cancer Institute over the past six years.

MATERIALS AND METHODS

Between 2004 and 2010, 227 consecutive patients including 116 males (51%) and 111 females (49%) with renal cell carcinoma (RCC) were referred to our Institute and treated with curative intent by radical nephrectomy. Other 26 patients, 14 males (53.8%) and 12 females (46.2%) with normal contralateral kidneys underwent partial nephrectomy with the same curative intent. These last patients were excluded of this study because most of them were operated after 2009 which hampered the analysis of their survival. After obtaining institutional board approval, pathological data of these patients were prospectively evaluated. Data collected by tumor registrars on all tumors included standard tumor registry data elements, such as patient age, gender, diagnosis date, initial therapy, follow-up, subsequent therapy and outcome. Patient follow-up data were gathered from medical charts at our institution and when necessary through contact with the patient family. We retrospectively reviewed the charts to determine dates of recurrence and death, as well as cause of death. Mean and median patient age was 58 and 60 years at diagnosis (range 2 to 83). Follow-up was evaluated in 223 of 227 patients. Median follow-up was 28 months (range 0 to 88). We excluded from the analysis of survival curves 4 patients with less than 1 month of clinical follow-up.

Study inclusion criteria were: surgical resection of pathologically confirmed renal cell carcinoma, adequate tissue available for pathological

reevaluation, and adequate radiographic and/or clinical follow-up data available. Exclusion criteria were: pathologically confirmed urothelial carcinoma or any benign lesion and tissue unavailable for accurate reevaluation.

To facilitate statistical comparisons the patients were grouped into four groups of staging: Stage I (T1 N0 M0), Stage II (T2 N0 M0), Stage III (T3 N0 M0, T1, T2, T3 N1 M0) and Stage IV (T4 N0, N1 M0 any T N2 M0 any T any N M1).

The weight and tumor size were recorded from surgical pathology reports. Pathological material were re-reviewed by a single pathologist (ABSS) blinded to clinical outcome data.

The data captured were histological type according to the 2004 WHO classification. The pathological variables studied were histological subtype, conventional Fuhrman grade, presence of sarcomatoid features, adrenal gland infiltration, invasion of the perinephric fat, vascular embolization, collecting system invasion, presence or absence of tumour necrosis (0%, 1% to 49%, or 50% or greater) and regional lymph node metastasis. These factors were gathered together in the universally used 2002 AJCC TNM staging classification system. Not all the parameters chosen for analysis were available for all patients. For example, not all patients underwent lymphadenectomy, thus preventing the histological analysis of lymph nodes in these cases. Also some histological parameters could not be evaluated in all patients by lack of information on pathological material reviewed by the pathologist.

The data obtained were recorded on a standard research form and filled in a database. Analysis was performed using SPSS®. The associations of qualitative factors with death and/or presence of progressive disease were done with the chi-square test. Independent factors related to death and/or progressive disease were identified by linear regression analysis with $p < 0.05$ considered statistically significant. Disease-free survival (DFS) was defined as time to the date of progression of disease and/or to the date of death from disease. The Kaplan-Meier technique was used to evaluate DFS and the log rank test was used to compare survival curves with $p < 0.05$ as the significance cutoff.

RESULTS

When our patients were stratified according clinical stage there were 73 (32.1%) with stage I, 68 (29.9%) with stage II, 66 (29%) with stage III and 15 (6.6%) with stage IV. We were unable to evaluate the clinical staging in the medical records of five patients.

When tumors were stratified according to size there were 22 tumors (9.7%) with 4 cm or less in diameter, 79 tumors (34.8%) larger than 4 cm and lesser than 7 cm and 124 tumors (54.6%) larger than 7 cm in diameter. We were unable to measure the diameter of the tumor in 2 patients.

Histological findings confirmed renal cell carcinoma (RCC) in all 227 patients. Of the 227 specimens 165 (72.7%) were identified as clear cell carcinoma, 24 (10.6%) as chromophobe tumor, 11 (4.8%) as papillary tumor type I, and 13 (5.7%) as papillary tumor type II. One group of 12 patients (5.3%) presented sarcomatoid features and were studied separately. Sarcomatoid RCC, initially considered a distinct subtype, is now recognized as a high-grade transformation that can arise in any subtype of RCC. The remaining 2 cases were a neuroendocrine tumor and a tumor of unclassified mesenchymal lineage. 46 patients (20.3%) had metastatic disease, 33 (14.5%) died of RCC and 11 (4.8%) were alive but showing progression of the disease when the survey was done.

Regarding Fuhrman grade 24 patients (10.6%) were classified as grade I, 97 (42.7%) as grade II, 61 (26.9%) as grade III and 38 (16.7%) as grade IV. In the specimens of seven patients we were unable to classify according to Fuhrman grade due to reasons explained in the section Materials and Methods.

Invasion of adrenal gland occurred in no more than 7 patients. Perinephric fat invasion, collecting system invasion and vascular embolization were present respectively in the specimens of 62 (27.3%), 38 (16.7%) and 69 (30.4%) patients.

Among the specimens, 108 (47.6%) showed tumor necrosis, which was identified in 77 of 165 cases (46.7%) of clear cell, 6 of 11 (54.5%) of papillary type I, 8 of 13 (61.5%) of papillary type II, 6 of 24 (25%) of chromophobe and in 9 (75%) of 12 specimens with sarcomatoid

features. A total of 118 patients (52%) had no tumor necrosis, 61 (26.9%) had less than 50% and 47 (20.7%) had 50% or greater. In one patient it was not possible to assess the presence or absence of tumor necrosis.

Lymph node involvement was present in 22 of 130 patients. Metastases occurred in 46 of our 227 patients. Death and/or progressive disease results significantly correlated with histological subtype ($p = 0.006$), Fuhrman grade ($p < 0.0001$), tumor necrosis ($p = 0.009$), perinephric fat invasion ($p = 0.002$), vascular embolization ($p = 0.0002$), presence of lymph node involvement ($p < 0.002$), tumor size ($p = 0.0006$), TNM stage ($p < 0.00001$) and presence of metastasis ($p < 0.00001$). Presence of adrenal invasion or collecting system invasion was not significant ($p = 0.14$ and $p < 0.083$). Only histological subtype, tumor necrosis, lymph node involvement and presence of metastasis proved to be independent predictive variables of disease-free survival on multivariate analysis ($p = 0.011$, 0.042 , 0.025 and $p < 0.0001$, respectively, Table-1). Histological subtype, Fuhrman grade, the variable percent of tumor necrosis (TN), TN presence/absence, perinephric fat invasion, vascular embolization, presence of lymph node involvement, tumor size, TNM stage and presence of metastases, were also significantly associated with overall survival (OS).

Figure-1 shows the disease-free survival of patients in relation to histological subtype. Patients without tumor necrosis had better survival rates after 5 years than those with tumor necrosis ($p < 0.0001$, Figure-2). Log rank analysis also showed differences in 5-year disease survival curves according to the presence of lymph node involvement ($p < 0.0001$, Figure-3) and presence of metastasis ($p < 0.0001$, Figure-4)

COMMENTS

The Brazilian National Cancer Institute receives many patients with large tumors who are not candidates for partial nephrectomy. In our evaluation we found only 26 patients undergoing partial nephrectomy. However most of them (18) were operated from 2009 until the end of the study. This has damaged our analysis of patient

Table 1 - Predictive prognostic factors for death and / or cancer progression. Univariate (chi-square) and multivariate (linear regression) analysis.

Prognostic factors	No. Pts.	Patients dead or with progressive disease (%)	Chi-Square	Univariate Analysis (p value)	Multivariate Analysis (p value)
Histological subtype					
Clear cell	165	34 (20.6%)			
Chromophobe	24	0 (0%)			
Papillary I	11	1 (9%)			
Papillary II	13	4 (30.8%)			
Presence of sarcomatoid features	12	6 (50%)	14.51	0.006	0.011
Fuhrman grade					
I	24	2 (8.3%)			
II	97	14 (14.4%)			
III	61	11(18%)			
IV	38	18 (47.4%)	21.46	< 0.0001	0.275
Tumoral Necrosis					
0%	118	15 (12.7%)			
≤ 50%	61	14 (22.9%)			
≥ 50%	47	15(31.9%)	29.35	0.009	0.042
Perinephric fat invasion					
Absent	136	20 (14.7%)			
Present	62	21 (33.9%)	9.52	0.002	0.201

Collecting system invasion					
Absent	135	25 (18.5%)			
Present	38	12 (31.6%)	3.00	0.083	0.410
Vascular embolization					
Absent	148	19 (12.8%)			
Present	69	24 (34.8%)	14.26	0.0002	0.658
Lymph node					
Absent	98	19 (19.4%)			
Negative	108	17 (15.7%)			
Positive	22	10 (45.5%)	12.31	<0.002	0.025
Tumor size					
< 4 cm	22	2 (9.0%)			
> 4 cm < 7 cm	79	7 (8.7%)			
> 7 cm	124	37(29.8%)	14.98	0.0006	0.832
Stage					
I	73	4 (5.4%)			
II	68	15 (22%)			
III	66	13(19.7%)			
IV	15	13(86.7%)	50.94	<0.00001	0.561
Metastasis					
Absent	181	2 (1.1%)			
Present	46	44 (95.7%)	202.91	< 0.00001	0.000

Figure 1 - Rate of disease-free survival after 5 years according to the various histological subtypes. The higher percentage of survival occurred in patients with chromophobe tumors and lower survival rate in patients with sarcomatoid features. (Kaplan-Meier log rank test $p < 0.0001$).

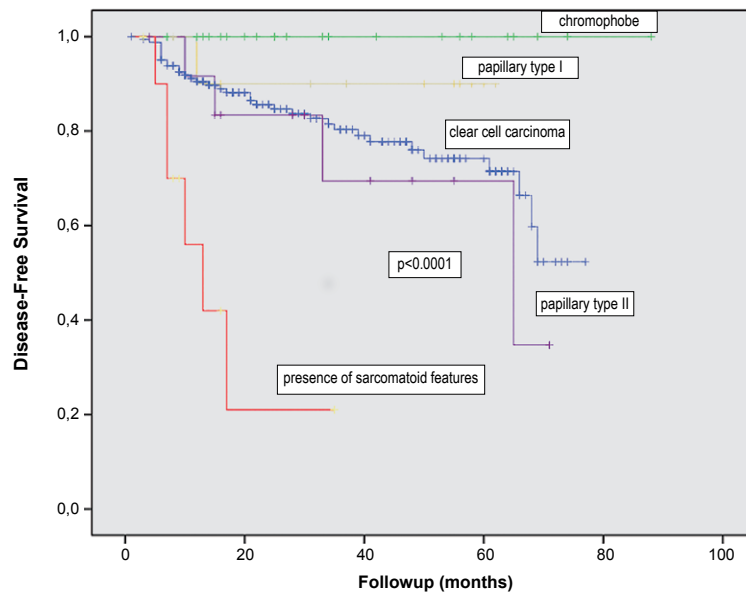


Figure 2 - 118 patients without tumor necrosis had higher 5-year survival than 61 with percentage of tumor necrosis less than 50% and 47 with percentage of tumor necrosis greater than 50%. (Kaplan-Meier log rank test $p < 0.0001$).

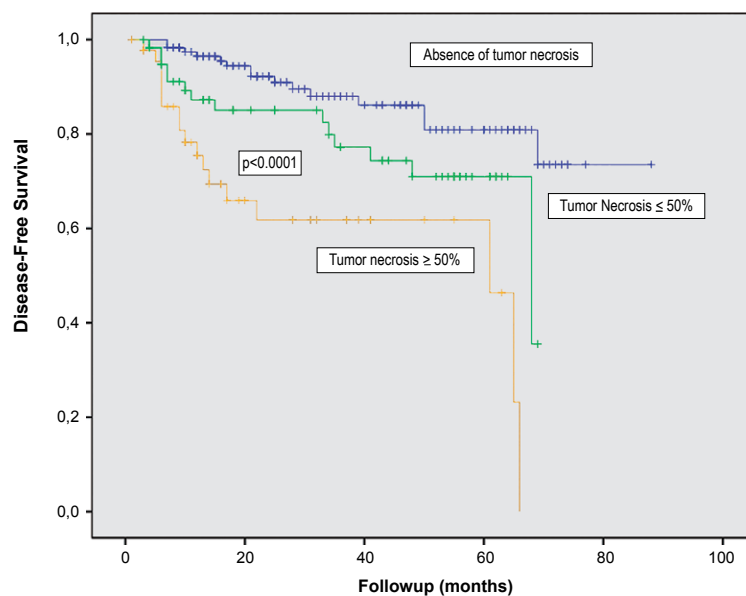


Figure 3 - 108 patients with negative lymph nodes had higher 5-year survival than 22 with positive lymph nodes. The 98 patients in whom no lymph nodes were assessed also had a disease-free survival at 5 years shorter than that of patients with negative lymph nodes. (Kaplan-Meier log rank test $p < 0.0001$).

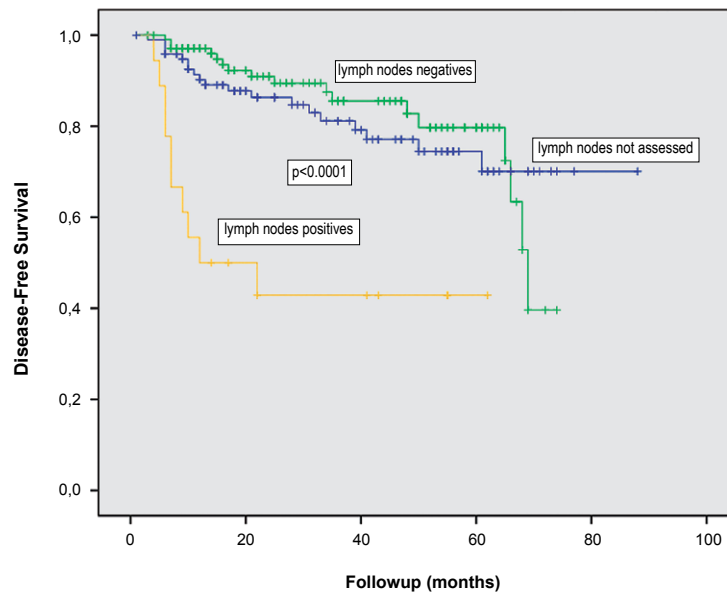
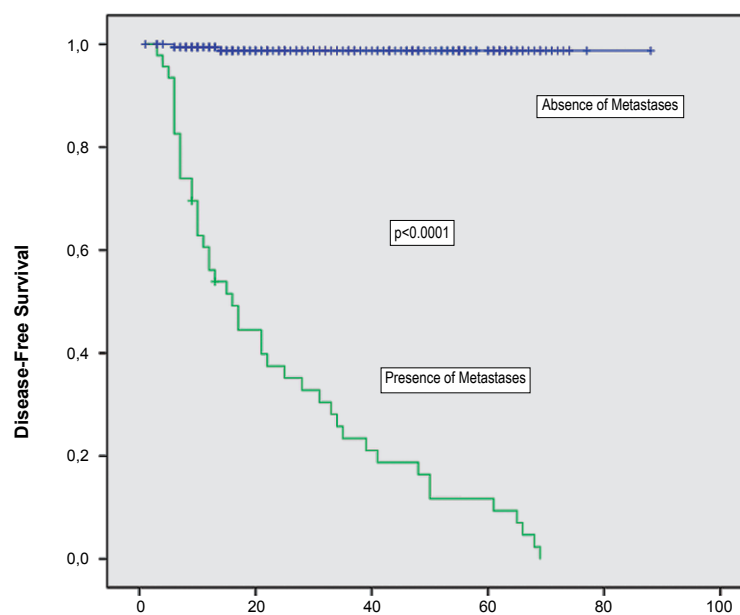


Figure 4 - Among 227 patients, 46 who had metastases had a worse 5-year survival rate. (Kaplan-Meier log rank test $p < 0.0001$).



survival. We prefer to wait some time to publish our data regarding partial nephrectomy in patients with kidney cancer. Partial nephrectomy is indicated for patients with bilateral tumors or a solitary functioning kidney. The success rate is excellent with a cancer-specific survival ranging from 78% to 100%. Although radical nephrectomy remains as standard treatment for localized tumors and normal contralateral kidney, growing number of authors has shown excellent results in these cases with nephron-sparing surgery since preservation of renal function is a prognostic factor for overall survival. For some authors partial nephrectomy must be the established treatment for T1a tumours (< 4 cm) and an emerging standard treatment for T1b tumours (4-7 cm) provided that the operation is technically feasible and the tumour can be completely removed (5).

Our disease-free survival rates after 5 years for patients with clear cell, chromophobe, papillary type I and II RCC were 72.5%, 100%, 90%, and 70% respectively. The presence of a sarcomatoid component was significantly associated with death of our patients with disease-free survival rate of 21.7% after 35 months of follow-up (Figure-1). Many studies have shown a better prognosis for patients with chromophobe, papillary and conventional (clear cell) RCCs, respectively (6,7). In a large series, Patard et al. (7) found a trend toward a better survival when clear cell, papillary, and chromophobe histologies were considered prognostic categories. However, in multivariate analysis, TNM stage and Fuhrman grade, but not histology, were retained as independent prognostic variables. Unlike in our series, histological subtype proved to be an independent predictive variable on multivariate analysis but this did not occur with Fuhrman grade and TNM stage (Table-1).

Death and/or progressive disease results significantly correlated with the size of the tumors but this parameter has not proved to have statistical significance on multivariate analysis. A recent study from Memorial Sloan-Kettering Cancer Center found that among patients with proven RCC, each 1 cm increase in tumor size increased the odds of having a tumor of high grade by 25% (8).

Only 7 of our patients presented adrenal invasion. Of them 3 died of the disease. The comparative analysis of survival of these patients was impaired due to the small number of patients. Han et al. (9) identified 27 patients with direct adrenal involvement from a universe of 1,087 undergoing nephrectomy. In this study, invasion of the adrenal gland remains a significant independent predictor of decreased survival on multivariate analysis.

Of our 62 patients with perinephric fat invasion, 21 (33.8%) died of RCC and the 5-year disease-free survival rate was 40%. Death and/or progressive disease results significantly correlated when we compared patients with presence or absence of perinephric fat involvement. However these results were not significant for disease-free survival on multivariate analysis (Table-1). Thompson et al. (10) studied 162 patients with perinephric fat invasion of whom 95 (59%), died of RCC. The estimated 5-year disease-free survival rate was 50.9% for these patients.

We found a trend toward a worse prognosis in patients with collecting system invasion ($p < 0.083$). According to Anderson et al. (11), the presence of urinary collecting system invasion is independently associated with higher overall and disease-specific mortality in patients undergoing nephrectomy for pT3 RCC.

Tumor thrombi were identified at several sites in 69 patients. These patients had worse prognosis than those who did not have tumor thrombi. However, this parameter has no proved significant on multivariate analysis. According to Feifer et al. (12), patients with venous branch invasion have an outcome inferior to that of patients with pT1-2 disease. This confirms the adverse prognosis of venous branch invasion and supports pathological up-staging. The prognosis of venous branch invasion would be similar to that of renal vein invasion.

The presence of tumor necrosis, lymph nodal involvement and distant metastasis were the most powerful prognostic factors for survival in our patients with RCC.

The survival rate at 5 years was 82.5%, 71.6% and 62.5% for our patients without tumor necrosis, with less than 50% tumor necro-

sis and more than 50% tumor necrosis, respectively (Figure-2). In our study the percentage of tumor necrosis was an independent predictor of disease-free survival. While prior series showed that pathologically confirmed tumor necrosis is a marker of aggressive disease and decreased survival, (13-15) others failed to show an association with decreased survival. (16-18). In the largest series, Sengupta et al. (14) examined more than 3,000 patients who underwent partial or radical nephrectomy and found an association between the presence of tumor necrosis with decreased disease specific survival. On multivariate analysis controlling for stage, size and grade the presence of tumor necrosis conferred almost 2 times the risk of death from RCC. In another recent study, Katz et al. (19) identified tumor necrosis in 30% of 841 patients. Those patients with 50% or greater necrosis in the primary tumor were more than 2 times more likely to die of RCC when controlling for nuclear grade, subtype, stage, size and age than patients with less than 50% TN. However, the presence or absence of TN alone failed to remain an independent predictor for survival.

A critical yet unresolved issue in the surgical management of RCC is the role and extent of lymphadenectomy. Some of the difficulty in recommending LN dissection is that there is no standardized and validated template to use. In addition, there is controversy regarding the definition of adequate regional or extended lymphadenectomy for patients undergoing nephrectomy. Whitson et al. (20) in a large series of patients showed an association between increased lymph node yield and improved disease specific survival of patients with lymph node positive nonmetastatic renal cell carcinoma who underwent lymphadenectomy. In our series, lymphadenectomy was performed in 130 of our patients of whom 22 had lymph node involvement. When lymph nodes were negative for malignancy, disease-free survival at 5 years was 80% while in patients with lymph node involvement, rate of disease-free survival dropped to 43.3%. In patients in whom we had no access to the lymph nodes because the lymphadenectomy was not carried out, the rate of disease-free survival reached 70% (Figure-3). Perhaps several of these patients should have

positive lymph nodes which would explain this intermediate survival rate. Another hypothesis for the lower survival of this group of patients compared with patients with negative lymph nodes would be the removal of micrometastases, undetected by conventional histology, during the lymphadenectomy.

Regarding the presence of metastases, only 14% of patients who developed metastases were alive after 5 years (Figure-4). Several studies have identified various sites of metastatic disease to have prognostic significance; however, the number of involved sites appears to be a good surrogate for these individual sites. In multivariate analysis, the number of metastatic sites appears to be a strong independent prognostic factor (21).

CONCLUSIONS

Histological subtype ($p = 0.006$), Fuhrman grade ($p < 0.0001$), tumor necrosis ($p = 0.009$), perinephric fat invasion ($p = 0.002$), vascular embolization ($p = 0.0002$), presence of lymph node involvement ($p < 0.002$), tumor size ($p = 0.0006$), TNM stage ($p < 0.00001$) and presence of metastasis ($p < 0.00001$) were significantly associated with death and/or progressive disease on univariate analysis. However, only histological subtype, tumor necrosis, lymph node involvement and presence of metastasis proved to be independent predictive variables of disease-free survival on multivariate analysis ($p = 0.011$, 0.042 , 0.025 and $p < 0.0001$, respectively). Therefore, the presence and rate of tumor necrosis should always be informed by the pathologist and lymphadenectomy should always be performed in all patients.

CONFLICT OF INTEREST

None declared.

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Ureteroscopy in patients with coagulopathies is associated with lower stone-free rate and increased risk of clinically significant hematuria

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ABSTRACT

Purpose: Patients with coagulopathy are at increased risk of peri-operative hemorrhage. The aim of the present study was to compare ureteroscopy (URS) in these high risk patients to those with normal bleeding profile.

Materials and Methods: Twelve patients with coagulopathies (Group I) undergoing 17 URS were included in the study [3 for biopsy of ureteral lesions and 9 for Holmium Laser Lithotripsy (HLL)]. A patient had Child B (MELD 11) cirrhosis, 6 patients were on warfarin, 3 patients on ASA, 1 patient on ASA and clopidogrel, and the last patient was on heparin. URS in Group I was performed without correction of coagulopathy. Group II consisted of 32 patients with normal bleeding profile who underwent 34 URS concurrently.

Results: Group I included 4 ureteral biopsies in 3 patients with suspicious ureteral lesions and 13 URS for HLL in 9 patients with nephrolithiasis. There were no significant differences between the two groups in terms of patient age, sex, percent of renal stones, median operative and fluoroscopy times. When compared with Group II, Group I had significantly larger median stone size (9.2 vs. 14.0 mm, $p = 0.01$) and significantly lower stone-free rate after first URS (94.1% vs. 69.2%, $p = 0.04$). However, after second URS, stone-free rates were comparable in both groups (92.3% vs. 100%, $p = 0.9$). Two (16.7%) patients with coagulopathy were readmitted due to gross hematuria. There were no post-operative complications in Group II.

Conclusions: Although URS in selected patients with coagulopathies is safe, it is associated with significantly lower stone-free rates and higher readmissions due to gross hematuria.

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INTRODUCTION

Anticoagulants and antiplatelet agents are commonly used for prophylaxis of cardiovascular, cerebrovascular or venous thrombotic disease and post-implantation of mechanical valves and stents (1). Peri-operative management of these

anti-coagulated patients presents a dilemma to the surgeon since patients on chronic anticoagulation therapy have multiple comorbidities thus increasing risks of adverse thromboembolic events following perioperative cessation of anticoagulation (2). Furthermore, surgery may have thrombogenic nature and a potential hypercoagulable state that

may result from a rebound increase in clotting factors after discontinuation of these drugs (3). Withdrawal of antiplatelet agents in the perioperative period is associated with higher risks than the maintenance of these medications (4). Several studies have reported coronary stent thrombosis after premature discontinuation of antiplatelet agents (5-7), resulting in increased post-operative myocardial infarction, peri-operative cardiac mortality, and overall mortality (5,6,8). Therefore, peri-operative maintenance on anti-platelet agents is recommended for low-hemorrhagic risk procedures after drug-eluting stents (9). Extracorporeal shock wave lithotripsy (SWL), percutaneous nephrolithotomy (PCNL), laparoscopic or open stone surgery are contraindicated in patients with coagulopathies (10). Traditionally, bleeding diathesis is corrected and the anticoagulation therapy is withheld prior to any urological intervention to minimize surgical hemorrhage (11). However, despite pre-operative correction and apparently normal clotting parameters, patients with coagulopathy have a higher rate of complications and lower efficacy of SWL (12). The safety of URS and holmium laser lithotripsy in patients with coagulopathies without correction of the abnormality have been reported in 3 retrospective studies (10,13,14). Therefore, the aim of the present study was to expand the indications for URS in patients with coagulopathies and compare their outcome with concurrent patients with normal clotting parameters.

MATERIALS AND METHODS

Retrospective review of prospectively collected data of patients undergoing URS by a single surgeon (SA) between July 2009 and January 2011 was performed. Twelve patients with coagulopathies undergoing 17 URS comprised Group I [3 for biopsy of ureteral lesions and 9 for Holmium Laser Lithotripsy (HLL)]. Thirty-two concurrent patients with normal clotting parameters who underwent URS and HLL served as the control group (Group II). All patients had routine pre-operative evaluation that included complete blood count, prothrombin time, partial thromboplastin test and International Normal-

ized Ratio (INR). Pre-operative patient information including age, sex, stone/tumor size and location, co-morbidities, and indications for anticoagulation of patients with coagulopathies were collected. Intra-operative information such as operative time, fluoroscopy time, use of access sheath, stone-free status and any complications were recorded immediately post-operatively on research data forms. Post-operative outcome and complications especially hemorrhagic and thromboembolic events were recorded from office and hospital charts.

TECHNIQUE

All ureteroscopies were performed under general anesthesia in lithotomy position. All patients received broad spectrum antibiotic prophylaxis. Under sterile conditions, cystoscopy was performed and Sensor™ (Microvasive Boston Scientific, Natick MA, USA) was inserted. At this point semi-rigid URS was performed to identify and address ureteral stones/tumors. Ureteral lesions were biopsied using Piranha™ cold-cup biopsy forceps (Boston Scientific, Natick MA, USA). Ureteral stones were lithotripsied using Holmium: YAG laser with either 200µ or 365µ laser fibers at 10W setting. Stones were fragmented and basked out using Zero Tip™ basket (Boston Scientific, Natick, MA, USA). Whenever possible, stones were fragmented and removed rather than pulverized. When the rigid ureteroscope did not reach the stone, then a second wire was placed, a 14-16 Peel-Away (Cook Urological, Inc., Spencer, Indiana, USA) ureteral access sheath was placed and a 7.5F Storz Flex-X™ ureteroscope was used for proximal ureteral stones and renal stones. At the end of the procedure, a 6F double pigtail indwelling ureteral stent was placed. When the vision became poor or URS took longer than 2 hours, a staged URS was scheduled. When patients were deemed stone-free ureteroscopically (≤ 3 mm), stents were removed a week later. Otherwise, patients were followed by serial KUB to assure stone-free status (≤ 3 mm) prior to removal of the ureteral stent. When significant residual fragments remained, a repeat flexible URS and basketing of stone fragments was scheduled a month later.

Statistical Analysis

Data were analyzed using the commercially available Statistical Package of Social Sciences for Windows (SPSS, Chicago, IL), version 17. Descriptive data were presented in terms of percentages, range, medians and standard deviations. Continuous variables such as length of surgery, fluoroscopy time and stone size were compared with the Mann-Whitney U test. Fisher's exact test was used for categorical variables with two-tailed $p < 0.05$ being statistically significant.

RESULTS

Twelve patients with coagulopathies with a median age of 63.5 years were included in Group I (9 males and 3 females). In terms of coagulopathy, a patient had Child B (MELD 11) cirrhosis with thrombocytopenia, 6 patients were on warfarin [4 for Deep Vein Thrombosis (DVT), 1 for atrial fibrillation, 1 for mechanical aortic valve], 3 patients were on acetylsalicylic acid (ASA) and another patient was on combination of ASA and clopidogrel for coronary artery disease and coronary stents, and the last patient was on low molecular weight heparin (Tinzaprin) for recent DVT/PE (Table-1).

A total of 17 URS with HLL or biopsies of suspicious ureteral lesions were performed in 9 and 3 patients, respectively. URS in Group I was performed without correction of coagulopathy or suspension of their anti-coagulation therapy. For the 9 patients with coagulopathies undergoing URS and HLL, the median maximum stone diameter was 14 mm (5 - 22 mm). However, 6 out of the 9 patients had significant stone burden including a lower pole partial staghorn (Table-1). When visibility was poor, a staged URS was performed to obtain stone-free status. Therefore, 2 out of 9 patients (22.2%) underwent a second URS and one patient required a third URS to achieve stone-free status. In one of the three patients with ureteral lesions undergoing URS and biopsy, the first biopsy was inconclusive. Therefore, a repeat URS with biopsy was performed.

Group II consisted of 32 patients undergoing 34 URS and HLL for 45 stones. There

were no significant differences between the two groups in terms of patient age, sex, percent of renal stones, median operative time and fluoroscopy time (Table-2). However, Group I patients had significantly larger median stone size when compared with Group II (14.0 vs. 9.2 mm, $p = 0.01$) (Table-2). Due to poor vision, 3/12 (25%) patients in Group I and 2/32 (6%) patients in the control Group II underwent second URS to achieve stone-free status ($p = 0.11$). Stone-free rate after first URS was significantly lower in Group I compared with Group II (69.2% vs. 94.1%, $p = 0.04$). However, after the second URS, the stone-free rates were comparable in both groups (92.3% vs. 100%, $p > 0.05$). Calcium oxalate monohydrate represented the most common stone composition in both groups (67% and 43% respectively) followed by uric acid stones.

No patient had significant gross hematuria during the immediate post-operative period in both groups. However, the median post-operative level of hemoglobin significantly decreased in patients with coagulopathies when compared with controls (0.8 vs. 0.2 g/dL; $p = 0.001$).

Two patients (22 %) from Group I (patients 3 and 4) on warfarin therapy were readmitted for management of gross hematuria. Patient #3 in Group I was readmitted on post-operative day 47 post URS and biopsy of invasive TCC. Patient #4 in Group I had an INR of 3.14 and was readmitted on post-operative day 6 post URS and HLL. Both patients underwent continuous bladder irrigation and their anti-coagulants were withheld till hematuria resolved. They did not require transfusions. There were no post-operative complications in Group II.

DISCUSSION

Ureteroscopy and holmium laser lithotripsy achieve a high stone-free rate of over 97% in ureteral calculi with only 6% of patients requiring an additional procedure (15). With increasing experience and miniaturization of flexible ureteroscopes, indications for URS have expanded to include large renal stones, children, pregnant women, and patients with coagulopathy (16). This is because endoscopic procedures with

Table 1 - Characteristics of patients with coagulopathies (Group I) and indications of URS.

Pt. No.	Age	Sex	Type of coagulopathy	Anticoagulant/bleeding diatheses	Indications of URS	No of URS	Stone composition / pathology
1	59	F	DVT	Warfarin	Partial staghorn stone (22 X 22 mm)	2	Uric acid dihydrate
2	62	M	DVT	Warfarin	UPJ stone (20X13 mm)	1	Struvite
3	86	M	Atrial fibrillation + DVT	Warfarin	Mid-ureteral mass (40X 20 mm)	1	High grade invasive TCC
4	47	M	Mechanical aortic valve	Warfarin	Lower pole stones (18 & 12 mm)	3	Calcium oxalate monohydrate
5	78	M	Recent atrial fibrillation	Warfarin	Obstructing UPJ stone (11X6mm) + renal gravel	1	Uric acid dihydrate
6	58	M	Coronary disease and stent	ASA	Distal ureteral stone (10X5 mm)	1	Carbonate apatite + Calcium oxalate
7	70	M	Coronary disease and stent, ESRD	ASA	Mid ureteral lesion (10mm)	2	Cytological atypia Ureteritis
8	52	M	Recent DVT/ PE	LMW heparin (Tinzaprin)	Lower pole kidney stones (14X10 and 9X7 mm) with UPJ stone (8X6 mm)	2	Calcium oxalate monohydrate
9	62	F	Child B, MELD 11 hepatic cirrhosis	Thrombo-cytopenia	UPJ stone (10 mm) and lower pole stone (5 mm)	1	Calcium oxalate monohydrate
10	63	M	Bilateral DVT	Warfarin	Upper ureteral stone (5 mm)	1	Calcium oxalate monohydrate
11	44	F	Coronary stent	ASA + Clopidogrel	Upper ureteral lesion	1	Chronic inflammation
12	59	M	Recent MI and CABG	ASA	Lower pole kidney stone (8 mm)	1	Calcium oxalate monohydrate

ASA: acetyl salicylic acid; **DVT:** Deep Vein Thrombosis; **ESRD:** End Stage Renal Disease; **PE:** Pulmonary embolism; **MI:** myocardial Infarction; **CABG:** Coronary Artery Bypass Graft; **LMW:** low molecular weight; **UPJ:** uretero-pelvic junction; **PE:** pulmonary embolism; **PLT:** platelets; **POD:** post operative day; **TCC:** Transitional Cell Carcinoma

Table 2 - Comparison of patients undergoing URS and laser lithotripsy.

Variable	Group I (n = 9)	Group II (n = 32)	P- Value
Median Age (yrs)	60.0	53.5	0.27
Male gender	7 (78%)	19 (59%)	0.49
Percent Renal Stones (# renal/ total)	8/14 (57%)	18/45 (40%)	0.35
Median stone size (mm)	14	9.2	0.01
Stone-free rate After 1st URS	69.2%	94.1 %	0.04
Stone-free rate After 2nd URS	92.3 %	100%	0.9
Median URS time (min.)	60	60	0.21
Median Fluoroscopy time (Sec)	63	114	0.24

Group I: patients with coagulopathies; **Group II:** patients with normal clotting parameters.

low risk of hemorrhage such as URS and HLL can be performed without discontinuation of anticoagulation therapy (17). Thus, URS and HLL may be the only option for these patients with coagulopathies since they are often poor candidates for SWL or PCNL due to hemorrhagic and thromboembolic complications (10,13).

In the present study, 6 out of 9 patients (patients 1, 2, 4, 5, 8, 9) in Group I undergoing URS and laser lithotripsy had significant stone burden (Table-1). Traditionally, these patients would be treated with PCNL with correction of the coagulopathy. Discontinuing and re-initiating anticoagulation therapy in these high risk patients may have increased risk of hemorrhagic and thromboembolic complications. PCNL with reversal of anticoagulation has been previously described in 27 such high risk patients (2). However, two patients (7%) developed post-operative hemorrhage with one patient requiring angio-embolization. Another patient (4%) developed DVT with pulmonary embolism on POD 4 requiring IVC filter since he had developed hemorrhage when anticoagulation was initiated (2). Furthermore, the expense of bridging therapy (with low molecular weight heparin or intravenous heparin) is considerable (10). Therefore, the present study expands

the indications for URS in patients with coagulopathies to those who are traditionally treated with PCNL with reversal of their anti-coagulation. This would be ideal for patients who cannot safely undergo withholding of anticoagulation.

The first series describing URS in patients with coagulopathies was by Kuo et al. (Table-3) (14). Eight patients with stone disease and 1 patient with upper tract TCC were treated by URS with the holmium laser (14). Six out of 7 patients who underwent laser fragmentation for calculi were stone free at 1 month, and no tumor recurrence was noted in the patient with TCC (follow-up of 4 months). One patient only had a post-operative bleeding complication related to the procedure, involving an episode of oliguria secondary to a small ureteral clot that was resolved with diuretics. Watterson et al. reported on a series of 25 patients who were anticoagulated either pharmacologically or by underlying systemic diseases (13). The overall stone-free rate after a single ureteroscopic procedure was 93%. There were no hemorrhagic complications in patients undergoing laser lithotripsy. One patient who underwent electrohydraulic lithotripsy developed a retroperitoneal hematoma necessitating transfusion (Table-3). Therefore, electrohydraulic litho-

Table 3 - Comparison between the present series and previous published studies.

Study	No of Patients	Type of Study	Remarks	Complications
Kuo et al. (14)	8 patients for urolithiasis + 1 upper pole TCC	Retrospective over 11 month period	Thrombocytopenia was corrected in 1 patient before URS	1 Ureteral bleeding and 2 non-urological complications.
Watterson et al. (13)	25 (30 URS for 29 stones)	Retrospective in 2 tertiary stone centers over 5.5 years	URS and HLL (20 ureter+ 9 kidney) Thrombocytopenia was corrected in one patient before URS	Retroperitoneal Hemorrhage in one patient (EH lithotripsy)
Turna et al. (10)	37 URS+ HLL Renal calculi	Retrospective in 2 centers over 7 years	URS on active anticoagulants	Post operative hematuria > 3 days in 3 patients
Present series	17 URS (HLL in 9 and biopsies in 3 patients)	Retrospective over 18 months	URS on active anticoagulants	Post operative hematuria in 2 patients requiring CBI

CBI: continuous bladder irrigation; **HLL:** Holmium laser lithotripsy; **TCC:** Transitional cell carcinoma; **URS:** Ureteroscopy; **EH:** Electrohydraulic

tripsy must be avoided in this highly selected group of patients with coagulopathies (18). The Holmium: YAG laser has the ability to fragment calculi of all compositions including calcium oxalate monohydrate and it is an ideal intracorporeal lithotripter for ureteral calculi with a high success rate and low morbidity (19). Moreover, the Holmium: YAG laser has haemostatic properties that would be beneficial for treating patients with bleeding disorders (20). Therefore, it is ideal for fulguration of bleeders during biopsy of ureteral lesions in patients with coagulopathies.

Turna et al. compared a group of 37 patients on aspirin, clopidogrel, or warfarin with a cohort of matched controls without coagulopathy and found similar stone-free rates (81.1% versus 78.4%, $p = 0.7$) (10). However, the peri-operative hemoglobin change was significantly higher in the anticoagulated group (6 g/L vs. 2 g/L, $p < 0.0001$). In that study, there were no procedures terminated because of poor visibility. The authors reported 3 cases of hematuria of more than 3 days in patients with coagulopathies (Table-3).

In the present study, after the first URS, the stone-free rate was significantly lower in Group I when compared with Group II (69.2% vs. 94.1%, $p = 0.04$). This could be related to the fact that patients in Group I had significantly larger median stone size (14 vs. 9.2 mm, $p = 0.01$). Furthermore, two thirds (6 out of 9) of patients had significant stone burden that are ideally managed by PCNL. In the present study Group I, 2 out of 9 (22.2%) patients underwent a second URS and one patient required a third URS to achieve stone-free status. However, after a second URS, the stone-free rates were comparable in both groups (92.3% vs. 100%, $p > 0.05$). Similarly, in the study by Watterson et al., there was a second URS in 5 patients. Furthermore, in that study, a thrombocytopenic patient had correction of thrombocytopenia prior to URS. Thus, although URS and HLL are safe in these highly selected patients with coagulopathies, they may require more than one URS session for stone clearance. Larger sample size is required to verify these results.

Previous reports of URS and laser lithotripsy in patients with coagulopathies did not report stone composition. In the present series, most of the stones in both groups (67% in Group I and 43% in Group II) were composed of calcium oxalate monohydrate, which is one of the hardest stones to fragment (21). Therefore, this may have contributed to the lower stone-free rate in Group I.

Except for one patient reported by Kuo et al., there are no other reports in the literature about safety of URS and ureteral biopsy in patients with coagulopathies (14). In the present study, 3 patients underwent ureteral biopsies safely and efficiently while they were on anticoagulants. One of them developed a late hematuria (after 47 days). This was an 86-year old man on warfarin for repeated bilateral DVT and atrial fibrillation in addition to past medical history of hypertension, diabetes mellitus, and chronic renal failure. His preoperative INR was 2.89. After his diagnostic URS and biopsy, he underwent external beam radiotherapy for his 4 cm mid-ureteral invasive TCC. Therefore, his delayed hematuria could be related to other factors than the procedure itself such as the invasive TCC, indwelling ureteral stent, or radiation ureteritis. The other 2 patients underwent 3 ureteral biopsies on 3 occasions without complications indicating the safety of ureteroscopic biopsies in these patients with coagulopathies.

There are several limitations of the present study. Although the data were collected prospectively, this still remains a retrospective review of highly selected small cohort of patients with coagulopathies undergoing URS. Furthermore, the cohort with coagulopathies was diverse with multiple different therapies (antiplatelet and anticoagulation (Coumadin, LMW heparin)) undergoing two different procedures (biopsy and lithotripsy). Another limitation was that the INR on the day of the URS was not confirmed to be in the therapeutic level. It was only checked in the pre-operative evaluation.

CONCLUSIONS

Although URS in selected patients with coagulopathies is safe, it is associated with sig-

nificantly lower stone-free rates and higher re-admission for management of gross hematuria. Prospective randomized studies with and without correction of the coagulopathy is needed to weigh the risks and benefits of correcting anticoagulation during ureteroscopy and laser lithotripsy or biopsy of ureteral lesions.

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ABBREVIATIONS

ASA: acetyl salicylic acid
CBI: Continuous Bladder Irrigation
DVT: Deep Vein Thrombosis
ESRD: End Stage Renal Disease
HLL: Holmium Laser Lithotripsy
INR: International Normalized Ratio
LMW: low molecular weight
PCNL: Percutaneous Nephrolithotomy
PE: Pulmonary Embolism
PLT: platelets
POD: post operative day
SWL: Extracorporeal Shockwave Lithotripsy
TCC: Transitional Cell Carcinoma
UPJ: uretero-pelvic junction
URS: ureteroscopy

CONFLICT OF INTEREST

None declared.

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EDITORIAL COMMENT

A clinical problem that troubles urologists is how they should treat patients on anticoagulants. Interruption of anticoagulation therapy for elective urologic procedures in these patients generates a complex situation in which competing risks of thrombosis and bleeding must be weighed up; when anticoagulation is discontinued patients

are at risk of cardiovascular complications, and when it is restarted they are at risk of hemorrhage (1,2). There is an increasing interest in the evaluation of the risk of operating on patients on ongoing anticoagulation due to technological advances (e.g. laser prostatectomy) and the growing aging population who suffers from cardiovascular co-morbid-

ities. However, the safety and efficacy of different procedures have not been well documented.

In the present study, Elkoushy et al. compared the outcomes of ureteroscopy (URS) in patients with coagulopathies with those with normal bleeding profile. It was found that although URS in selected coagulopathic patients was safe, it was associated with significantly lower stone-free rates and higher re-admission for gross hematuria. The main limitations of the study included its retrospective nature, the diversity of the study popu-

lation (patients under different drugs with different properties) and the small number of patients enrolled (e.g. one could argue that the difference in patients who underwent 2nd URS due to poor vision did not reach statistical significance due to the small sample size). However, this study is useful because the authors add their experience to the limited existing literature and provide information which help urologists to better inform their patients about the potential risks and benefits of URS without stopping anticoagulation.

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Cytoprotective role of the aqueous extract of *Terminalia chebula* on renal epithelial cells

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ABSTRACT

Purpose: Kidney stone is one of the most prevalent diseases worldwide. Calcium oxalate (CaOx) has been shown to be the main component of the majority of stones formed in the urinary system of the patients with urolithiasis. The present study evaluates the antilithiatic properties of *Terminalia chebula* commonly called as “harad” which is often used in ayurveda to treat various urinary diseases including kidney stones.

Materials and Methods: The antilithiatic activity of *Terminalia chebula* was investigated on nucleation and growth of the calcium oxalate crystals. The protective potency of the plant extract was also tested on oxalate induced cell injury of both NRK-52E and MDCK renal epithelial cells.

Results: The percentage inhibition of CaOx nucleation was found 95.84% at 25µg/mL of *Terminalia chebula* aqueous extract which remained almost constant with the increasing concentration of the plant extract; however, plant extract inhibited CaOx crystal growth in a dose dependent pattern. When MDCK and NRK-52E cells were injured by exposure to oxalate for 48 hours, the aqueous extract prevented the injury in a dose-dependent manner. On treatment with the different concentrations of the plant extract, the cell viability increased and lactate dehydrogenase release decreased in a concentration dependent manner.

Conclusion: Our study indicates that *Terminalia chebula* is a potential candidate for phytotherapy against urolithiasis as it not only has a potential to inhibit nucleation and the growth of the CaOx crystals but also has a cytoprotective role.

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Key words:

urolithiasis; kidney; phytotherapy; calculi

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INTRODUCTION

Urolithiasis is a very painful disease that has afflicted a wide sector of human population since ancient times (1). Calcium-containing stones are the most common comprising about 75% of all urinary calculi, which may be in the form of pure calcium oxalate (50%) or calcium phosphate (5%) and a mixture of both (45%). Cal-

cium oxalate stones are found in two different varieties, calcium oxalate monohydrate (COM) or Whewellite, and calcium oxalate dihydrate (COD) or Weddellite. COM, the thermodynamically most stable form, is observed more frequently in clinical stones than COD and has a greater affinity for renal tubular cells, thus responsible for the formation of stones in the kidney (2). Crystal growth and agglomeration may be due to supersaturation with respect to stone forming constituents or the

presence of various inhibitory or stimulatory biomolecules or even pH (3).

Renal tubular fluid is supersaturated with calcium and oxalate ions, which can nucleate to form crystals of calcium oxalate monohydrate. Microcrystals of COM, the most common crystal in renal stones, irreversibly bind to cell surface microvilli, are subsequently internalized and can stimulate proliferation (4). Therefore, this indicates that renal epithelial cells can bind and internalize calcium oxalate crystals.

Extracorporeal shock wave lithotripsy (ESWL) is currently the first line treatment for upper urinary tract calculi. This treatment is not without side effects and kidney damage during ESWL is a clinically significant problem (5). Therefore, it is worthwhile to look for an alternative cure and phytotherapy is proving to be a promising alternative.

Since civilization, medicinal plants are part of human society to combat diseases. Traditional knowledge of healers and venders are in great demand in the developed as well as developing countries for primary healthcare because of their wide biological and medicinal activities, higher safety margins and lesser cost (6).

Terminalia chebula locally named as "harad" in India has been extensively used in ayurveda. It is used to treat urolithiasis and is actively used in various drug formulations of kidney stone treatments like neeri (product information by AIMIL PHARMACEUTICALS (INDIA) LTD.). It is extensively explored for antimicrobial (7), antioxidant (8), anticarcinogenic (9), hypocholesterolemic (10) and diuretic (11,12) activities by various research groups.

The present study is aimed to investigate the efficacy of *Terminalia chebula* on nucleation and growth of calcium oxalate in vitro and further examining the potency of the same on oxalate induced injury in MDCK and NRK 52E cells.

MATERIALS AND METHODS

Plant material

The fruits of *Terminalia chebula* used in this study were collected from Shimla, India which

were identified and then authenticated by microscopical and physiochemical data.

Preparation of plant extracts

The air-dried fine powdered plant fruits were infused in distilled water until complete exhaustion. The extract was then filtered using Whatman No. 1 filter paper and the filtrate was evaporated in vacuo and dried using a rotary evaporator at 40° C. The final dried samples were stored in labeled sterile bottles and kept at -20° C and were referred to as aqueous extract (13). The various concentrations of the plant extract tested for their inhibitory potency were 25 µg/mL, 50 µg/mL, 100 µg/mL, 200 µg/mL, 400 µg/mL and 1000 µg/mL, which were prepared at the time of experiment.

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

Nucleation Assay of calcium oxalate

The method used was similar to that described by Hennequin et al. with some minor modifications (15). Solutions of calcium chloride and sodium oxalate were prepared at the final concentration of 3mmol/L and 0.5mmol/L, respectively, in a buffer containing Tris-HCl 0.05mol/L and NaCl 0.15mol/L at pH 6.5. 3 mL of calcium chloride. The solution was mixed with test sample at different concentrations of 25 µg/mL, 50 µg/mL, 100 µg/mL, 200 µg/mL, 400 µg/mL and 1000 µg/mL. Crystallization was started by adding 1.5 mL of sodium oxalate solution in 1.5 mL calcium chloride. The temperature was maintained at 37° C. The absorbance of the solution was monitored at 620nm every 1 min. The percentage inhibition produced by the aqueous extract was calculated as $(1 - (T_{si}/T_{sc})) \times 100$, where T_{sc} was the turbidity slope of the control and T_{si} the turbidity slope in the presence of the inhibitor.

Growth Assay of calcium oxalate

Inhibitory activity against CaOx crystal growth was measured using the seeded solution-depletion assay, described previously by Nakagawa et al (16). Briefly, an aqueous solution of 10mM Tris-HCl containing 90mM NaCl was adjusted to pH 7.2. Stone slurry (1.5 mg/mL) was prepared in 50mM sodium acetate buffer (pH 5.7). CaOx monohydrate crystal seed was added to a solution containing 4mM calcium chloride (CaCl_2) and 4mM sodium oxalate ($\text{Na}_2\text{C}_2\text{O}_4$). The reaction of CaCl_2 and $\text{Na}_2\text{C}_2\text{O}_4$ with crystal seed led to deposition of CaOx on the crystal surfaces, thereby decreasing free oxalate that is detectable by spectrophotometry at $\lambda 214$ nm. When various extracts at different concentrations of 25 $\mu\text{g/mL}$, 50 $\mu\text{g/mL}$, 100 $\mu\text{g/mL}$, 200 $\mu\text{g/mL}$, 400 $\mu\text{g/mL}$ and 1000 $\mu\text{g/mL}$ is added into this solution, depletion of free oxalate ions will decrease if the test sample inhibits CaOx crystal growth. Rate of reduction of free oxalate was calculated using the baseline value and the value after every 1 min. for 20 mins. incubation with or without test sample. The relative inhibitory activity was calculated as follows: % Relative inhibitory activity = $((C-S)/C) \times 100$, where C is the rate of reduction of free oxalate without any test sample and S is the rate of reduction of free oxalate with a test sample.

Cell Culture

MDCK and NRK 52E cells were obtained from National Centre of Cell Sciences (NCCS, Pune, India). The cells were maintained as monolayer in DMEM with 2.0 mM L-glutamine adjusted to contain 3.7 g/l sodium bicarbonate and 4.5 g/l glucose. Medium was supplemented with 1% penicillin (100 units/mL), streptomycin (10,000 $\mu\text{g/mL}$) and 10% fetal bovine serum. Cells were cultured in 25 cm^2 tissue-culture treated flasks at 37° C and 5% CO_2 in humidified chambers.

Oxalate-induced Cell Injury

MDCK and NRK 52E cells were incubated in DMEM containing 1mM sodium oxalate in the presence of different concentrations of the aqueous extract of the test sample (10, 25, 50 and 80 $\mu\text{g/mL}$) for 48 hours (14,17). Cell injury was assessed by measuring the cell viability through

trypan blue and monitoring the lactate dehydrogenase (LDH) leakage into the medium.

Cytotoxicity Trypan blue assay

The cytotoxicity of the crude extract of T. chebula was assessed by determining the cell viability using trypan blue exclusion method. For the determination of cell viability, cells were plated at a density of 4×10^4 cells/well and cultured for 48 hours. The medium was replaced with serum-free medium and the cells were treated with various concentrations of the plant extracts (10, 25, 50 and 80 $\mu\text{g/mL}$) for a further 48 hours. The percentage viability for the cells was calculated as (live cells/total cells)*100.

Lactate dehydrogenase leakage assay

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

Statistical Analysis

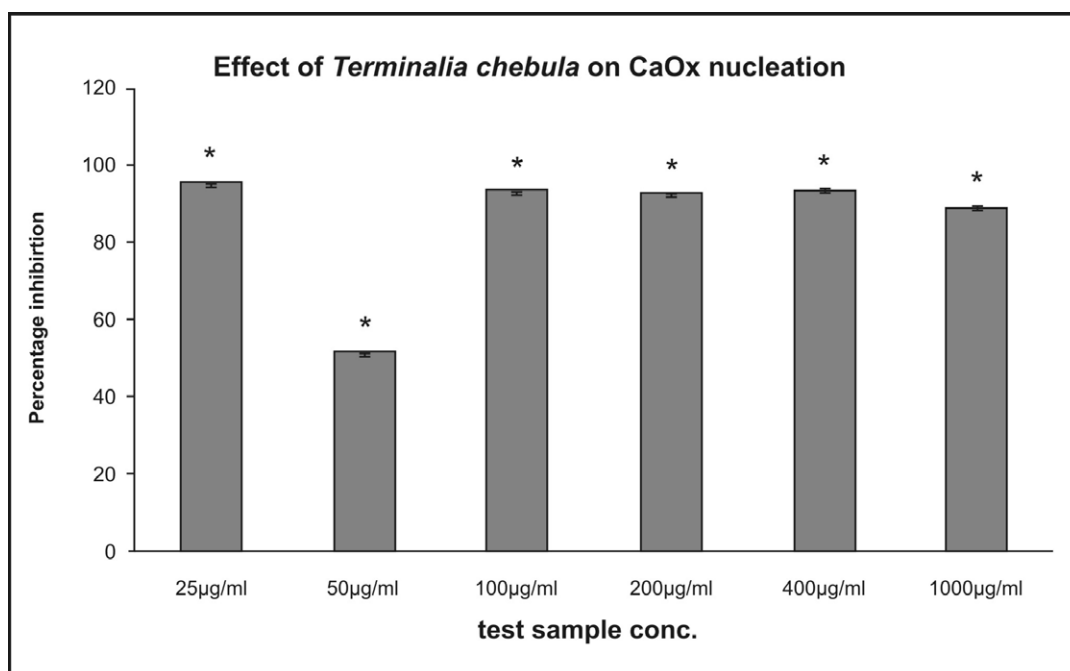
Data were expressed as mean values of three independent experiments (each in triplicate) and analyzed by ANOVA ($p < 0.05$) to estimate the differences between values of extracts tested.

RESULTS

Inhibition of Nucleation of CaOx Crystals by Terminalia chebula Extract:

Figure-1 displays the effect of aqueous extract of Terminalia chebula on the nucleation of calcium oxalate crystals. With respect to the control (with no plant sample), the percentage inhibition shown by aqueous extract at 25 $\mu\text{g/mL}$ was 95.8% with almost constant inhibition at 100 $\mu\text{g/mL}$, 200 $\mu\text{g/mL}$ and 400 $\mu\text{g/mL}$ in the range of 91-94%.

Figure 1 – Effect of crude extract of Terminalia chebula on nucleation of CaOx. Data are mean \pm S.D. of three independent observations. * $p < 0.05$.



Inhibition of CaOx Crystal Growth by Terminalia chebula Extract:

Figure-2 displays the effect of Terminalia chebula on the growth of calcium oxalate crystals. The aqueous extract displayed concentration dependent percentage inhibition when compared to the control (with no plant extract). The percentage inhibition with 25 µg/mL was found to be 39.9%, which increased to 105.8% with 1000 µg/mL aqueous extract.

Diminution of Oxalate-Induced Renal Tubular Epithelial Cell Injury by Terminalia chebula Extract:

Figures-3 and 4 depicts the protective effect of the aqueous extract of Terminalia chebula towards the renal tubular epithelial cells, MDCK and NRK-52E respectively with respect to cell viability. The oxalate induced a significant injury to the cells which could be ascertained by a decrease in viability which was greater in MDCK as

compared to NRK-52E. However, the injury due to oxalate was significantly reduced in those cells treated with the T. chebula extracts. As the concentration of the extract increased from 10 µg/mL to 80 µg/mL, the percentage viability improved showing that the plant has an inhibitory activity towards the oxalate which caused injury to the renal cells in a concentration dependent manner. The plant extract alone (80 µg/mL, containing 0.4% DMSO) had no effect on the cell injury in the absence of oxalate indicating that even at the highest concentration of DMSO used there was no cytotoxicity to the cells. The concentration dependent percentage viability was seen in both the cell lines. The viability increased from 41.3%, as in oxalate injured cells to 60.4% in the presence of 80 µg/mL plant extract when tested with NRK-52E. A similar pattern was observed with MDCK where the viability increased to 71.3% when treated with 80 µg/mL plant extract as compared to 52.9% viability in oxalate injured cells.

Figure 2 - Effect of crude extract of *Terminalia chebula* on growth of CaOx. Data are mean \pm S.D. of three independent observations. * $p < 0.05$

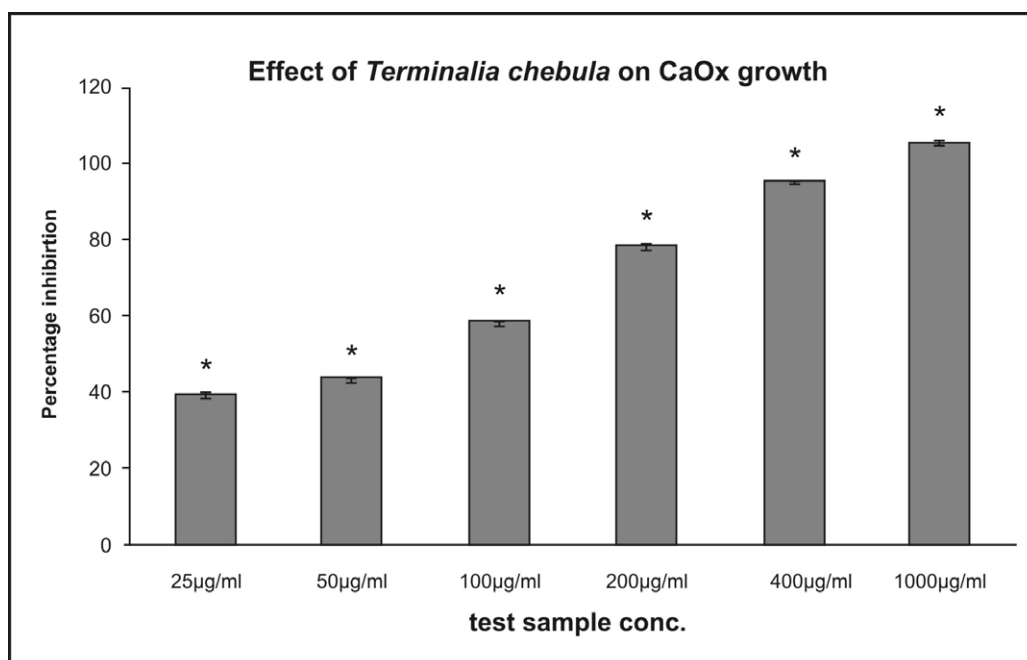


Figure 3 – Effect of *Terminalia chebula* on the viability of MDCK. Data are mean \pm S.D. of three independent observations. * $p < 0.05$ versus untreated control, ** $p < 0.05$ versus oxalate control.

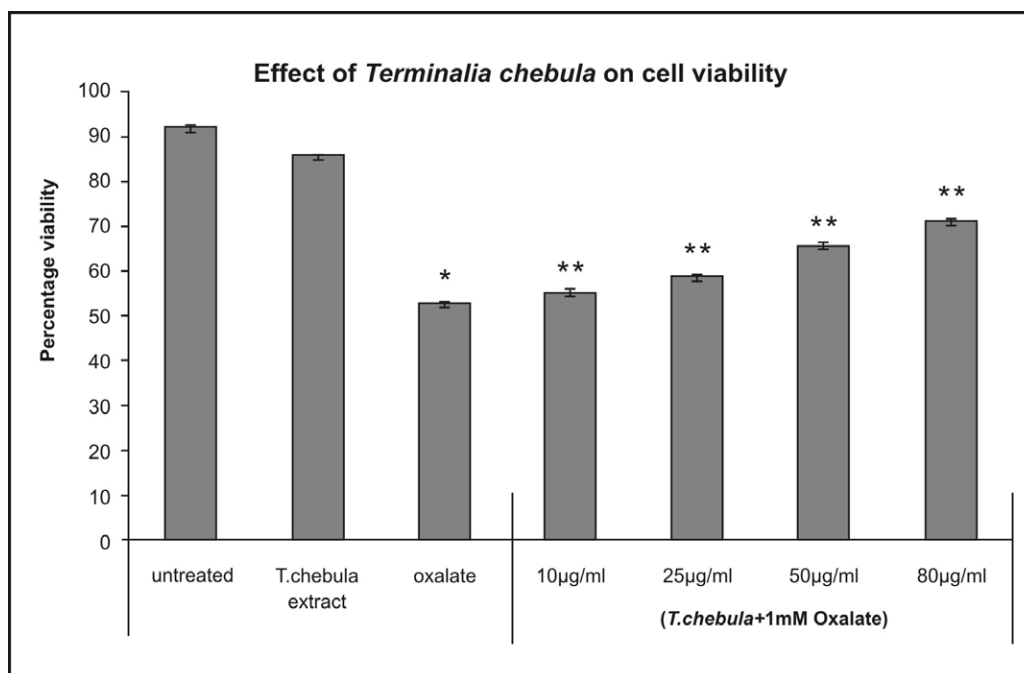


Figure 4 – Effect of *Terminalia chebula* on the viability of NRK-52E. Data are mean \pm S.D. of three independent observations. * $p < 0.05$ versus untreated control, ** $p < 0.05$ versus oxalate control.

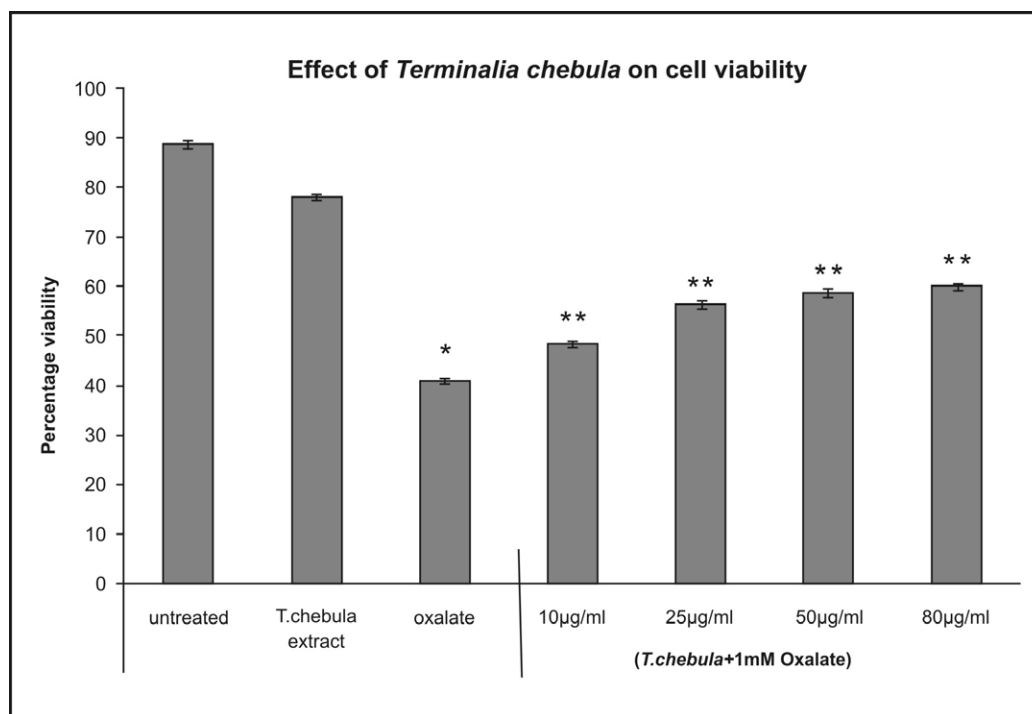


Figure 5 – Effect of *Terminalia chebula* on the % LDH release of MDCK. Data are mean \pm S.D. of three independent observations. * $p < 0.05$ versus untreated control, ** $p < 0.05$ versus oxalate control.

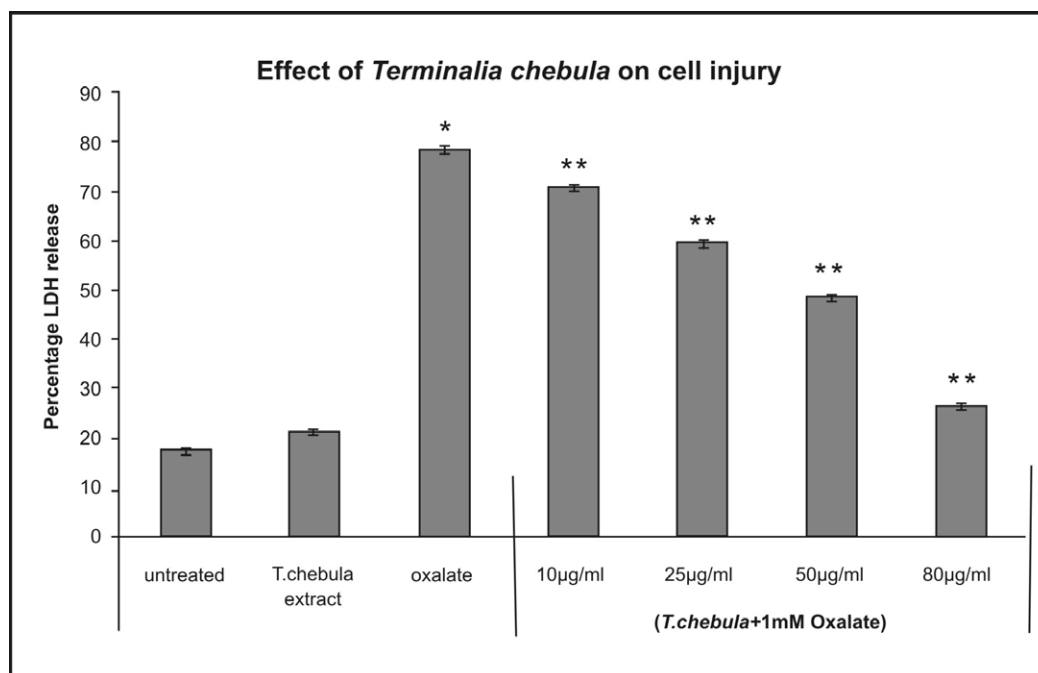
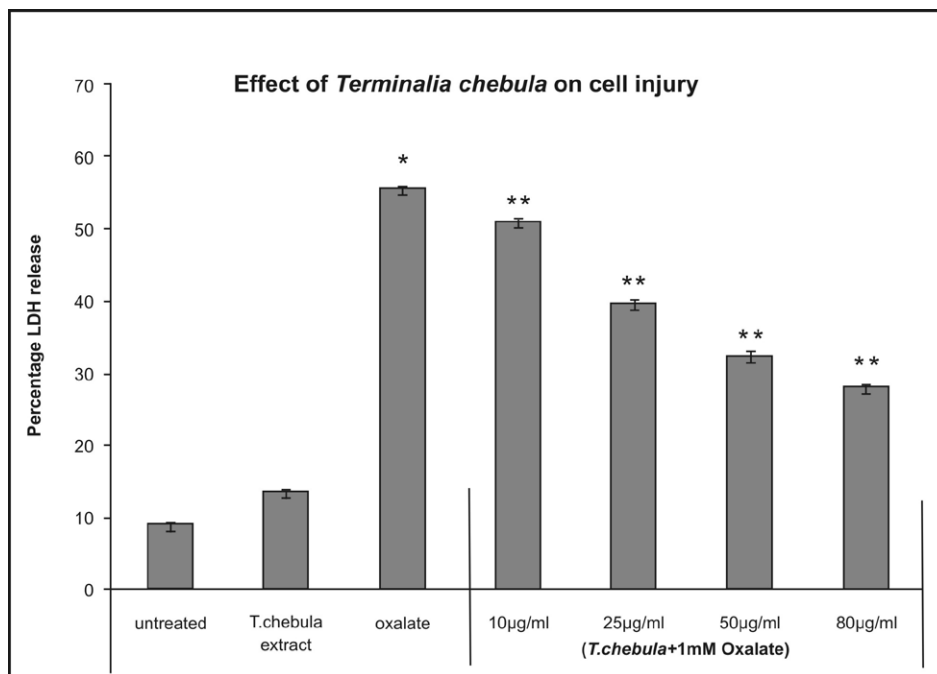


Figure 6 – Effect of Terminalia chebula on the % LDH release of NRK-52E. Data are mean \pm S.D. of three independent observations. * $p < 0.05$ versus untreated control, ** $p < 0.05$ versus oxalate control.



Lactate dehydrogenase is a stable cytosolic enzyme that is released when the cell is lysed or there is any injury on the cell membrane. A significant increase in LDH release was seen when both the cells were exposed to oxalate alone. When cells were treated with the plant extract at varying concentrations (10, 25, 50 and 80 µg/mL) along with oxalate (1mM) for 48h, a reduction in oxalate-induced cell injury was observed as assessed by a decreased LDH release (Figures 5 and 6). Again it was observed that the plant extract alone had no significant effect on the measures of cell injury in the absence of oxalate. The percentage LDH release for MDCK and NRK-52E is dependent upon concentration after treatment with oxalate and the plant extract with respect to control. The LDH release was significantly decreased to the level of 28.2% in the treatment with 80 µg/mL test sample in NRK-52E, though the LDH release with oxalate injured cells was found to be 55.8% with respect to untreated cells. MDCK cells also showed the same pattern with 78.6% LDH

release in oxalate injured cells which decreased significantly to 26.7% with the highest concentration of the plant sample.

DISCUSSION

There has been a long standing quest for potent inhibitors of calcium oxalate growth as it is the most common urinary stone associated with renal injury. Recent evidence suggests that in many calcium oxalate stone formers the earliest changes may be calcium salt deposition in the medullary interstitium. In marked hyperoxaluric states, primary hyperoxaluria directs calcium oxalate crystal adhesion to renal epithelial cells (19).

Stones larger than 5mm fail to pass through and hence can be treated through ESWL but the chance for stone recurrence is still about 50%. In addition, ESWL might show some significant side effects such as renal damage, hypertension or renal impairment. Therefore, phytotherapeutic agents could be useful as either an alternative or

a complementary therapy in the management of urolithiasis with some possible mechanisms of action including an increased excretion of urinary citrate, decreased excretion of urinary calcium and oxalate or could be attributable to diuretic, antioxidant or antibacterial effects (20).

The present investigation deals with the effects of putative litholytic medicinal plant, *Terminalia chebula* on CaOx crystals. Fruits of *T. chebula* are a popular folk medicine and have been studied for its diuretic activity but the scientific basis of its activity was not yet established. In this study, the inhibitory potency of the plant was tested on nucleation and growth of calcium oxalate crystallization in vitro. The aqueous extract inhibited the CaOx growth to the tune of 105.8%. Further, a protective effect on renal epithelial cells was shown by the aqueous plant extract in a concentration-dependent manner. When MDCK and NRK-52E cells were injured by exposure to 1mM oxalate for 48h, the plant extract prevented the injury in a dose-dependent manner. The oxalate injury to MDCK cells were more intense as compared to that of NRK-52E. The percentage LDH release in NRK-52E was less at 80 µg/mL than MDCK. Therefore, *T. chebula* seems to be more responsive towards NRK-52E than MDCK.

Several traditional Chinese medicines/plants that are used in Kampou medicine also have demonstrated their abilities to inhibit calcium oxalate crystallization. Dietary factors appear to affect the ability of urine to inhibit COM crystallization. In this regard, lemon juice has been found to inhibit the rate of crystal nucleation and aggregation (20).

Various plants are being evaluated for their antiurolithiatic potency through their activity on renal epithelial cell lines which are accepted as a powerful tool to establish the mechanism of nephrolithiasis. Atmani et al. used MDCK cell lines as a model to study the adhesion of radioactive COM crystals in the presence and absence of aqueous extract of *Herniaria hirsuta* and found that the crystal attachment was inhibited in a concentration dependent manner (19). In vitro effect of an aqueous extract of *Phyllanthus niruri* L., a plant used in Brazilian folk medicine for the treatment

of urolithiasis, on a model of COM crystal endocytosis by MDCK cells, was investigated by Campos and Schor. The extract exhibited a potent and effective non-concentration-dependent inhibitory effect on the COM crystal internalization. This response was present even at very high (pathologic) COM concentrations and no *Phyllanthus niruri* L. induced toxic effect could be detected (21). The fruits of *Ammi visnaga* L. commonly called as Khella have traditionally been used in Egypt to relieve pain of kidney stone passage by drinking a tea prepared from the crushed or powdered fruits of khella. A study was undertaken to evaluate its effect on renal epithelial injury using LLC-PK1 and Madin-Darby-canine kidney (MDCK) cells. Over the past few decades two continuous renal epithelial cell lines have been most used or studying nephrolithiasis, the Madin-Darby canine kidney collecting duct tubular epithelial cells (MDCK) and porcine kidney proximal tubular epithelial cells (LLC-PK1). MDCK cells have been widely used as a model system for the distal/collecting duct and LLC-PK1 cells have retained many characteristics of the proximal tubule. Vanachayangkul et al. evaluated the effect of aqueous khella extract on oxalate induced renal injury and found that the cell injury (LDH release) was significantly reduced in cells treated with the extract (22).

The antilithiasic potency of various plants like *Dolichos biflorus* (23), *Trachyspermum ammi* (24), *Tribulus terrestris* (2) and *Achyranthes aspera* (25) and the inhibitory effect of various biomolecules on renal stones (26) have been evaluated in our lab.

The inhibitory role of various plant species from west and south of Algeria in calcium oxalate growth was investigated in vitro by Beghalia et al. (27). They further postulated that the plant extracts may contain substances that inhibit COM crystal aggregation and also the binding of the crystals to the renal epithelial surface. This could explain a decrease in LDH release observed in the cells treated with the plant extract compared to those treated with oxalate alone. The cDNA microarray was used to evaluate gene expression in urolithiasis by exposing the COM crystals to NRK-52E cells (28).

CONCLUSIONS

This study demonstrated that *Terminalia chebula* extract showed cytoprotective properties towards the MDCK and NRK-52E cells by reducing the LDH leakage and increasing the cell viability. At the same time, it also has an ability to inhibit the calcium oxalate crystals in vitro. In the light of these studies, it is a valuable candidate for further pharmacological analysis.

ABBREVIATIONS

COM: calcium oxalate monohydrate
 COD: calcium oxalate dihydrate
 CaOx: calcium oxalate
 ESWL: extra corporeal shock wave lithotripsy
 MDCK: madin darby canine kidney
 NRK-52E: normal rat kidney epithelial
 DMSO: dimethyl sulfoxide
 DMEM: dulbecco's modified eagle's media
 LDH: lactate dehydrogenase

CONFLICT OF INTEREST

None declared.

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EDITORIAL COMMENT

The authors address an interested in vitro manuscript, containing a new fact, studying a possible option of lithiasis treatment. The subject of the article is particular, exposing MDCK and NRK-52E renal epithelial renal cells to oxalate induced cell injury. The manuscript concluded that the plant extract, *Terminalia chebula*, has a protect action against urolithiasis, inhibiting nucleation and the growth of the CaOx crystals.

The urolithiasis clinical treatment has been extensively studied with different herbs, otherwise, others studies demonstrated a potent inhibitory effect on CaOx crystal adhesion and endocytosis of folk plants, as *Phyllanthus niruri*, from South America, Africa in vitro and vivo models (1,2).

This well done paper demonstrated a nice result, but it needs to be done in animal models, human cells to provide a future clinical study to be a consistent and reproducible data.

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EDITORIAL COMMENT

This is a very interesting paper which addresses a subject that is not commonly seen in the urologic literature. It has been written in a satisfactory and scientific way since the type of therapy described is more commonly found in lay press and as such does not contain strong scientific substract.

I am sure that this study brings scientific information that could lead to further studies in this area similarly as some substances used as cancer latest generation chemotherapeutic agents which were originally found in plant species. The

Brazilian species of *Phyllanthus niruri* L. Popularly known as “chá de quebra pedra” (Ref. 21 in the article) is an example of many plants of our flora that could have been submitted to randomized studies to try to bring answers to the prevention and eventually treatment of stone disease. This management represents a major source of loosing work force and complications for patients without mentioning the great money expenditure with sophisticated equipments like ESWL that time has proven to be not as effective as initially proposed.

The authors must be congratulated for the style and content of this manuscript.

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Laparoscopic treatment of lymphoceles after renal transplantation

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ABSTRACT

Objective: Lymphocele formation following renal transplantation is a frequent complication and may affect as many as 49% of patients. Operative treatment of symptomatic post transplant lymphocele (PTL) consists of wide drainage of the fluid collection into the abdominal cavity by excising its wall, connecting the lymphocele cavity to the intraperitoneal space. Laparoscopic fenestration seems to be the best treatment as it combines satisfying success rates with a minimally invasive approach. The aim of the study was to review a single center experience on the laparoscopic treatment of symptomatic PTL and detail relevant aspects of the surgical technique.

Materials and Methods: The data of 25 patients who underwent laparoscopic surgical treatment for a symptomatic lymphocele following kidney transplantation were retrospectively reviewed. Demographic data and surgical results were assessed. Detailed surgical technique is provided.

Results: Between 1996 and 2008, 991 patients received a kidney transplant at our institution. Twenty-five patients (2.52%) developed a symptomatic lymphocele and laparoscopic drainage was performed. The indications for surgical drainage were graft dysfunction (84%), local symptoms (16%) or both (32%). The mean time until surgical therapy was 14.2 ± 6 weeks. Mean hospital stay was 1.5 ± 0.2 days. Postoperative complications occurred in only 2 patients (8%) (one ureteral injury and one incisional hernia) and required reoperation. After a mean follow-up of 36.2 ± 4 months, only 1 patient had a symptomatic recurrence.

Conclusions: Laparoscopic fenestration is an effective surgical technique to treat symptomatic lymphocele following kidney transplantation with low recurrence rate and long standing results.

ARTICLE INFO

Key words:

Kidney transplantation;
lymphocele; laparoscopy;
Surgical Procedures;
Minimally Invasive

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INTRODUCTION

Lymph is the result of blood plasma extravasation from the capillary vessels into the interstitial space. Its role is related to the maintenance of adequate human body organs and tissues perfusion and depends on the balance between

hydrostatic and colloid osmotic pressures both inside and outside the capillary vessels (1).

Lymphocele is a pseudocystic entity with lymph content covered with a hard fibrous capsule. It can be a complication of any surgery involving the lymphatic system. The term "lymphocele" (lymphatic collection without an epithelial

lining) originated from the Japanese literature.

Medical and surgical factors are involved in the etiology of the lymphocele. The surgical factor appears to be the most important, specially the lesion of the allograft's lymphatic vessels, and also of the perivascular ones near the anastomosis (2,3).

Liquid collections around the renal allograft are very frequent and may occur in up to 49% of patients. The significant majority of post transplant lymphoceles occurs within the first 3 months after surgery (4). Although most collections are asymptomatic and resolve spontaneously, some may lead to more serious complications. Some of these patients may have general, vascular or obstructive uropathy repercussions that are secondary to the collection, and require early treatment to preserve allograft function (5). These repercussions cause symptoms that result from compression of the pressure created by the lymphocele on the ureter, bladder, or vascular structures, or from infection of these collections. Intervention is indicated when the compressive effect causes graft dysfunction or other symptoms including deep vein thrombosis, abdominal pain, or edema of the lower extremity and genitalia (6).

In this scenario, laparoscopy allows a minimally invasive approach combining image magnification, precise lymphocele drainage and minimal morbidity when proper surgical technique is implemented.

We reviewed our single center 12 - year experience with symptomatic post transplant lymphocele to evaluate the efficacy and safety of laparoscopic procedure for this problem. A comparison with other centers results is provided.

MATERIALS AND METHODS

After approval by the Institutional Review Board, 991 adult patients who underwent kidney transplantation at our institution between March 1996 and July 2008 were evaluated. The information regarding patients and associated surgical procedures were recorded.

In this study, only patients who presented either allograft dysfunction or local symptoms

with perirenal and perivesical fluid collection surrounding the kidneys or ureter were considered. They were observed through ultrasonographic evaluation in order to confirm the presence of lymph and the absence of urine. A sample of the fluid collection was acquired through ultrasound guided percutaneous puncture and sent for biochemical analysis. Creatinine level similar to serum confirmed its lymphatic nature. Computer tomography (CT scan) provided better evaluation of lymphocele collection and its anatomical relations to the surrounding structures.

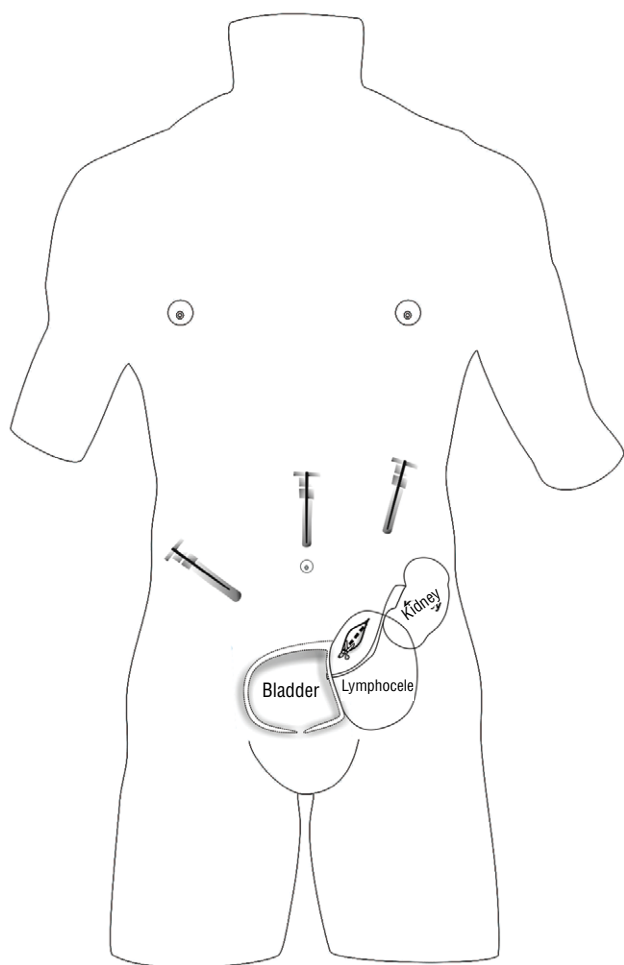
All drainage procedures were performed under general anesthesia and a single dose of antibiotics (first generation cephalosporin) was given for prophylaxis.

A transperitoneal three-port technique was used. Pneumoperitoneum was obtained with CO₂ insufflation up to a pressure of 12mmHg following the insertion of a Verress needle through the supraumbilical crease. One 10-mm camera port was placed in the supraumbilical location followed by a second 5-mm working port along the mid clavicular line near the costal margin on the transplant ipsilateral side and a third 5-mm port in the opposite flank side.

The essential steps of the procedure include: identification of the limits of the lymphocele, laparoscopic needle aspiration for confirmation; and finally precise incision of the lymphocele cavity. The common wall between the lymphocele and the peritoneal cavity was excised with electrocautery after determining the location of the kidney and other vital structures. The fenestration created between the extraperitoneal space containing the transplanted kidney associated lymphocele and the peritoneal cavity was made as large as possible but with great care to avoid the inferior and lateral peritoneal surfaces where the ureter and renal hilum could be accidentally injured. In this scenario, critical care was taken when performing the lymphocele wall incision (Figure-1). The cutting line was always longitudinal in a cranial to caudal fashion adding extra care to prevent ureteral and vascular injuries.

Patients were encouraged to ambulate and were started on a diet on the first day after surgery.

Figure 1 - Transperitoneal three-port position and lymphocele wall incision.



RESULTS

During the study period, 991 adult renal transplants (523 cadaveric, 468 living) were performed (Table-1). The incidence of symptomatic lymphoceles was 2.52%. There were 25 lymphoceles treated (14 women and 11 men). Preoperative confirmation of the nature of the fluid collection was obtained in all cases by percutaneous aspiration and fluid analysis including creatinine and cytology with differential cells and bacterial culture. There was no evidence of infection in any case of our cohort. The indications for surgical drainage were graft dysfunction (21 pts. - 84%), local symptoms (4 pts. - 16%) or both (8

pts. - 32%) (Table-1). The mean time from transplantation to surgical therapy for lymphocele was 14.2 ± 6 weeks.

The operative time (including anesthesia time) was 88 ± 6 min. There were no intraoperative complications and all cases could be completed with pure laparoscopic technique. Mean hospital stay was 1.5 ± 0.8 days (Table-2).

Clinical follow-up included serum creatinine measurements and abdominal ultrasound after definitive surgical treatment. One patient (4%) developed symptomatic recurrence after 45 days following lymphocele treatment and underwent reoperation. An open technique approach was chosen in this case as technical difficulty was anticipated since the patient had had several abdominal surgical interventions other than the kidney transplantation and lymphocele drainage. Preoperative imaging allowed for location near to the graft hylum. Confirmation of proper site was carried out only with palpation and careful aspiration. This patient underwent uncomplicated repeated fenestration without subsequent recurrence. Two patients (8%) had postoperative complications (one ureteral injury and one incisional hernia) that required reoperation. The patient with ureteral injury developed symptomatic abdominal fluid collection one week after the procedure. In the reoperation, a lateral opening at mid third of transplanted ureter 1 cm away from the drainage incision could be noted. Correction was undertaken with ureteral edges revitalization and single stitches with absorbable suture. A double J catheter was placed under fluoroscopic control as well.

Incisional hernia developed 2 weeks after laparoscopy. An intestinal segment could be noted through the hernia ostium at the time of reoperation. All patients restored renal function after definitive treatment.

DISCUSSION

Perinephric fluid collections are common in the early postoperative period. Whereas asymptomatic fluid collections do not require aggressive diagnostic workup or treatment, symptomatic lymphoceles require definitive treatment (7).

Table 1 - Patient demographics.

Number of transplants (living/cadaveric donors)	991 (468/523)
Number of post transplant lymphoceles	25 (2.52%)
Number of lymphoceles (living/cadaveric donors)	8/17
Mean interval from transplantation (weeks)	14.2 ± 6
Mean diameter of lymphoceles (centimeters)	15.4 ± 2.8
Number women/men	14/11
Mean age (years)	38 ± 9
Operative time (minutes)	88 ± 6
Intraoperative complications	No
Conversion	No
Hospital stay (days)	1.5 ± 0.2
Follow up (months)	36.2
Number of symptomatic recurrences	1 (4%)
Postoperative complications	2 (8%)

Table 2 - Intraoperative data and outcome.

Operative time	88 ± 6 min
Intraoperative complications	No
Conversion	No
Hospital stay (days)	1.5 ± 0.2
Follow up (months)	36.2
Number of symptomatic recurrences	1 (4%)
Postoperative complications	2 (8%)

Symptomatic lymphoceles are of much less frequency but are easily recognized and diagnosed (8). When symptoms occur they are typically related to compression of adjacent structures. The most frequent symptoms associated with lymphoceles are lower abdominal pain, lower extremity or scrotal swelling, deep vein thrombosis, or graft dysfunction secondary to ureteral or bladder outlet obstruction (1). In

84% of the patients of our series increased serum creatinine level was the first symptom indicative of a lymphocele. Lower extremity edema, genital edema, deep venous thrombosis before detection of the lymphocele were the leading symptoms in only 16% of the patients.

In our series of 991 patients studied over 12 years, the incidence of lymphocele was 2.52%, which is comparable to the rate of 1% to 12% of patients observed in other reviews (8). The occurrence of lymphoceles after surgery for kidney transplantation does not seem to be the result of any single factor (9). A number of aetiological factors are described and a large variety of combinations are likely to be causal (9). Like illustrated previously there are good evidences to suggest that the extraperitoneal location of renal transplants and subsequent lack of communication with the absorptive peritoneal surface contribute to significant elevated incidence of lymphocele after renal transplantation. Other factors can contribute to lymphocele formation like delayed graft function (10), repeat transplantation (11), acute graft rejection (12), cadaveric

donor kidney (3), sirolimus immunosuppression (13), and other host disease factors such as adult polycystic kidney disease (14).

Thromboembolic prophylaxis with low-dose subcutaneous heparin can be another aetiological factor. This may be a consequence of the higher vulnerability of the lymphatic system to the effects of anticoagulants due to the lower concentration of clotting factors and the lack of platelets (9).

Although it has been suggested that implantation to the external iliacs is a risk factor for lymphocele formation, we routinely use the internal or external iliacs and did not find this to be a problem (15).

In this series, there was a significant difference in the number of lymphoceles in the kidneys from cadaveric donors. It could be explained by bigger inflammatory process in these patients, where surgery performed in urgency and not routinely like living donors.

Renal transplant recipients with increased serum creatinine levels and clinical symptoms indicative of a lymphocele should undergo diagnostic ultrasound or computerized tomography.

Before the laparoscopic era the only alternative to open transperitoneal lymphocele drainage was percutaneous aspiration with subsequent observation or sclerosant therapy. They introduce the possibility of infection and they can keep open lymphatic vessels due to continuous aspiration (16).

Closed percutaneous drainage systems can be used for a defined period of time. They also carry a recurrence rate of 40-50%. A number of sclerosants have been used as an adjunct to percutaneous drainage (3). Although sclerosant therapy has a better success rate than simple aspiration, recurrences remain common, and complications including acute renal failure have been reported (17). No large series have established the benefit of one method of sclerosis over the others (4).

Operative treatment of symptomatic post transplant lymphocele consists of lymphocelectomy with creation of a large internal window for intraperitoneal fluid drainage. Open lymphocelectomy has long been the standard. A literature review including 129 patients who underwent

open drainage between 1980 and 1998 revealed a complication rate of 4% and a recurrence rate of 15% (18). It is believed that the high incidence of symptomatic recurrences following open drainage is a result of bowel adhesions obstructing the internal window and thus causing extraperitoneal fluid accumulation.

Since laparoscopic fenestration of post transplant lymphocele was first described by McCullough et al. (19) in 1991, multiple groups have shown this approach to be safe and effective (4,6,7). The general benefits of laparoscopic surgery include less aggressiveness to the abdominal wall with quick recovery, favorable cosmetics, reduced blood loss, low postoperative morbidity and short hospital stay.

The relative contraindications to a laparoscopic surgery include bowel adhesions secondary to multiple previous abdominal surgeries, the need for a concomitant open procedure, and lymphocele anatomy in close proximity to the renal hylum (6).

Since laparoscopy has been employed to treat post transplant lymphocele, a number of complications have been described (4,8). Injury of the urinary tract, particularly of the transplanted ureter, has been described as a major drawback of laparoscopic lymphocele drainage (4), and an incidence rate as high as 20% has been reported (16). Laparoscopic drainage of small lymphoceles in close proximity to the renal hylum bears an increased risk of iatrogenic ureter or vessel injury.

Recent reviews note different results with regard to complications associated with laparoscopic lymphocelelectomy. Hsu et al., in a multicenter review of experienced centers, found a complication rate of 5% with only a single urinary tract (bladder) injury (8). Cadrobbi et al. (20) found a 7% incidence of urinary tract injury (renal pelvis, ureter, bladder) with laparoscopic fenestration, but the rate was lower in centers with significant experience.

Proper identification of the renal hylum and the transplanted ureter can be difficult during laparoscopic fenestration. The successful use of intraoperative transcutaneous or laparoscopic ultrasound has also been described (4). The device seems to be a useful adjunct to identify

structures prior to fenestration and to document complete drainage of the fluid collection. However, the same authors admitted that this tool was not always helpful for identification of the transplant ureter. We believe that meticulous and careful procedure rather than the use of endoscopic ultrasound ensures safety during laparoscopic lymphocele drainage.

Laparoscopic drainage is thought to have a lower incidence of symptomatic recurrences compared with open drainage (10). In the literature the recurrence rate following laparoscopic lymphocele fenestration ranges from 4% to 6% (4,6,8). In our series, the recurrence rate after laparoscopy was 4% (1 of 25). We believe that it was due to a failure to localize and adequately open a little inferior component of the lymphocele.

Based on our single patient with incomplete drainage of an unappreciated second cavity, we have begun to use laparoscopic transcutaneous ultrasound routinely if there is any question about lymphocele anatomy, in an effort to help prevent further recurrences.

Laparoscopic lymphocele drainage is a safe and effective procedure. Given the benefits of minimally invasive surgery, including reduced postoperative morbidity and shorter hospital stay, we conclude that laparoscopic drainage should be considered first-line therapy for patients with symptomatic post renal transplant lymphoceles.

CONFLICT OF INTEREST

None declared.

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EDITORIAL COMMENT

The authors present their experience on the treatment of 25 patients submitted to a kidney transplant with symptomatic lymphoceles. It has been observed a clear reduction of the incidence of lymphoceles in the last decade. There is not an isolate etiology for its occurrence. However, a careful dissection of the iliac vessels with careful taking to ligate the lymphatic vessels is certainly an important step on its prevention.

Before performing the laparoscopic drainage, some steps should be reinforced. A computer tomography is fundamental in planning the surgery, the collection located lateral to the graft

or deep in the iliac fossa may represent a major difficult when performing the procedure. The authors have observed just one case of ureteral injury, probably in a patient with a lymphocele deep in the pelvis.

Another important aspect, mentioned by the authors, is to perform a percutaneous aspiration one day before the procedure, to exclude an infected lymphocele. These patients may have an infected collection without any clinical signs.

The laparoscopic access is safe and effective for the treatment of symptomatic lymphocele as shown by the authors.

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Testicular torsion and weather conditions: analysis of 21,289 cases in Brazil

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ABSTRACT

Purpose: The hypothesis of association between testicular torsion and hyperactive cremasteric reflex, worsened by cold weather, has not been proved. Thirteen studies in the literature evaluated this issue, with inconclusive results. The aim of the present study was to evaluate the seasonality of testicular torsion in a large subset of patients surgically treated in Brazil, and additionally to estimate the incidence of testicular torsion.

Materials and Methods: Brazilian Public Health System Database was assessed from 1992-2010 to evaluate hospital admissions associated with treatment of testicular torsion. Average monthly temperature between 1992-2010 was calculated for each region.

Results: We identified 21,289 hospital admissions for treatment of testicular torsion. There was a higher number of testicular torsions during colder months ($p = 0.002$). To estimate the incidence of testicular torsion, we have related our findings to data from the last Brazilian census (2010). In 2010, testicular torsion occurred in 1.4:100,000 men in Brazil.

Conclusions: Testicular torsion occurred at an annual incidence of approximately 1.4:100,000 men in Brazil in 2010. Seasonal variations do occur, with a significant increase of events during winter. Our findings support the theory of etiological role of cold weather to the occurrence of testicular torsion. Strategies to prevent these events can be based on these findings.

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spermatic cord torsion; testis; male; child; climate; seasons; Brazil

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INTRODUCTION

Testicular torsion is an infrequent event characterized by testicular rotation around its cord. It causes interruption of blood supply and necrosis if treatment is delayed. It can occur at any age although it is more common during childhood and adolescence, and incidence slowly decreases with age. Testicular torsion normally presents as sudden severe pain followed by testicular swelling and reddening of the scrotal

skin. Other symptoms as abdominal pain, nausea and vomiting may occur. Surgical treatment includes orchidopexy or orchidectomy, according to testicle viability (1). Even though diagnosis and treatment are straightforward in most cases of testicular torsion, etiology is still controversial (1). The theory of hyperactive cremasteric reflex, which supposedly could be worsened by cold weather, has not yet been proved (2). Thirteen studies in the literature have evaluated seasonality of testicular torsion, with a total of 4,659

patients evaluated (3-15). Different conclusions were drawn from these studies regarding the validity of the hyperactive cremasteric reflex theory. The aim of the present study was to evaluate the seasonality of testicular torsion in a large subset of patients surgically treated from 1992 to 2010 in Brazil, and additionally to estimate the incidence of testicular torsion in this large population series.

MATERIALS AND METHODS

We used the Brazilian Public Health System Information Database (DATASUS) from 1992 to 2010 as the primary data source for our study (16). DATASUS represents the primary effort of the federal government to collect data from the national health system. This database includes information from all public health hospitals throughout the country. Using the DATASUS public database between 1992 and 2010, hospital admissions associated with surgical treatment of testicular torsion have been searched. A total of 21,289 men admitted to public hospitals who underwent surgical treatment of testicular torsion were identified.

Men were divided according to month of diagnosis/treatment, year and region of the country. Brazil is divided in five main regions: South, Southeast, Central-West, Northeast and North. These regions have very distinctive climate

among each other, being the Southern temperatures colder than the Northern. One index city of each region was chosen, and average monthly temperature between 1992 and 2010 was calculated for each city based on data collected from historical weather data. Demographic data from the Brazilian population were obtained from the last national census, realized in 2010 (17).

We described trends in the rate of testicular torsion and identified relations between weather temperature and incidence of testicular torsion in Brazil and its distinctive regions from 1992 to 2010. Statistical analysis was performed using SPSS 13.0 (SPSS for Mac OS X, SPSS, Inc., Chicago, Illinois). Groups were compared with Pearson's chi-square test and ANOVA. Statistical significance was determined at $p < 0.05$.

RESULTS

From 1992 to 2010 we identified 21,289 hospital admissions for surgical treatment of testicular torsion. Patients were distributed according to month and year of incidence and geographic region of the country - North, Northeast, Central-West, South and Southeast regions of Brazil (Table-1). These data were also corrected according to total population of each region. Statistical analysis was performed between regions, and there were similar rates of testicular torsion ($p = 0.38$).

Table 1 - Distribution of men surgically treated for testicular torsion in each region of the country (1992-2010) and its male population (2010), Brazil.

	n	% of total cases	Male Population (millions)	% of Male Brazilian population	p
South	3,340	15.69	13.44	14.38	
Southeast	8,565	40.23	39.08	41.83	
Central-West	1,368	6.43	6.98	7.47	
Northeast	6,606	31.03	25.91	27.74	
North	1,410	6.62	8.00	8.57	
TOTAL	21,289	100.00	93.41	100.00	0.38

Variation between the mean hottest (February) and mean coldest (July) months in Brazil from 1992 to 2010 was 4.38°C (ranged from 20.5°C to 24.9°C). Temperature variation was higher in the southern vs. northern regions (6.5°C vs. 3.1°C, $p < 0.0001$). There was a significant increment in number of testicular torsion operated during colder months (Figure-1F, $p = 0.002$). When considering the distinctive geographic regions of Brazil, there were significant differences between incidences of testicular torsion in average hottest vs. coldest months of the year in both the northern ($p < 0.001$, Pearson = 18.7, OR = 1.1) and southern ($p < 0.0001$, Pearson = 160.3, OR = 1.4) regions, but differences were more significant in the South and Southeast regions (Figures 1D-E).

When historical series were evaluated according to number of cases per year in each geographic region, there was a trend toward re-

duction of incidence of men with testicular torsions operated at the public health system in the Southeast region of Brazil (Figure-2).

To estimate incidence of testicular torsion, we have related our findings to data from the last Brazilian census. In 2010, there were 65,384,893 men in the Brazilian public health system. Average number of testicular torsions in Brazil was of 1,120/year (from 1992 to 2010) and of 920 in 2010, representing an overall incidence of approximately 1.4 torsions/100,000 men in 2010.

DISCUSSION

Intravaginal torsion of the spermatic cord is an emergency condition, with risk of irreversible ischemic injury to testicular function if not promptly treated. It is caused by the torsion of

Figure 1 (A-F). A) Distribution of testicular torsion cases in Brazilian public health system from 1992-2010 according to month of the year, average monthly temperature (°C) and geographic regions (A-E); Brazil (F).

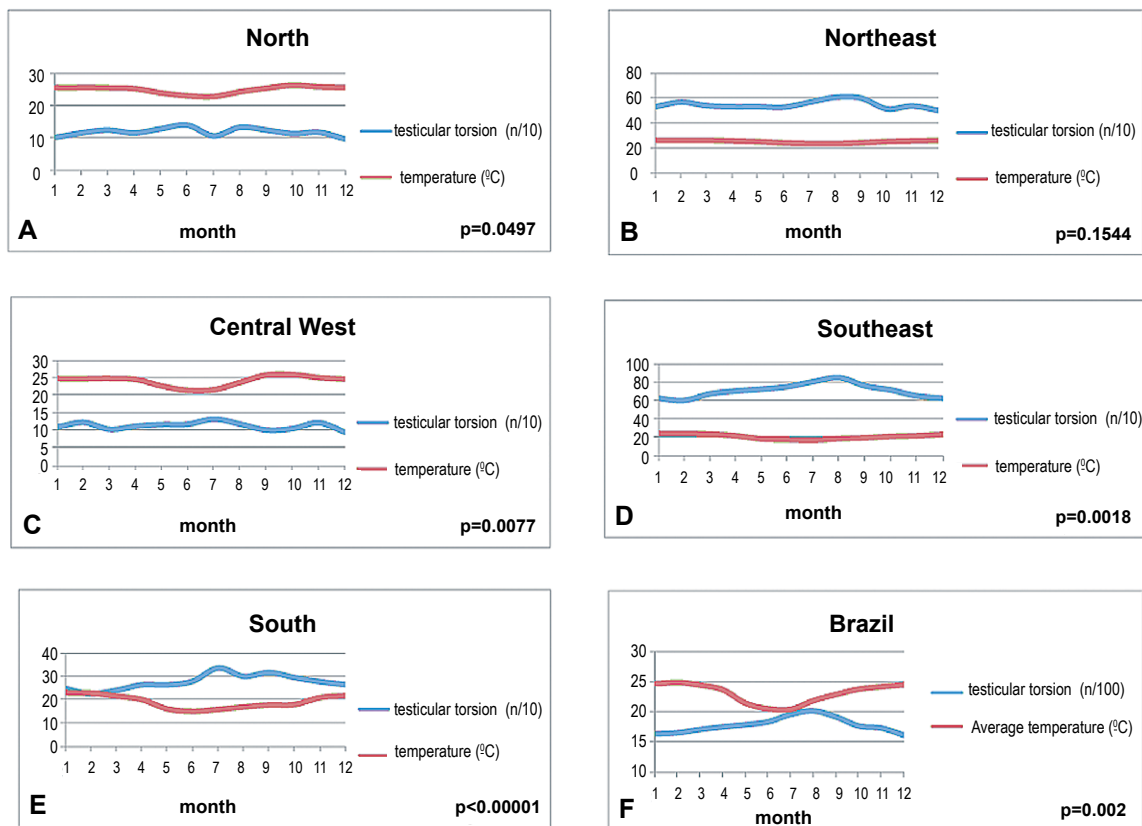
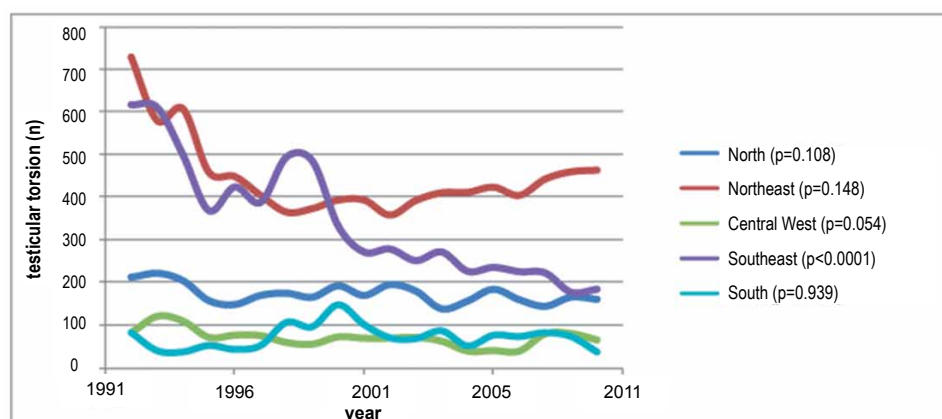


Figure 2 - Historic series of testicular torsion in the public health system in Brazil, 1992-2010 (geographic regions).

the spermatic cord within the space of the tunica vaginalis. It may result from the lack of normal fixation of an appropriate portion of the testis and epididymis to the fascial and muscular layers that surround the cord within the scrotum. The free space between visceral and parietal layers of the tunica vaginalis can extend proximally up to the cord for a variable distance. When it extends to the spermatic cord, an abnormality denominated “bell-clapper deformity” is characterized, leading to an over-mobility of the testis that hangs freely within the tunical space (1,18,19). However, the presence of bell-clapper abnormality is only a predisposing factor to testicular torsion, since not all men with such deformity develop testicular torsion (19). Other risk factors associated with testicular torsion include trauma, exercise and hyperactive cremasteric reflex.

The association between cold weather and cremasteric reflex is well established *in vitro* and the theory of hyperactive cremasteric reflex in the setting of cold weather is postulated as another predisposing factor to testicular torsion (2). There are thirteen studies in the medical literature evaluating this issue, with distinctive conclusions (Table-2) (3-15). To test the hypothesis of association between cold weather and testicular torsion, we have evaluated this large series of men with testicular torsion.

Our study has some important findings. First, there was a clear increment in hospital ad-

missions to surgical treatment of testicular torsion during the coldest months of the year in Brazil. In the Southeast of Brazil were climatic amplitude was higher and more precise data available, this association was even more evident and in the coldest months of the year there were significant increments in the occurrence of testicular torsion. These findings corroborate to hypothesize an association between testicular torsion and cold weather. Previous reports on the seasonality of testicular torsion have been conflicting. Shukla et al. were the first authors to hypothesize an association between cold weather and testicular torsion in 1982 (3). Since then, a total of 9 studies have found a positive association between low ambient temperature and testicular torsion (3-11) whereas 4 did not (12-15). All studies that found a positive association were conducted in cold regions or regions with high temperature amplitude. On the other hand, there are four studies that have not observed seasonal variation of testicular torsion. In a series of 2,876 men, Cost et al. have not observed seasonal variations of testicular torsion (15). However, this study included men from all across the United States, a country with very different weather conditions, without distinctions according to regions' temperature (15). Williams et al. did not find a statistically significant association but a clear trend towards seasonality was reported by the authors (14). Preshaw found similar rates of testicular torsion throughout the year among 272 men evalu-

Table 2 - Studies evaluating the association between seasonality and testicular torsion (3-15).

Author	Year	n	Country	Statistic association with seasonality?	Total n: 4,659
Shukla et al. (3)	1982	46	Ireland	yes	
Mabogunje (4)	1986	131	Nigeria	yes	
Anderson and Williamson (5)	1988	624	England	yes	
Hoshino et al. (6)	1993	39	Japan	yes	
Ugwu et al. (7)	2003	57	Nigeria	yes	
Al-Hunayan et al. (8)	2004	75	Kuwait	yes	
Srinivasan et al. (9)	2007	58	USA	yes	
Lyronis et al. (10)	2009	39	Greece	yes	
Molokwu et al. (11)	2011	173	Scotland	yes	
				TOTAL	1,242
Driscoll et al. (12)	1983	134	Scotland	No	
Preshaw (13)	1984	272	Canada	No	
Williams et al. (14)	2003	135	USA	No	
Cost et al. (15)	2011	2,876	USA	No	
				TOTAL	3,417

ated (13). Interestingly, this study was conducted in Calgary, Canada, where there is a cold and dry weather, but there is a long cold season, greater extremes of daily temperature and little climatic amplitude throughout the year.

Previous authors have raised the relevance of indoor/outdoor temperature, reporting that nearly 55% of testicular torsions occurred indoors (12). In this scenario, in places with extreme weather conditions where indoor/outdoor temperatures tend to differ more, it might be difficult to evaluate the association between testicular torsion and climatic conditions. In Brazil, particularly in the Southeast region, climatic conditions vary, but temperatures are normally agreeable. In this setting, heaters are not commonly available, and during winter indoor and outdoor temperatures are more similar to each other. Further studies evaluating a large number of cases in a smaller region, with more constant climatic variations might bring more information

about this issue. It seems that not only temperature, but also other climatic factors as humidity might also play an important role (4).

We could estimate the incidence of testicular torsion in a large population series. From 1992 to 2010, annual incidences ranged from 896 to 1715 cases (mean \pm standard deviation = 1120 ± 232 cases/year). Incidence in Brazil during 2010 could be estimated of 1.4 torsions/100.000 men. This incidence is lower than estimated for colder countries of 27-48 torsions/100.000 men (5,20) and even in hotter middle-east countries, with incidence of 7.9 torsions/100.000 men estimated (8). We are not sure about the factors associated with these remarkable low rates of testicular torsion in Brazil. Possible explanations could be the underreporting of cases, demographic characteristics or possibly the hot weather. Maybe colder countries have higher incidences of testicular torsion (5,20).

There was also a trend toward reduction in the number of annual cases in the Southeast re-

gion of Brazil when comparing the number of annual cases in the last decades. However this data might be biased as Brazil has been experiencing remarkable development in the last decades, leading to an increase in access to private health system (17). The same bias can be related to the observation of a difference of the annual incidence of testicular torsion in each region of the country corrected by its population (Table-1). Economic and social characteristics of the population within the five regions of Brazil are very distinctive, and access to private health services vary. Therefore, no further conclusions can be withdrawn from these data.

Our study has several limitations. DATA-SUS registries do not provide clinical information, limiting further analysis. In addition maybe some patients treated surgically with an initial diagnosis of testicular torsion could have other conditions and some information might be incorrect, since we could not exclude: 1-) men who underwent surgery and intraoperative diagnosis was other than testicular torsion; 2-) men discharged with scrotal pain and with a missed diagnosis of testicular torsion. However, we believe that; 1-) These false positive and false negative cases might compensate each other; 2-) Since we are mainly evaluating seasonal variations, these possible biases might not vary according to weather conditions. However these biases could impair our incidence calculi. Nevertheless, as testicular torsion incidence had not been previously estimated in any tropical country, we believe that, even though not perfect, our data are very important.

Furthermore, only public health system data can be assessed, and this information evaluates only 70% of the Brazilian population. Besides, only average temperatures of a determined month during the study period were considered, and not the exact temperatures in the moments that each torsion occurred. However, to the best of our knowledge this is the larger epidemiologic study about testicular torsion, and we believe these findings can help to explain factors associated with testicular torsion.

CONCLUSIONS

In conclusion, testicular torsion occurred at an annual incidence of 1.4:100,000 men in Brazil in 2010. Seasonal variations do occur, with a significant increase in incident cases during colder months. Our findings support the theory of etiological role of cold weather in intravaginal torsion of testis. Maybe strategies to prevent these events can be thought in a near future based on these findings.

CONFLICT OF INTEREST

None declared.

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EDITORIAL COMMENT

Given the limited and relatively contemporary literature on the issue, although with important limitations (once well recognized), the study of Korkes et al. is an important epidemiological study regarding the suspicion of testicular torsion related to weather conditions based on DATASUS and IBGE, including the databases restrictions. The rational was previously proposed in the literature since 1982 (1) and observed for the first time in 1986 in a tropical country (2).

While the authors present the largest study in the literature, there is no demographic data about studied patients and neither diagnosis confirmation. In studies of acute scrotum where surgical exploration of the testes was performed without a preoperative ultrasound evaluation, true testicular torsion was found in only 33% of cases (3). Among the countless potential biases

intrinsic to the study design, it can be the case of analyzed events considering the Brazilian public health system limited circumstances.

The practice of medicine contains countless examples of elegant medical theories that contradict the best available evidence; to increase the critical reasoning abilities of readers of the urological literature and to raise the level of evidence are fundamental tasks (4).

Future well-controlled and prospectively designed studies are warranted to confirm the hypothesis originally proposed by Shukla et al. in 1982 (1) and to expand the knowledge on the issue, recognizing and balancing important potentially confounding effects, including other climatic factors as humidity, indoor/outdoor ambience as well as detailed factors related to patients as cultural and surgical practices.

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Beneficial effect of ubiquinol, the reduced form of coenzyme Q10, on cyclosporine nephrotoxicity

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ABSTRACT

Background: Cyclosporine (CyA) nephrotoxicity is partly due to some oxidative stress. Ubiquinol, the reduced form of coenzyme Q10 (rCoQ10), has recently gained attention for its anti-oxidative potential. The aim of this study is to evaluate the effect of rCoQ10 on a CyA nephrotoxic rat model.

Materials and Methods: Six-week-old male Wistar rats were divided into three groups (five animals each). Group 1 received a medium only. Group 2 received 30 mg/kg/day of CyA only. Group 3 received both the same dose of CyA and 600 mg/kg/day of rCoQ10. CyA and rCoQ10 were both given orally for four weeks. Systolic blood pressure (BP), daily urinary albumin secretion (u-Alb), serum creatinine (s-Cr) level, and super-oxide anion (SO) level in the renal tissue were measured and compared among those three groups. Immunohistochemistry using an antibody for the transforming growth factor-beta (TGF-beta) was also examined.

Results: BPs, u-Albs, s-Crs, and SO levels of groups 1, 2, and 3 were 114 ± 3 , 132 ± 4 , and 129 ± 5 mmHg, 2.6 ± 0.5 , 42.1 ± 7.2 , and 22.8 ± 3.4 micro-g/day, 1.1 ± 0.2 , 1.7 ± 0.2 , and 1.3 ± 0.2 mg/dl, and 224 ± 84 , 1251 ± 138 , and 512 ± 109 RLU/g kidney respectively. U-Albs, s-Crs, and SO levels were significantly ameliorated by rCoQ10. Micro-vacuolar changes and TGF-beta positive deposits in the proximal renal tubular cells of CyA group rats disappeared in those of CyA and rCoQ10 group rats.

Conclusion: RCoQ10, an antioxidants, may have potential for preventing CyA nephrotoxicity.

ARTICLE INFO

Key words:

oxidative stress; antioxidants; cyclosporine; transforming growth factor-beta

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INTRODUCTION

Cyclosporine (CyA), a potent immunosuppressant, has a nephrotoxic side effect. Decrease of glomerular filtration rate caused by renal arteriolar spasms and renal interstitial fibrosis induced by transforming growth factor-beta (TGF-beta) upregulation, two major characteristics of CyA nephrotoxicity, are both evoked by some CyA-derived oxidative stress (1,2). Therefore, it is expected that antioxidants have benefits against

the nephrotoxic side effects of CyA. Recently, ubiquinol, the reduced form of coenzyme Q10 (rCoQ10), a dietary supplement, has gained attention for its anti-oxidative potential (3). The following animal study was carried out to evaluate the effect of rCoQ10 on CyA nephrotoxicity.

MATERIALS AND METHODS

Animals were handled in an accredited facility according to the institutional guidelines for

animal care and the research protocols conformed to the principles outlined by the ethical committee for animal study in the University of Tokyo.

Six-week-old male Wistar rats were divided into three groups (five animals each). Group 1 received a medium (i.e. olive oil) only. Group 2 received 30 mg/kg/day (i.e. experimentally nephrotoxic dose (4)) of CyA only. Group 3 received both the same dose of CyA and 600 mg/kg/day of rCoQ10 (Kaneka, Osaka, Japan). CyA was dissolved in olive oil at a concentration of 20 mg/ml and given orally once a day using a gastric tube. RCoQ10 was mixed with standard chow at a rate of 0.6% and fed to each rat. Both were given for four weeks.

Systolic blood pressure (BP) of each rat was monitored every week using a tail-cuff method (BP-98A, Softron, Tokyo, Japan). Twenty-four-hour urine of each rat was collected using metabolic cages on the last day and urinary albumin excretion (u-Alb) was measured by an enzyme-linked immunosorbent assay (AKRAL-120, Shibayagi, Gunma, Japan). On the last day, blood sample was obtained from each rat and serum creatinine (s-Cr) level was measured by traditional Jaffe's method using alkaline picrate (5). Whole blood CyA (WB-CyA) level was also examined by the fluorescence polarization immunoassay method. Superoxide anion levels in the whole kidney were checked by a lucigenin chemiluminescence method as previously reported (6).

Immunohistochemistry of the kidney using TGF-beta antibody (sc-146, Santa Cruz Biotechnology Inc., Santa Cruz, California, U.S.A.) was also performed.

Statistical analyses were done using ANOVA and subsequently Fisher's PLSD test. The data are presented as mean \pm SEM.

RESULTS

In 6-week-old male Wistar rats, 30 mg/kg/day of CyA for 4 weeks increased BP compared with control (113.6 ± 2.6 vs. 132.4 ± 3.8 mmHg, $p < 0.001$). U-Alb was elevated with CyA administration (2.6 ± 0.5 vs. 42.1 ± 7.2 micro-g/day, $p < 0.001$) and s-Cr was also increased by CyA (1.1 ± 0.2 vs. 1.7 ± 0.2 mg/dl, $p < 0.001$). RCoQ10 could not ameliorate hypertension (129.2 ± 4.6 mmHg, NS vs. CyA group); however, it could improve albuminuria (22.8 ± 3.4 micro-g/day, $p < 0.001$ vs. CyA group) and s-Cr elevation (1.3 ± 0.2 mg/dl, $p < 0.01$ vs. CyA group, Table-1). No significant difference was seen between WB-CyA levels of groups 2 and 3 (528 ± 94 vs. 498 ± 85 ng/ml).

Although CyA administration enhanced renal oxidative stress in 6-week-old male Wistar rats (224 ± 84 vs. 1251 ± 138 RLU/g kidney, $p < 0.0001$), rCoQ10 diminished SO generation from kidney (512 ± 109 RLU/g kidney, $p < 0.0001$ vs. CyA group, Table-1).

Micro-vacuolar changes and TGF-beta positive deposits observed in the proximal tubular cells of CyA group both disappeared by rCoQ10 administration (Figure-1).

DISCUSSION

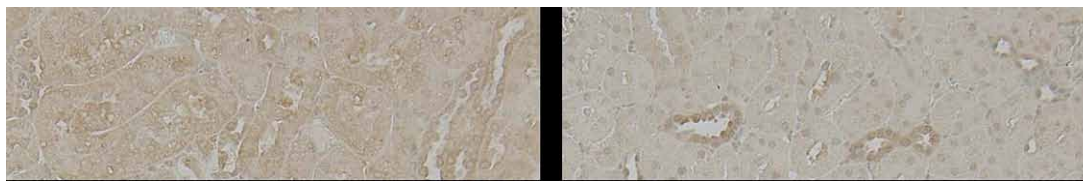
In the present study, we demonstrated that rCoQ10, ubiquinol, ameliorated albuminuria

Table 1 - Changes of systolic blood pressure (BP), daily urinary albumin excretion (u-Alb), serum creatinine (s-Cr) level, super-oxide anion (SO) level in the kidney.

	BP (mmHg)	u-Alb (micro-g/day)	s-Cr (mg/dl)	SO (RLU/g kidney)
Control (n = 5)	113.6 ± 2.6	$2.6 \pm 0.5^{**}$	1.1 ± 0.2	224 ± 84
CyA (n = 5)	$132.4 \pm 3.8^*$	$42.1 \pm 7.2^{**}$	$1.7 \pm 0.2^*$	$1251 \pm 138^{\$}$
CyA + CoQ (n = 5)	$129.2 \pm 4.6^*$	$22.8 \pm 3.4^{**}$	$1.3 \pm 0.2^{\#}$	$512 \pm 109^{\@}$

* $P < 0.001$ vs. Control; ** $p < 0.001$ with each other; # $p < 0.01$ vs. CyA; \$ $p < 0.0001$ vs. Control & CyA + CoQ; @ $p < 0.002$ vs. Control

Figure 1 - Immunohistochemistry Renal tissue stained with a TGF-beta antibody (sc-146, Santa Cruz Biotechnology Inc., Santa Cruz, California, U.S.A).



Left: Group 2: Micro-vacuolar changes and TGF-beta positive deposits are observed in the proximal tubules; **Right:** Group 3: Both of them are not observed.

and s-Cr elevation associated with the reduction of oxidative stress upregulation in the kidneys of 6-week-old male and CyA-loaded Wister rats. RCoQ10 also diminished TGF-beta expression in the proximal renal tubular cells.

There are several animal and clinical studies suggesting that antioxidative agents have some preventive effect of CyA nephrotoxicity (7-12). In 2004, Dlugosz et al reported the benefit of CoQ10 on renal transplant recipients for the first time (13); however, they used ubiquinone, the oxidized form of CoQ10. Thus, the novelty of our present study is that we could demonstrate the apparent beneficial effects of ubiquinol, the reduced form of CoQ10, with its anti-oxidative potential on CyA nephrotoxic rat model. Actually, there are two forms of CoQ10, the oxidized (ubiquinone) and the reduced (ubiquinol). Both can be taken orally with few side effects (3). Ubiquinone is changed to ubiquinol when it reaches lymphatics through small intestine (14). As a result, more than a half of total CoQ10 in the body is the reduced form in many human tissues (15). Orally administered ubiquinol significantly increases the amount of the reduced form of CoQ10 (16).

Antihypertensive effect of CoQ10 (including ubiquinol) is still controversial (15,17,18). For example, Mori et al reported that ubiquinone, the oxidized form of CoQ10, showed no independent effect on blood pressure and could not reduce urinary protein in patients with chronic renal disease (17). In our present study with rCoQ10, no significant reduction of BP was observed. Our previous animal study revealed that CyA induced hypertension might be introduced by the activation of intrarenal renin-angiotensin axis (4).

Therefore, reno-protection by rCoQ10 would be accomplished through another route. Antihypertensive treatment itself is essential for the complete control of CyA nephrotoxicity.

TGF-beta is known as one of chemical mediators for tissue fibrosis (19,20). Renal interstitial fibrosis is the irreversible histological damage observed in some long-term CyA-treated renal allografts (21). RCoQ10 might have potential to prevent chronic renal injury and improve the long-term outcome of renal transplantation by diminishing TGF-beta expression.

Since there was no significant difference between WB-CyA levels of groups 2 and 3, rCoQ10 might have had no interaction with CyA.

In conclusion, we demonstrated some beneficial effect of rCoQ10 against CyA nephrotoxicity probably due to its antioxidant action.

ABBREVIATIONS

CyA: cyclosporine
rCoQ10: reduced form of coenzyme Q10
BP: systolic blood pressure
u-Alb: daily urinary albumin secretion
s-Cr: serum creatinine
WB-CyA: whole blood cyclosporine
SO: super-oxide anion,
TGF-beta: transforming growth factor-beta

CONFLICT OF INTEREST

None declared.

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EDITORIAL COMMENT

The evaluation of antioxidants substances is one of the most promising research lines on the 21th century. The well known harmful effects of free radicals and their involved mechanisms on oxidative stress are even more studied nowadays. The constant search for products or substances with the objectives to stop or break this depletory process has shown lots of progression, culminating on many potential active principles for future drugs to treat this kind of cell damage.

The article discusses the potential beneficial effects of ubiquinol over the oxidative stress caused by Cyclosporine, one potent imunossuppressor commonly used by patients submitted to kidney transplantation. The study performed on Wistar rats has shown some beneficial of ubiquinol over the nephrotoxicity exercised by Cyclosporine. Understanding the correct mechanisms of oxidative stress on many different situations and how to combat it, may open a space for new researches for the development of drugs or medicines to help to improve the global survival of patient/graft dependent of imunossuppressors.

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Laparoscopic ureteropyeloanastomosis in the treatment of duplex system

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ABSTRACT

Purpose: Duplex system is one of the most common anomalies of upper urinary tract. Anatomical and clinical presentation determine its treatment. Usually, the upper moiety has a poor function and requires resection, but when it is not significantly impaired, preservation is recommended. Laparoscopic reconstruction with upper pole preservation is presented as an alternative treatment.

Materials and Methods: Four female patients with duplex system, one presenting with recurrent urinary tract infection and the others with urinary incontinence associated to infrasphincteric ectopic ureter, were treated. Surgical procedure involved a laparoscopic ureteropyeloanastomosis of the upper pole ureter to the pelvis of the lower moiety, with prior insertion of a double J stent.

Results: Surgical time varied from 120 to 150 minutes, with minimal blood loss in all cases. Follow-up varied from 15 to 30 months, with resolution of the clinical symptoms and preservation of the upper moiety function.

Conclusion: Laparoscopic ureteropyeloanatomosis is a feasible and safe minimally invasive option in the treatment of duplex system.

ARTICLE INFO

Key words:

laparoscopy; kidney; congenital abnormalities

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INTRODUCTION

Pyeloureteral duplication or duplex system is one of the most common anomalies of the upper urinary tract (1). Embryologically, a complete duplex system arises from two separate ureteric buds and is more common in girls than boys, in a ratio of 6:1. It has a broad spectrum of clinical presentations and significance. Girls may present with urinary incontinence associated to normal voiding after toilet training, due to the ectopic orifice of the ureter of the upper unit, which opens in the urethra distally to the external sphincter, or in the introitus. This ureter may also end in an intravesical ureterocele that obstructs

this unit and, depending on its size and location, all other units and the bladder neck. The ureter of the lower pole generally ends in the bladder but has a short intravesical tunnel, often leading to vesico-ureteral reflux (VUR). While the lower unit is usually preserved anatomically and functionally, that of the upper moiety is frequently dilated or dysplastic (2).

Many treatment alternatives are available, depending on the functional and anatomical status of the affected units, as well as the size, location and degree of obstruction of the ureterocele or ectopic ureter, and the presence of urinary incontinence. If renal function in one moiety is very poor, as frequently observed in the upper

unit, polar nephrectomy is the most appropriate procedure. However, when the anatomical and functional impairment of this moiety is not significant, it may be preserved, therefore requiring a reconstruction either by a proximal ureteropyeloanastomosis, a distal uretero-uretero anastomosis or a vesicoureteral reimplantation (3-5).

In the last decade, laparoscopy has become a safe and effective modality for the treatment of pediatric urologic anomalies that require ablative or reconstructive techniques (6-8). Laparoscopic polar nephrectomy is now considered the standard of care of duplex systems, when the upper moiety is to be removed (9,10). Laparoscopic intervention in duplex systems to preserve the upper unit is naturally more demanding, but with the technical improvements and growing experience with laparoscopic pyeloplasty, more centers are able to perform this procedure (9,11,12).

We report our experience with the laparoscopic treatment of duplex systems associated to ectopic ureters and preservation of the affected renal unit.

MATERIALS AND METHODS

In this retrospective series, we report four female patients with duplex kidneys who underwent laparoscopic ureteropyeloanastomosis. An informed consent was obtained from all patients or parents.

None of our patients had antenatal diagnosis, and were referred to our department at late age, without diagnosis of duplex kidney. Medium time of diagnosis was 12 years of age, varying from 8 to 19 years. No patient had previous surgical treatment. Two younger patients presented continuous urinary leakage associated to normal voiding, another young patient presented with recurrent urinary tract infections, while the older patient had intermittent urinary leakage associated to normal voiding; the patient with recurrent urinary tract infections was on antibiotic prophylaxis with trimethoprim from the first consultation until stent removal after the surgery. Blood pressure and serum creatinine were measured in all patients before and after surgery, during follow-up consultations.

The diagnosis of duplex system was suspected by clinical data and ultrasound examination, and confirmed in all cases by computerized tomography (CT) or intravenous pyelogram (IVP). Preservation of the upper moiety was based subjectively on the degree of its pyelocalicial dilatation, the thickness of its parenchyma and degree of its function, as evaluated by contrast excretion, either by DMSA exam or IVP (other patients, with significant anatomical or functional impairment of the upper moieties, were submitted to polar nephrectomy). A micturating cystography was also performed in all patients, to exclude vesico-ureteric reflux.

A three or four ports laparoscopic transperitoneal approach was performed in all patients, with previous double-J stenting of the ureter of the lower unit and insertion of a Foley catheter into the bladder. After colonic mobilization, the kidney was exposed and both ureters were isolated at the lower pole of the kidney. The ureter of the lower moiety was easily identified by the presence of the inserted double-J stent. The ureter of the upper moiety, usually dilated, was separated and carefully dissected as far down as possible, care being taken to preserve the integrity of the ureter of the lower unit. The ureter of the upper moiety was then sectioned at its lowermost segment and its lower stump left open in the absence of VUR. The upper stump was dissected upwards, until very close to the vascular pedicle of the kidney, where the pelvis of the lower unit was also exposed. A stay suture could have been inserted through the abdominal wall, in order to stabilize the lower pole and facilitate dissection of the hilar structures. The ureter of the upper moiety was again sectioned and spatulated close to the pelvis of the lower unit, while the later was incised longitudinally. An end-to-side free-hand anastomosis between both structures was then performed with running 4.0 or 5.0 Vicryl suture (Figure-1). In three cases, the double-J stent was maintained in the pelvis of the lower unit, while in one it was transposed to the ureter of the upper unit. When the anastomosis was completed, the stay suture was transected and a Penrose or suction drain inserted through one of the accesses, being removed the next day. The Foley catheter

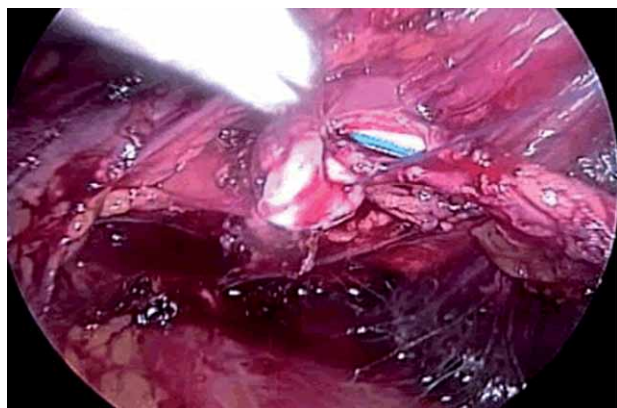


Figure 1 – Intraoperative view of end-to-side ureteropyeloanastomosis (case 4).

was usually removed in the second post-operative day, prior to discharge of the patient. The double-J catheter was removed after 4-5 weeks. Control urinalysis was made after completion of antibiotic prophylaxis.

Patients were operated on by two of the authors (M.H. and F.T.D.), both experienced in reconstructive laparoscopy.

Post-operative evaluation was based on clinical data (cessation of urinary leakage), as well as routine US control, in order to evaluate dilatation of both moieties, followed by DMSA, IVP or CT evaluation to functionally evaluate the upper moiety every six months.

RESULTS

Clinical data of patients are summarized in Table-1. All had evidence of unilateral pyelo-ureteral duplication with a dilated, but functioning upper moiety, and a normal lower moiety. None had preoperative VUR to any of the units. All cases were operated laparoscopically and the operative time (OT) varied from 120 to 150 minutes. Blood loss was minimal in all cases, and there was no post-operative morbidity. All patients were discharged after the removal of the bladder catheter. The first patient had no more UTIs, nor flank pain, while the other patients had immediate cure of the urinary incontinence.

Follow-up varied from 15 to 60 months, with a medium time of 40.2 months, and post-operative IVP or CT showed functioning upper and lower poles with good drainage of both moieties in all patients (Figures 2-5). Patient number 4 had a one year post-operative renal scan with a renal function of 51% on the operated kidney and no obstruction after furosemide. Blood pressure and serum creatinine did not change after surgery.

DISCUSSION

Ureteropyeloanastomosis is an alternative treatment of duplex system, when the up-

Table 1 – Clinical data of patients.

Case	Sex	Age	Side	Symptoms	Past History	Physical Exam	Diagnosis
1	♀	8 years	R	Recurrent UTI Flank pain	-	Normal	Suprasphincteric ectopic ureterocele
2	♀	8 years	L	Incontinence	-	Continuous urinary vaginal leakage	Infrasphincteric ectopic ureter
3	♀	19 years	L	Incontinence	Pregnancy and vaginal delivery	Continuous urinary vaginal leakage	Infrasphincteric ectopic ureter
4	♀	14 years	L	Incontinence	-	Continuous urinary vaginal leakage	Infrasphincteric ectopic ureter

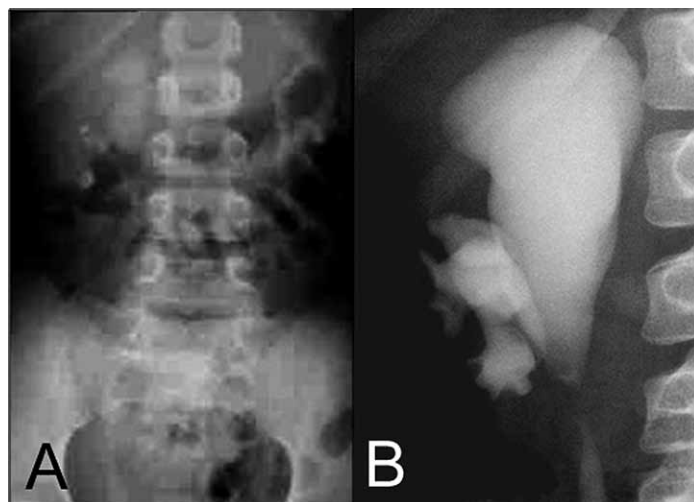


Figure 2 – A) Pre-operative IVP in case 1; B) Post-operative IVP 6 months after procedure

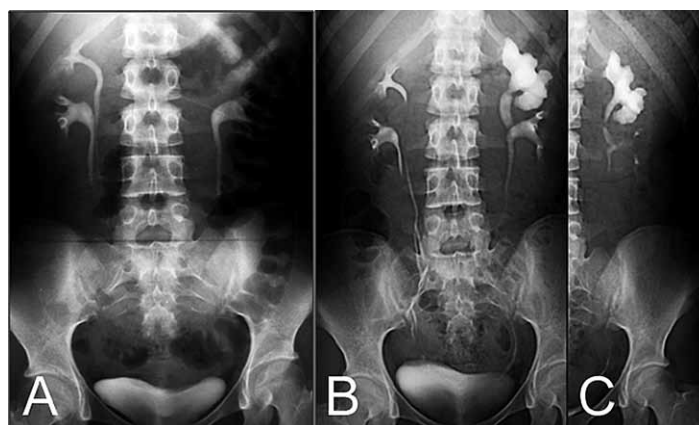


Figure 3 – A) Pre-operative IVP in case 2; B and C) Post-operative IVP 4 months after procedure.

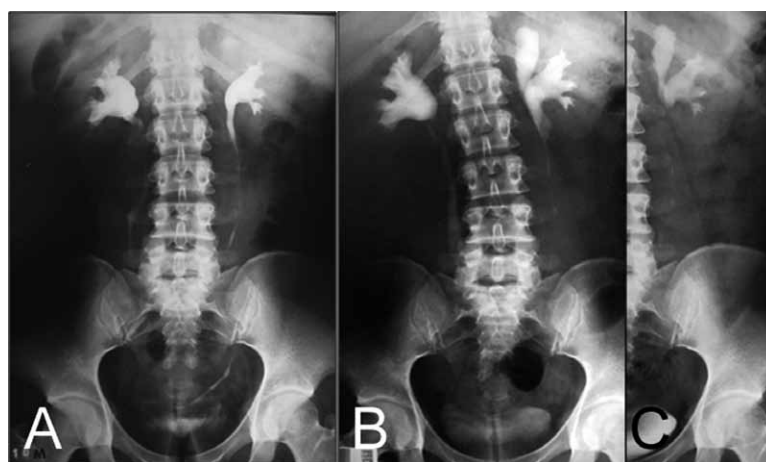


Figure 4 – A) Pre-operative IVP in case 3; B and C) Post-operative IVP 6 months after procedure.

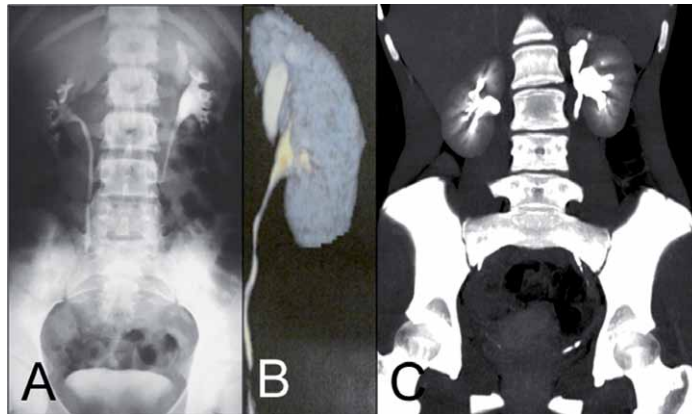


Figure 5 – A) Pre-operative IVP in case 4; B) Pre-operative CT reconstruction; and C) Post-operative CT 6 months after procedure.

per moiety is to be preserved. According to Diaz-Ball et al. (5) ureteropyeloanastomosis for duplex system was first performed by Kummel in 1913, for the treatment of ectopic ureterocele. Laparoscopic procedures for duplex system treatment were introduced by Suzuki et al. in 1993 (13). Until now, there are some case series with laparoscopic reconstruction for duplex system, but clinical presentation is generally different, usually due to urinary infection, upper tract obstruction or reflux. There are few reports on laparoscopic treatment for urinary incontinence due to ectopic ureter in duplex system (14,15). Ramalingam et al. (11) described three cases of laparoscopic ureteropyeloanastomosis, two for duplex system associated to ureteropyelo-junction (UPJ) obstruction and one for duplex system associated to reflux. Kutikov et al. (12) was the first to describe laparoscopic ureteropyeloanastomosis in the treatment of duplex system in 6 children: four girls with ureteral ectopy and incontinence, one with bilateral ectopy and a boy with ureteral ectopy to the prostate.

Treatment of incontinence due to ectopic ureter with functioning upper pole can be performed at the level of the kidney (ureteropyeloanastomosis), lower ureter (uretero-ureteroanastomosis) or bladder (ureteral reimplantation). The first two procedures can be performed only when there is no VUR to any of the ureters, while

the latter is the ideal procedure when VUR is present. Ureteropyeloanastomosis allows the removal of the distal dilated segment of the ureter of the upper unit and also precludes the “yo-yo” reflux (urinary reflux from the healthy ureter to the massively dilated lower ureteral segment of the upper unit), which can lead to urinary stasis and infection when uretero-ureteroanastomosis is performed (3). Khoubehi et al. (16) described a laparoscopic ureteropyelostomy for symptomatic “yo-yo” reflux in an adult with partial duplication. Furthermore, ureteropyelostomy is devoid of the risk of postoperative VUR or obstruction that can occur after reimplantation of duplex ureters. It is important to emphasize that VUR to the lower pole recipient ureter must be excluded; therefore a cystography is mandatory preoperatively. A renal DMSA scintigraphy can be performed if there is doubt on the upper pole function (1-3). In our series, the preoperative imagenological evaluation ensured us on the residual upper pole function, which was then preserved with the procedure.

Ureteropyeloanastomosis can be technically demanding, mainly when pelvic and ureteral dilation is small or absent. There is also a potential risk of bleeding when working close to renal vessels as well as an impairment of the healthy lower pole if an anastomotic stricture occurs (3). Double-J catheter can be inserted before,

during or after the anastomosis. In our series, retrograde stenting of the lower pole ureter was performed at the beginning of the procedure, facilitating its identification and dissection during the procedure. Also, it facilitates the longitudinal incision of the pelvis of the lower moiety, without damaging the opposite wall (17,18). After completion of the posterior lip of the anastomosis, transposition of the stent to the ureter of the upper unit, as performed in one of our cases, did not influence the end-result. The decision to transpose the stent was aleatory, not based on the intraoperative appearance of the anastomosis or the local condition of the recipient renal pelvis. The distal ureteral segment of the upper pole can be excised as distally as possible, but care must be taken to avoid damage to the lower pole ureter. Its stump may be left open as it is ectopic and as long as there is no urinary drainage due to VUR (4,12). In all of our cases it was left open, with no adverse consequences.

Since our patients did not have antenatal diagnosis, their medium age of treatment was 12 years, in contrast to that described in the literature (from six months to five years) (1,3,12,19). One of them had even been pregnant and had a normal delivery before duplex system with infrasphincteric ectopic ureter was diagnosed. All of them had sought medical care before being referred to our service, but were treated clinically due to lack of correct diagnosis. The laparoscopic ureteropyeloanastomosis, with preservation of the upper moiety, was successful in all our patients, despite their age, and we recommend it in the treatment of younger children, who are regularly submitted to other laparoscopic procedures (6,20).

CONCLUSIONS

Laparoscopic ureteropyeloanastomosis is a feasible treatment for duplex kidneys associated to a functioning upper moiety. In our series, we had a success rate of 100%, without morbidity or mortality. It can be considered as an option of minimally invasive treatment in cases with upper pole preservation.

ABBREVIATIONS

UTI: urinary tract infection
VUR: vesico-ureteral reflux
CT: computerized tomography
IVP: intravenous pyelogram
OT: operative time

CONFLICT OF INTEREST

None declared.

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EDITORIAL COMMENT

A duplex renal system is one of the most common urological anomalies that would be seen in practice. Clinical presentation can be highly varied and management must be tailored case by case. Traditionally, a poorly functioning upper pole moiety was dealt by performing a partial nephrectomy in an open fashion. Nowadays, many centers utilize laparoscopic partial nephrectomy as the treatment of choice for managing such cases. However, a chal-

lenging scenario is one where the upper pole moiety has clinical significant function and preservation is desired. Dr. Hisano and colleagues describe their approach of a laparoscopic ureteropyelotomy and present data supporting their clinical success. Their group has delineated step by step how laparoscopic ureteropyelotomy is feasible and an excellent option for preserving the upper pole moiety and addressing the ectopic ureter. The laparoscopic ureteropyelostomy is a technique that one should consider for this often seen problem.

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Long-term follow-up of penile curvature correction utilizing autologous albugineal crural graft

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ABSTRACT

Purpose: Peyronie's disease is an acquired connective tissue disorder of the penile tunica albuginea with fibrosis and inflammation. The disease produces palpable plaques, penile curvature and pain during erections. Usually it results in impairment of the quality of life. Our objective is to review the long-term results of the albugineal grafting harvested from the penile crura for the treatment of severe penile curvature.

Materials and Methods: Thirty-three patients with Peyronie's disease were submitted to a grafting with tunica albuginea from the penile crura for the correction of penile curvature. The results were evaluated after 6 months of the procedure. Variables studied were overall satisfaction with the procedure, correction of the penile curvature, erectile capacity, penile shortening and the presence of surgical complications.

Results: Mean follow-up after surgery was 41 months. Complete correction of the curvature was achieved in 30 patients (90%). The mean preoperative curvature was 91.8 degrees and median plaque length was 2 cm (ranged from 1 to 5 cm). Three patients (9%) experienced recurrence of the penile curvature and required a new procedure. In 30 men (90%) the procedure fulfilled their expectations and in 31 patients (93.9%) their opinions were that sexual partners were satisfied with the penile correction. Penile shortening or augmentation was referred in 6 (18.1%) and 1 (3%) patient, respectively.

Conclusion: Our series demonstrated that grafting the albugineal defect after incision of the tunica albuginea with tunica from the crus for the correction of penile curvature is safe and results in satisfactory straight erections during a long-term follow-up.

ARTICLE INFO

Key words:

Peyronie Disease; Tunica albuginea; Penile Induration

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INTRODUCTION

Penile curvature is the result of a congenital penile defect in tunica albuginea, trauma, or Peyronie's disease (PD). While congenital curvatures affect patients in their early sexual life, PD is more frequent after the fortieth decade. PD is an acquired connective tissue disorder of the tunica albuginea, and the pathophysiology is not

perfectly understood (1,2). The development of fibrosis and inflammation of the tunica albuginea produces palpable plaques, penile curvature and pain during erections. Sexual dysfunction due to erectile impairment or penile curvature can accompany PD in some cases, and it results in negative impact of the quality of life in two thirds of the patients (3). In the presence of severe curvatures, the deformity of the penis interferes with penile

penetration, resulting in difficult coitus. Patients with this condition frequently report shame, embarrassment and interpersonal difficulties (4).

The etiology of PD is probably multifactorial. Repetitive and undetected microtraumas during coitus result in delamination between the layers of the tunica albuginea, microvascular injury, hemorrhage, and local activation of inflammatory and fibrotic pathways (5). PD is a relatively common situation that can affect almost 10% of the adult male population (6). PD is initially characterized by an inflammatory phase associated with painful erections, bending, or a change in plaque size. During this phase (6-18 months), the condition may progress, stabilize, or regress. As long as the inflammation in the plaque subsides, pain tends to disappear (7).

In patients with a slight curvature and no erectile dysfunction (ED), treatment is conservative (1). Medical treatment should be utilized in the inflammatory phase of the disease, although weak evidence of improvement is reported in the literature (2).

Surgical correction of PD is reserved for patients with curvature or narrowing of the tunica albuginea, which interferes with sexual intercourse after plaque stabilization. At least three months of stabilization is required. Currently surgery includes shortening of the convex tunica (shortening procedures), or incision of the plaque with subsequent grafting (lengthening procedures). In a case with PD and severe ED, insertion of a penile prosthesis is preferred. Lengthening procedures are more complex than shortening procedures, and preferentially utilized in patients with severe penile curvature, hourglass deformities, or unacceptable penile shortening (8,9). After the plaque incision for lengthening of the concave side, a graft should be sutured in the defect. Several grafting materials have been used like dermis, fascia temporalis, dura mater, tunica vaginalis, saphenous vein, bovine and human cadaveric pericardium, porcine small intestine submucosa, and synthetic materials (polyester and polytetrafluoroethylene) (10). The perfect substitute to fill the tunica defect is still under research; nevertheless we believe that the healthy tunica can be the ideal graft, as previously described (11).

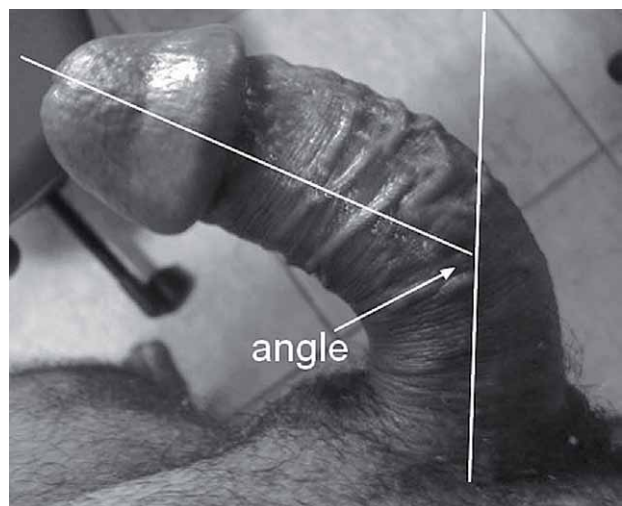
The aim of the present study is to review our long-term results of the surgical grafting with tunica albuginea retrieved from the penile crura for the treatment of symptomatic penile curvature.

MATERIALS AND METHODS

Between June 2002 and October 2009, 33 patients with Peyronie's disease were submitted to correction of the penile curvature with grafting of crural tunica albuginea as previously described (11). Surgical candidates were patients with stable Peyronie's disease for at least one year and unable to achieve sexual penetration due to curvature, and those in which medical management had failed, as stated previously (12). Patients had curvatures greater than 75 degrees determined with preoperative photography of erections (Figure-1), and all were candidates to a lengthening procedure. Medical treatment utilized before surgery included oral colchicine, vitamin E or tamoxifen, or intralesional verapamil. Patients should have good penile rigidity, with or without phosphodiesterase inhibitors.

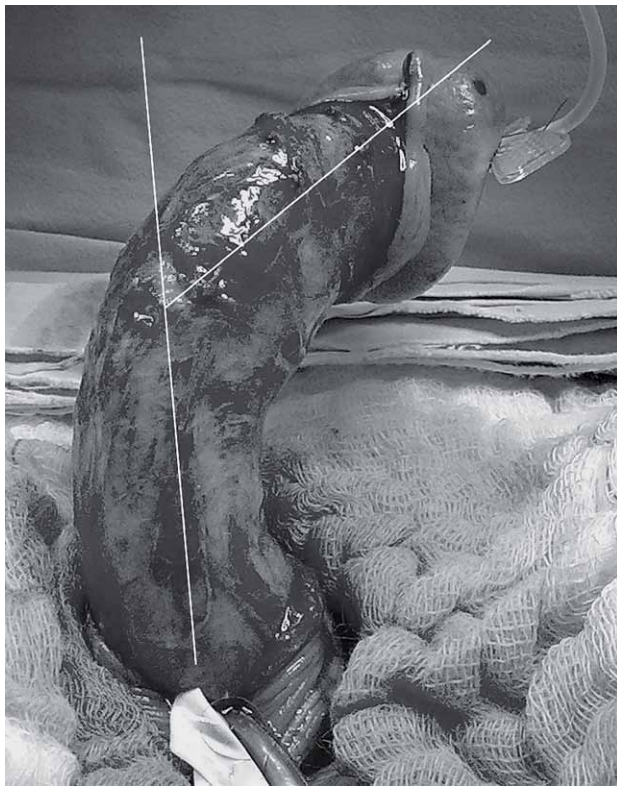
Briefly, the patient was placed in the lithotomy position, penile skin was incised (circumcision) and the penis was completely degloved. A proximal tourniquet was applied on the penis, and

Figure 1 - Measurement of the angle based on patient's picture.



saline solution was infused through a 19-gauge needle in the corpus cavernosum to produce an artificial erection (Figure-2). The dorsal neurovascular bundle and urethra, when necessary, were dissected free from the tunica albuginea (Figure-3). The exposed fibrous plaque and occasionally the septum of corpora cavernosa were incised under traction of the glans with the objective of obtaining the maximum length. At the edges of the gap, the smooth muscle was dissected from the tunica albuginea. A 3 cm perineal incision was made, one or both crura were identified, and a segment of the crural albuginea was dissected from the corpus cavernosum (Fig-

Figure 2 - Induced erection to observe the curvature.



ure-4). Bilateral crural graft was utilized if necessary. The donor area was watertight sutured with polyglactin 2-0 (Vicryl®). The graft was prepared removing all residual ischiocavernosus muscle. Grafting in the penis was performed with running sutures with polyglactin 4-0 (Vicryl®) (Figure-5). Artificial erection was performed to assure cur-

vature correction, and complementary plication of the tunica was utilized if necessary (Figure-6). Coitus was delayed for at least 30 days. All patients were operated by one surgeon (CTDR).

Patients were interviewed on 3 and 6 months and results were evaluated after 6 months from the procedure. Variables studied were overall satisfaction with the procedure, penile straightness, erectile capacity, penile shortening and the presence of complications. Data were presented as descriptive statistics.

The study was approved by the local IRB and written informed consent was obtained from all subjects.

Figure 3 - It is necessary to detach neurovascular bundle or urethra to permit the access to the Peyronie's plaque.



Figure 4 - After the perineal incision, we can see the urethra and penile crus.

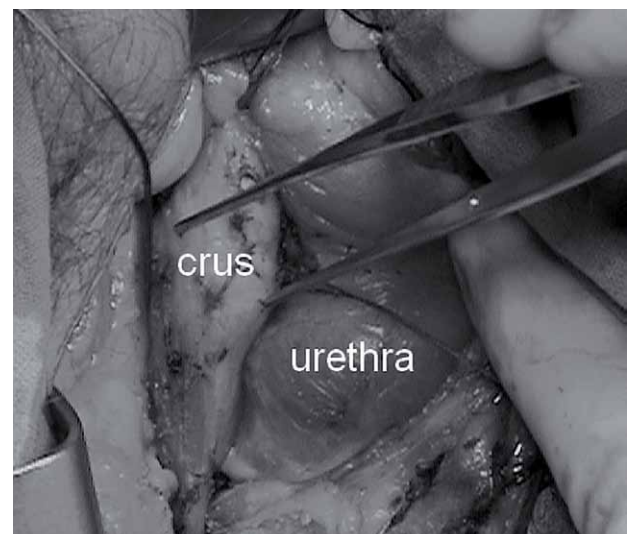
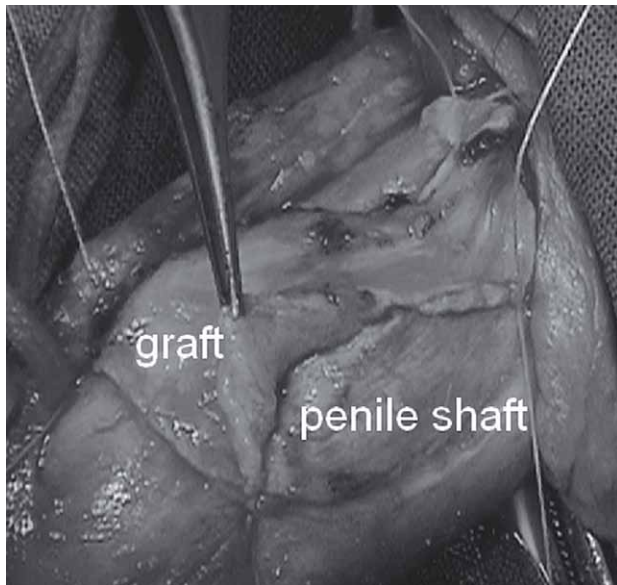


Figure 5 - The tunical graft sutured over the penile defect.

RESULTS

The mean and standard deviation age were 54.2 ± 15.1 years. Mean follow-up time after surgery was 41 months, ranging from 9 to 96 months. Complete correction of the curvature was achieved in 30 patients (90%). The mean and standard deviation of preoperative curvature were 91.8 and 18.7 degrees (ranged 75 to 160 degrees). Three patients (9%) experienced recurrence of the penile curvature and required a new procedure (plication of the tunica) and the final result was a straight penis. The mean and standard deviation of post-operative curvature was 4.5 and 6.2 degrees. The median size of the plaque was 2 cm (ranged from 1 to 5 cm).

Table-1 summarizes the results. Thirty patients (90%) answered that they would do the procedure again. Also, in 30 men (90%) the procedure fulfilled their expectations and in 31 patients (93.9%) their opinions were that sexual partners were satisfied with the penile correction.

Six patients (18.1%) complained of impairment of erectile function and received phosphodiesterase 5 inhibitors or intracavernosal therapy. Two patients (6.06%) developed refractory ED. Penile shortening or augmentation was informed by 6 (18.1%) and 1 (3%) patients respectively, while

Figure 6 - Artificial erection to check the procedure.

26 patients (78.8%) stated no difference in penile size. Six patients (18.1%) complained of hypoesthesia on glans and 4 (12.1%) had penile pain or discomfort on intercourse. No patient had wound infection, urethral injury or penile nodules.

The main reason for dissatisfaction with the procedure was recurrence of the curvature (1 patient), impairment of erectile function (1 patient), and glans hypoesthesia (1 patient).

DISCUSSION

Peyronie's disease has an important impact over the quality of life of the patients. Patients report negative effects in four major domains: physical appearance and self-image, sexual function and performance, pain and social stigmatization (4).

Surgical correction of the curvature is based on lengthening the penis, incision or excision of the plaque with grafting in the concave side, or shortening of the penis, using plication

Table 1 - Pre and post operative curvatures.

Curvatures (degrees)	Pre operative n	Post operative n
75 - 90	26	-
91 - 120	6	-
120 - 160	1	-
0 - 10	-	31
11 - 20	-	2

sutures in the convex side of the curvature. Tunnical shortening procedures, such as Nesbit corporoplasty, present low morbidity and are faster surgeries than grafting; however they result in significant loss of penile length (13). We prefer lengthening procedures in situations where penile shortening is expected with Nesbit plication, like curvatures with angle greater than 45 degrees or hourglass defect.

Penile shortening was subjectively informed by 18.1% of the patients. We explained carefully, before the surgery, that the objective of the procedure was to straighten the penis or correct the hourglass defect. In the medical literature, there is no consensus on how to measure the penile length in a curved penis with Peyronie's disease. We can stretch the flaccid penis or measure it in full erection injecting saline. The majority of the authors measure it from the pubis to the end of the glans penis. Some problems are envisioned with these length measurements. First, pubic fat can interfere in the measurement. Second, the strength utilized to pull the flaccid penis can interfere in obtaining reliable data. Third, when we measure a full erected curved penis, we would obtain a section of a perimeter of a circle (in the convex side), since the measurement in straight line is not a true measure of penile length. Fourth, the surgery is not developed to increase the penile length, and we explain that to the patients and that the main objective is to obtain a straight penis. Grafting the concave side will allow us to obtain an erection with the length of the convex side.

This study describes the long-term results of grafting with tunica albuginea, the grafting technique of choice in our service. After more

than 3 years of follow-up, we observed that 90% of our patients maintained a straight penis and were satisfied with the procedure.

The investigation for the ideal graft for penile reconstruction has demonstrated that the best tissue to substitute the sick albuginea is the patient albuginea (11,13). The albuginea of the penile crura can be retrieved in different sizes and from both sides, is ready to use, is autologous, and has no extra cost. Moreover, we think that normal function of the tunica albuginea like tensile strength and elasticity needs an architecture of collagen and elastic fibers, and crural graft fulfills these requirements. As a matter of fact, none of our patients developed penile bulging or narrowing.

The crural grafting technique avoids costs allografts or xenografts. In addition, any urologist familiarized with the perineal anatomy should be able to harvest the tissue, and no major morbidity is expected with this approach. Pain in the donor site is limited to the first days of surgery, and perineal scarring has minimal cosmetic effect.

Hypoesthesia of the glans had similar rates of other lengthening techniques, and it is probably related to the neurovascular bundle dissection. El-Sakka et al. reported a 10% rate of diminished glans sensation among patients that underwent a plaque incision and venous grafting (14), while Taylor and Levine (15) found 31% of diminished penile sensation with plication and pericardial grafting. In our series 18% of the patients complained of glans hypoesthesia.

Peyronie's disease is a risk factor for erectile dysfunction. However, there are two poten-

tial causes to increase erectile dysfunction with our technique. First, the perineal dissection of the crura could damage the penile neurovascular bundles. We avoid dissecting close to the ischium bone, keeping the harvesting of the tunica close to the ischiocavernosus muscle. Second, the diminution of the crural compartment of the corpus cavernosum can impair the haemodynamics of the penis. We did not find a higher rate of erectile dysfunction in our series, even when both crural tunics are harvested. We believe that crural portion of corpora cavernosa can be reduced without any harm. Even a penile prosthesis can be implanted after the procedure with a reduction of the prosthesis rod. Also, we could not find any

case of fibrotic plaques on the crura, and all patients had healthy albuginea.

CONCLUSIONS

Our series demonstrated that grafting the albugineal defect after incision of the tunica albuginea in lengthening surgeries of penile curvatures with crural tunica albuginea is safe and results in satisfactory straight erections in a long-term follow-up.

CONFLICT OF INTEREST

None declared.

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EDITORIAL COMMENTS

Peyronie's disease is characterized by different penile deformities caused by albugineal fibrosis and shrinkage. The disease is often associated with penile shortening. It has major influence on quality of life and is often associated with serious psychological consequences. The incidence is 3-10% among the male population; it can be commonly found in men between 40 to 70 years; however it can also affect young population under 30 years (1). According to Usta et al., Peyronie's disease is commonly associated with erectile dysfunction, ranging from 20 to 54% (2).

The surgery is indicated only to stable cases, which is not been clearly defined, but is generally accepted to be at least one year of disease and at least six months where there has been no change in the deformity and that pain has resolved. The surgical candidate should also describe a compromise or inability to engage in coitus secondary to deformity or inadequate rigidity. In addition, a patient who has medical treatment failure and has extensive plaque calcification is a proper candidate for surgery. Surgical treatment must be individualized, aiming not only at restoring penile function, but also restoring as much as possible the previous shape of the penis. Assessment of the erectile capacity is essential to decide the type of operation. Furthermore, patients' expectation should be considered and informed consent should be obtained.

Different surgical techniques have been proposed for penile curvature correction. In general they are divided in Tunical-shortening procedures (Nesbit and its modifications, dot-plication and Yachia procedures), Tunical-lengthening procedures which include excision, incision and grafting; and insertion of penile prosthesis grafting. Lengthening procedures are more complex than shortening procedures, and preferentially utilized in patients with severe penile curvature, hourglass deformities, or unacceptable penile shortening.

Different grafts materials were suggested to cover the tunical defect, and can be classified into 2 classes:

- Autologous graft:(dermal, tunica vaginalis flap, fascia lata, muscularis aponeurosis, bucal mucosa and veins, including deep dorsal vein, saphenous vein and tunica albuginea graft.

- Allograft tissue and synthetic materials: cadaveric or bovine pericardium, Dacron and Gore-Tex are the most reported.

At present time, none of them are the gold standard treatment. The ideal graft should be readily available, easily sutured, pliable, compliant, inexpensive, well tolerated and with minimal tissue reaction.

In general when Tunica albuginea is used, excision should be made in the corpora. One should have in mind that this could cause narrowing of the proximal corpus cavernosum. There for this procedure should only be used for small corporal defects. According to Teloken et al the defect should not be larger than 3.5 x 2.0 cm (3). In this study the median plaque size was 2 cm. Furthermore, this procedure may weaken the support of the penis and increase the complexity of future penile prosthesis implantation (3).

In this study the authors describe long term results of operative therapy for Peyronie's disease of 33 patients by using graft of the tunica albuginea retrieved from the crura of the penis. This method has been already described before, but there are only few long term results available in the literature.

All patients had severe curvature which unable them to have sexual intercourse. After the operation 90% of the patients were satisfied with the operation, which is comparable with previous published results of this operation technique, with shorter follow-up (4-6). Still 18% complained of hypoaesthesia of the glans which is known from the literature to be a common complication. Unfortunately there was no report about penile length before and after the operation.

In this study the authors demonstrated that this operation method is safe and achieved satisfactory results in the majority of the patients and therefore I want to congratulate them.

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Evaluation of the resistive index of prostatic blood flow in benign prostatic hyperplasia

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ABSTRACT

Objective: The aim of this work is to study the resistive index (RI) of prostatic blood flow by transrectal power Doppler sonography in benign prostatic hyperplasia (BPH) to determine its correlation with other parameters of BPH.

Materials and Methods: Eighty-two male patients aged 52-86 years with lower urinary tract symptoms (LUTS) due to BPH were included in the study. Patients with prostate cancer, neurogenic bladder, or with other pathology (e.g. prostatitis, bladder stone) were excluded from the study. All patients were evaluated by full history including International Prostate Symptoms Score (IPSS), general and local examination (DRE), neurologic examination, uroflowmetry, laboratory investigations including urine analysis, routine laboratory tests and serum prostate specific antigen (PSA). Transrectal ultrasonography was used to calculate the total prostatic volume. Transrectal Power Doppler Ultrasound (PUD) was used to identify the capsular and urethral arteries of the prostate and to measure the RI value.

Results: The mean prostate volume was 75.1 ± 44.7 g. The mean RI of the right and left capsular arteries were 0.76 ± 0.06 and 0.76 ± 0.07 , respectively. The mean RI of the urethral arteries was 0.76 ± 0.08 . There was a high significant correlation between the increase of the RI of the right and left capsular and urethral arteries and the degree of obstruction (P value < 0.001), severity of symptoms (P value < 0.001) and also the prostatic volume (P value < 0.001).

Conclusion: Resistive index of the prostatic blood flow can be applied as an easy and non-invasive tool to evaluate the lower urinary tract obstruction due to BPH.

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INTRODUCTION

Patients with benign prostatic hyperplasia (BPH) constitute a large number of urological patients. Management of these patients relies on history taking, clinical examination and investigational modalities which are additional tools for accurate diagnosis and later follow-up. Ultrasound examination opened the door for further modalities to follow (1). Since the introduction

of transrectal ultrasonography (TRUS) by Watanabe et al. in 1968, it became a valuable tool for diagnosis of most prostatic diseases (2). Over the last two decades this technology has been used successfully in evaluating prostatic disease (1). The color Doppler Ultrasound (CDUS) was later followed by power Doppler Ultrasound (PDUS) that allowed us to assess the vascular architecture of the prostate through a non-invasive modality (3). PDUS has high sensitivity for displaying

slow blood flow, besides the capability to depict the number, course and continuity of prostatic vessels (4). The resistance index (RI) of the prostate vasculature was able to discriminate patients with normal prostate and those with benign prostatic hyperplasia (BPH) and be useful as a new hemodynamic parameter (5). This study aimed to evaluate the relation between RI of prostatic blood flow by PDUS in BPH and its correlation with other parameters of BPH.

MATERIALS AND METHODS

Eighty-two male patients aged 52-86 years with lower urinary tract symptoms (LUTS) due to BPH were included in this study. Patients were randomly selected on the basis of their presentation to the outpatient clinic of Benha University Hospital, Egypt. Patients with prostatitis, prostatic abscess, histologically confirmed prostate cancer, neurogenic bladder and bladder stones were excluded from the study. All patients were assessed by full history taking with special emphasis on International Prostate Symptom Score (IPSS). Physical examination included general examination, digital rectal examination (DRE), neurological examination especially bulbocavernosus reflex (BCR), cremasteric reflex and anal reflex to screen for possible neurological insult. Uroflowmetry was done for all the patients at least twice. Urine analysis, routine laboratory tests and serum prostate specific antigen (PSA) were done for all patients. Imaging studies included plain X-ray KUB, abdomen and pelvic ultrasound to assess post-voided volume and to exclude other associated pathologies. Transrectal ultrasound (TRUS) of the prostate was done to estimate prostatic volume followed by power Doppler ultrasonography (PDUS) to identify the capsular arteries (Figure-1) and urethral arteries (Figure-2) of the prostate and to measure the RI in both right and left capsular arteries and urethral arteries. All sonographic examinations were done by the same radiologist.

Informed consent was given by all participants and the study protocol was approved by the research ethical committee of Benha Faculty of Medicine. Doppler indices were used to obtain

information involving blood flow and vascular impedance in prostatic vessels. The indices depend on ratios involving the peak systolic velocity (PSV), the end diastolic velocity (EDV) and mean velocity (MV) through one cycle. RI is one of the primary indices used clinically and is calculated through the following equation (6).

$$RI = \frac{(PSV) - (EDV)}{PSV}$$

All data were collected, tabulated and statistically analyzed by SPSS software version 0.16. P-value less than 0.05 was considered statistically significant.

Figure 1 - PDUS examination of the prostatic vasculature showing capsular arteries.

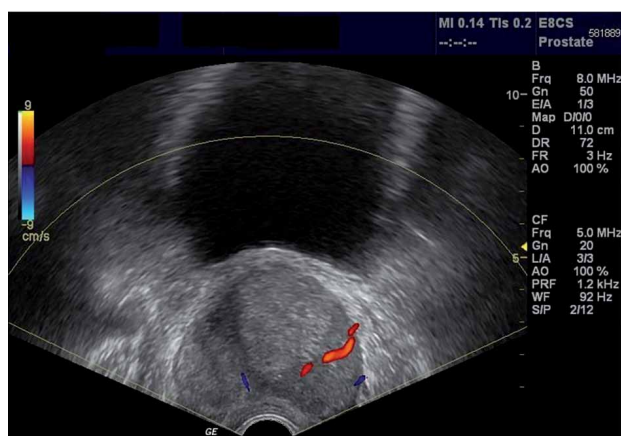
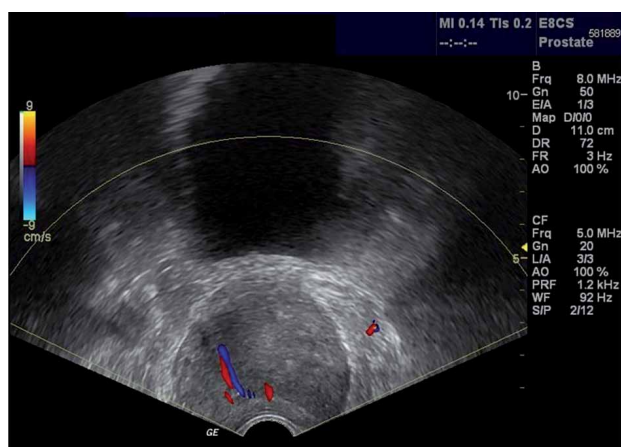


Figure 2 - PDUS examination of the prostatic vasculature showing urethral arteries.



TRUS examination: The procedure was performed after a preliminary cleaning enema in left lateral decubitus position with the knees bent and the bladder partially filled. TRUS examination was performed using a Toshiba machine with end-fire probe with the sonoline Elegra system. The power Doppler imaging was done with a centre probe frequency of 6.5 MHz and the wall filter set to low. The end of the transrectal probe was sheathed in a thin latex balloon (usually a condom), containing scanning gel.

RESULTS

Total number of patients enrolled in this study was 82. Age range was 52-86 years with mean age 66.8 ± 10.2 . Seven parameters were studied and the values of these parameters are sum-

marized in (Table-1). According to uroflowmetry results and pelvic and abdominal U/S, patients were classified into two groups: obstructed ($Q_{\max} < 15$ mL/s and PVR > 100 mL) and non-obstructed ($Q_{\max} > 15$ mL/s). Sixty two patients (76%) were obstructed and 20 (24%) patients were non-obstructed. There was a highly significant increase in the RI of right and left capsular arteries and urethral arteries correlated to increased obstructive pattern of the patients (Table-2).

According to IPSS, our patients were classified into 2 groups. First group included 21 patients that had mild and moderate obstructive symptoms (mild 0-7 and moderate 8-19 on IPSS) and the second group included 61 patients with severe obstructive symptoms (20-35 IPSS score). Mean RI of right & left capsular arteries and urethral arteries were measured in both groups and summarized in

Table 1 - Summary of results.

Parameters	Mean \pm SD	Range
AGE (years)	66.8 ± 10.2	52-86
IPSS	26.5 ± 10.7	5-35
Prostate volume (gm)	75.1 ± 44.7	14-253
Q_{\max} (mL/second)	13.3 ± 4.6	4.3-21.4
RI of right capsular artery	0.76 ± 0.06	0.62-0.90
RI of left capsular artery	0.76 ± 0.07	0.60-0.90
RI of urethral arteries	0.76 ± 0.08	0.57-0.94

Table 2 - Comparison of RI of Rt. & Lt. capsular and urethral arteries between obstructed and non-obstructed groups of patients (according to uroflowmetry).

	Non-obstructed N = 20	Obstructed N = 62	t	(P-value)
	Mean \pm SD (range)	Mean \pm SD (range)		
RI of right capsular artery	0.68 ± 0.03 (0.62-0.73)	0.79 ± 0.05 (0.63 - 0.90)	-10.3	< 0.001
RI of left capsular artery	0.67 ± 0.04 (0.60 - 0.74)	0.79 ± 0.05 (0.64 - 0.90)	-10.5	< 0.001
RI of urethral arteries	0.67 ± 0.04 (0.57 - 0.79)	0.79 ± 0.06 (0.67-0.94)	-8.6	< 0.001

(Table-3). There was a highly significant increase in the RI of right and left capsular and urethral arteries correlated to the increasing severity of symptoms of the patients according to IPSS. The size of the prostate was measured by TRUS and was considered normal when < 25g and enlarged if > 25g. Six patients were considered to have normal prostate and 76 patients had enlarged prostate. There was a significant increase in the RI of right and left capsular and urethral arteries correlated to the increase of the prostatic volume (Table-4).

DISCUSSION

With the advent of power Doppler imaging, RI measurement in patients with LUTS has become

a promising parameter for the diagnosis of BPH (1). The definite mechanism through which the RI is elevated in BPH is still unclear (1). It was assumed that a hyperplastic prostate tissue pushed the capsule out as it grew thus increasing the intraprostatic pressure as well as RI. The increase of the intraprostatic pressure is equally distributed throughout the whole prostate, so the increase of RI was found in both peripheral and transition zones (7).

There is a relative difference in the RI of prostatic vasculature in different prostatic pathologies. The mean RI value of 0.579 (range, 0.45 – 0.80) for cancer cases was slightly lower than the mean values for cases with atypia, inflammation, and benign disease, which were 0.601 (range, 0.49

Table 3 - Comparison of the RI of Rt & Lt capsular arteries and urethral arteries between patients with mild to moderate symptoms, and with severe symptoms according to IPSS.

	Mild & moderate N= 21	Severe symptoms N = 61	<i>t</i>	(P-value)
	Mean ± SD (range)	Mean ± SD (range)		
RI of Rt. capsular artery	0.68 ± 0.03 (0.62 - 0.73)	0.79 ± 0.04 (0.70 - 0.90)	10.8	< 0.001
RI of Lt. capsular artery	0.66 ± 0.03 (0.60 - 0.74)	0.79 ± 0.03 (0.68 - 0.90)	11.2	< 0.001
RI of Urethral arteries	0.67 ± 0.04 (0.57 - 0.79)	0.79 ± 0.06 (0.69 - 0.94)	8.6	< 0.001

Table 4 - Comparison of parameters between patients with a normal prostate size and those with enlarged prostate.

	Normal size prostate N = 6	Enlarged prostate N = 76	<i>t</i>	(P-value)
	Mean ± SD (Range)	Mean ± SD (Range)		
RI of right capsular artery	0.67 ± 0.04 (0.62 - 0.72)	0.77 ± 0.06 (0.63 - 0.90)	-4.1	< 0.001
RI of left capsular artery	0.67 ± 0.05 (0.60 - 0.74)	0.77 ± 0.08 (0.62 - 0.90)	-3.6	< 0.001
RI of urethral arteries	0.65 ± 0.04 (0.57 - 0.70)	0.77 ± 0.07 (0.61 - 0.94)	-3.8	< 0.001

– 0.86), 0.621 (range, 0.54 – 0.77) and 0.616 (range, 0.40 – 1.00), respectively. It is noteworthy that the subgroup of patients with the highest RI value measured at the relevant site was the one with inflammation compared with those with cancer, atypia, and benign disease (8).

The parameters used by different research groups to correlate RI with BPH were different. Hayami et al. assessed the value of power Doppler imaging in predicting the histological components of BPH and demonstrated that RI was the most reliable factor for predicting the ratio of glandular lumen to stromal elements. These values were considered useful for predicting the therapeutic response to different treatment options (9). Others pushed their research one step further. Turgut et al. used PDUS and RI in a trial to differentiate prostate cancer from BPH. The authors reported that the mean RI for cancer cases was slightly lower than the mean RI value for benign conditions despite the fact that the difference was statistically insignificant ($P > 0.05$) (8).

In the present study, out of 82 patients, 62 (76%) were diagnosed to be obstructed and the remaining 20 (24%) unobstructed based on uroflow-metric studies. There was a significant difference in RI of right capsular arteries between the obstructed and non obstructed groups ($P < 0.001$). There was a significant difference in RI of left capsular arteries between the obstructed and non obstructed groups ($P < 0.001$). Similarly, there was a significant difference in RI of urethral arteries between the obstructed and non obstructed groups ($P < 0.001$). There was a highly significant increase in the RI correlated to the increase of obstructive patterns of flow rates of the patients. Although there was a significant statistical value between RI of both right and left capsular arteries and urethral arteries when correlated to IPSS, uroflowmetry results and prostate volume, there was not a significant difference in mean RI among the individual arteries.

Kojima et al. showed a significant correlation between RI and urodynamic parameters obtained in peak flow rate (Q-max) of uroflowmetry and IPSS in their study. Out of 33 patients with obstruction, 28 (85%) had a RI of 0.7 or more, while 11 out of 24 patients (46%) without obstruction had a RI less than 0.7 (7).

In the study of Tsuru et al. (10), the RI of capsular arteries was (0.73 ± 0.08) and that of urethral arteries was (0.69 ± 0.08), and there was a significant correlation between IPSS and RI of capsular arteries. These results are similar to our results in capsular arteries but there was a noted difference from that obtained in urethral arteries and this may be explained by different methods of assessing intra-prostatic arteries and the great improvement of machine technology that we used. Shinbo et al. studied the RI as a risk for acute urinary retention in patients with BPH; they stated that RI is increased in patients with BPH and is related to the severity of bladder outlet obstructive symptoms (11). Other researchers didn't observe this strong correlation. Hayami and associates showed that there was a weak difference in RI between the obstructed and non-obstructed groups (0.73 ± 0.1 vs. 0.71 ± 0.03) (9).

Frauscher et al. showed that the RI was significantly elevated in BPH patients in comparison to the normal group (12). These results are similar to that obtained by the study done by Kojima et al. who found that there was a significant increase of the RI in 40 cases of BPH (0.72 ± 0.05) compared to 37 cases with a healthy prostate (0.64 ± 0.04), ($P < 0.0001$) (5). Kojima et al. demonstrated that the RI value of patients with normal prostatic volume was significantly lower than that of patients with an enlarged prostate (0.64 ± 0.04 vs. 0.72 ± 0.06 ; $P < 0.0001$) (7).

Jamal and Khadr showed that the RI increases significantly correlated to the increase in prostatic volume, and that there was a significant difference in RI between patients with normal prostate and those with BPH (0.64 ± 0.04 vs. 0.72 ± 0.06 ; $P < 0.0001$) (13). This result is in agreement with previous studies and similar to our results.

It is noteworthy to mention that although most of the researchers - whether in total agreement, partial agreement or who even didn't agree with our results - did not identify or correlate the individual capsular and urethral arteries, yet we analyzed signals from these vessels separately. This may be related to the advances in the machine technology.

The PDUS is three to four folds more sensitive for the detection of blood flow when compared to CDUS (14). We used this technology to

correlate the RI of prostate vasculature with BPH symptoms, prostate size and degree of obstruction.

CONCLUSIONS

The RI measurement using PDUS can be added to the modalities available for investigating BPH. Furthermore, its value can be correlated to the prostate size and degree of obstruction. Further research in this field will even allow the use of this modality to investigate other pathologies affecting the prostate and can be used also to evaluate the outcome of management.

CONFLICT OF INTEREST

None declared.

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EDITORIAL COMMENT

The authors analyzed the resistive index (RI) of prostatic blood flow by transrectal power Doppler sonography as a method to evaluate the lower

urinary tract obstruction due to BPH. They demonstrated a high significative correlation between the increase of the RI of the right and left capsular and urethral arteries and the degree of obstruction, severity of symptoms and prostatic volume.

The relative invasiveness of urodynamics and its indeterminate results in a significant number of cases have stimulated the development of non invasive methods for the diagnosis of bladder outlet obstruction (BOO). In this context, the use of ultrasound (US) as a surrogate method to establish this diagnosis has constituted an interesting field of research. Prostate volume, post void residual volume, intra-vesical prostatic protrusion, bladder weight, detrusor wall thickness, urethral prostatic

angle and resistive index of prostatic arteries are the main forms to predict the risk of BOO through US analysis. The association of some of these measures has led to accuracy rates over 90%. The lack of standardization of these measures is probably the main obstacle that has hampered the reproduction of the results in other urologic centers. Urologists should be aware of these facts in order to explore the maximum potential of this cheap, available, non invasive and innocuous diagnostic tool.

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EDITORIAL COMMENT

The authors present their experience with the use of prostatic resistive index (RI) to distinguish between men with obstructive versus non obstructive prostatic hyperplasia. Their findings suggest ultrasound RI to be a validated auxiliary tool to identify patients suffering from significant obstructive urinary flow.

Since the obstructed group presented a significant higher RI than non obstructed group, selection criteria before transrectal ultrasound assessment seem to have been adequate ($Q_{max} < 15$

mL/s and PVR > 100 mL). However, although the mean RI difference was statistically significant for all measured parameters (right artery, left artery and urethral artery, $P < 0.001$) numbers were frequently overlapped (see Table-2). As such, identifying obstructive cases on an individual basis may be rather difficult.

Also, it is hypothesized that an enlargement of the median lobe may not impact RI value as it does not impose increased resistance to capsular arteries. The association of intravesical prostatic protrusion (IPP) could add for a more precise diagnosis in this scenario (1,2).

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EDITORIAL COMMENT

The authors should be congratulated for this study about the resistive index of blood flow in the prostate of patients with BPH. There are few studies (1) describing the effects of the prostatic resistive blood flow in the urodynamic parameters, and the potential applications of this information can change our practice. The knowledge about it is still incipient and the resistive

index of blood flow of the prostate and bladder outlet can be a simple and useful tool to determine infravesical obstruction. (2)

However, this study opens an opportunity for the development of more trials including necessarily a comparison with pressure/flow parameters that could reveal more information about the sensitivity, specificity and positive predictive value of this method regarding infravesical obstruction.

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Primary fixation of mini slings: a comparative biomechanical study in vivo

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ABSTRACT

Introduction and Objectives: The mini sling concept for stress urinary incontinence is an anatomical approach that involves placing a midurethral low-tension tape anchored to the obturator internus muscles bilaterally. They overcome the blind passage of long needles and all the related complications. There are many different devices available and because these are outpatient procedures, primary fixation plays an important role in the outcome. The objective is to evaluate the primary fixation of the various devices of attachment of the commercially available mini-slings through biomechanical tests.

Materials and Methods: A total of 45 Wistar rats were divided in 3 groups of 15 rats each. They underwent 5 subcutaneous implantation of different mini slings and one polypropylene mesh (control), as follows: TVT-Secur® (Gynecare, USA), Type 1 polypropylene mesh (control); Ophira Mini Sling System® (Promedon, Argentina), Tissue Fixation System® (TFS PTY, Australia), Zipper Sling® and "T device" (Prosurge, USA). The abdominal wall was removed on bloc at different times after implant for biomechanical evaluation, which consisted in application of unidirectional force to the extremity of the fixation system or mesh, until it was completely removed from the tissue using a tension meter (Nexygen 3.0 Universal Testing Machine - LLOYD Instruments). The force was measured in Newtons (N).

Results: There was significant difference in the resistance to extraction among the different fixation systems. At 7 days the Ophira Mini Sling System® presented the best fixation and "T dispositive" the worst.

Conclusion: Ophira mini sling System® presented the best primary fixation at 7°, 14° and 30° days. The impact of this feature in the clinical setting needs to be verified.

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INTRODUCTION

The understanding of the physiopathology of Stress Urinary Incontinence (SUI) has constantly improved over the two last decades, since the presentation of the Integral Theory (1) and TVT introduction (2). In spite of the good

cure rate reported with TVT, severe complications such as visceral and vessels injuries have been described (3). As an alternative to TVT, Delorme introduced the transobturator tape (TOT) (4,5) with successful results. In spite of the low intra-operative complication rate of TOT, most complications were related to the blind passage of

needles through the obturator foramen (3), which led to the creation of small minimally invasive devices which avoid this passage and can be implanted under local anesthesia, on an outpatient basis and with minimal dissection. Due to a shorter insertion path, it is expected that some complications such as vesical perforation, vascular injuries, perineal fasciitis and reduction of postoperative pain in the area of adductor muscles can be reduced (6,7). It is possible to suppose that the greater adhesion of the mesh to host tissues and the lower the amount of implanted material lower the risk of extrusion and the rate of sexual discomfort, respectively.

Initial results with some mini sling systems were disappointed although they were appealing. The first results reported with TVT-Secur showed success rates 10% lower than could have been expected with other types of slings (8-10). Therefore, the proposal of biomechanical studies is justified in order to understand the physiopathological process associated to sling efficacy itself. Up to this moment, no studies have been published in literature comparing the tissue fixation capacity of the different types of mini slings.

In this original experimental study, the primary fixation of the different anchoring devices of some commercially available mini slings and experimental devices is evaluated "in vivo" through biomechanical tests.

MATERIALS AND METHODS

This study was approved by the Ethics Committee for Animal Research of the University of Campinas and there is no conflict of interest. Forty-five Wistar rats (weight between 150g and 200g), aged 8 weeks were divided into 3 groups of 15 rats each. Animals were intravenously anesthetized with sodium pentobarbital at 6% and were positioned in horizontal dorsal decubitus after abdominal trichotomy and asepsis with Povidone-iodine. A 2 cm transverse incision was then made in the lower abdomen. After the dissection, five different types of mini sling anchoring devices and one polypropylene mesh (control) were implanted between the subcutaneous cellular tissue and the abdominal muscle fas-

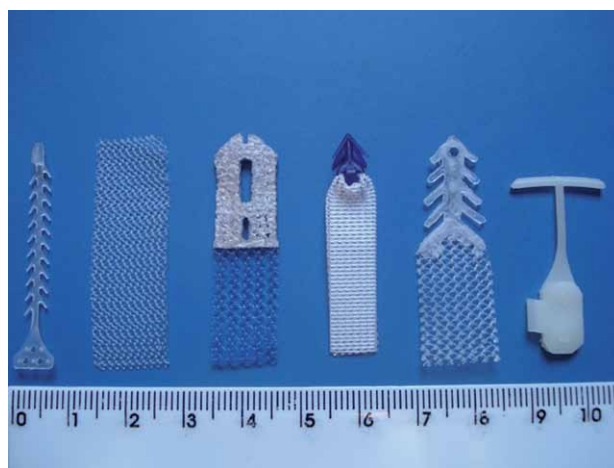
cia, namely: TVT-Secur® (Gynecare, USA), polypropylene mesh (PP-control), Ophira Mini Sling System® (Promedon, Argentina), Tissue Fixation System® (TFS PTY, Australia), Zipper Sling® and "T device" (Prosurg, USA) (Figure-1).

The five samples of each device were randomly implanted in each group (two per animal - one in each side of abdominal wall) (Figure-2). After implantation of the anchoring devices of the mini slings, the skin was sutured, taking care to avoid that the mesh was in direct contact with the skin suture.

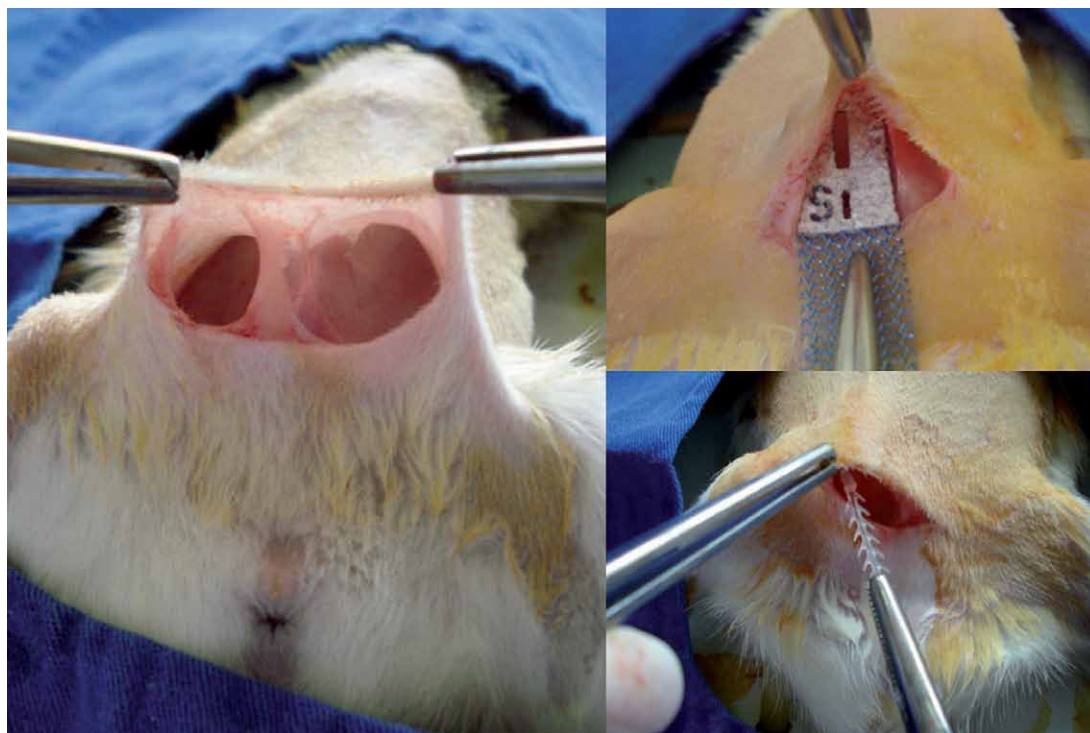
The evaluation of the tensile resistance was made in a fresh fragment of the abdominal wall of the rat. After an observation period (7, 14, 30 days) the animals were divided in 3 groups and euthanized, as follows: Group 1 (15 rats euthanized at day 7); Group 2 (15 rats euthanized at day 14); and Group 3 (15 rats euthanized at day 30).

The abdominal wall was removed and symmetrically divided into 2 blocks containing the implanted anchoring device. Subsequently, approximately 2 mm of the extremity of the devices were dissected to be pulled so that they

Figure 1 - Devices.



- 1° Ophira mini sling system® (Promedon, Argentina)
- 2° Polypropylene Mesh (Promedon, Argentina)
- 3° TVT-Secur® (Gynecare, USA)
- 4° Tissue Fixation System® (TFS PTY, Australia)
- 5° Zipper Sling® (Prosurg, USA)
- 6° Prosurg® (Prosurg, USA)

Figure 2 - Surgical Technique.

could be adapted to the fastener of the tension meter ("Nexygen 3.0 Universal Testing Machine" - LLOYD Instruments) which is specially intended for load tests in soft tissues (11). The opposite portion of the fragment, containing the abdominal wall without the device, was fixed to the lower fastener of the tension meter and a biomechanical study was performed, in order to measure their tissue adherence at different times.

Next, an increasing load was applied to the extremity of the anchoring device or mesh until it was completely removed from the tissue (Figure-3). The load was measured in Newtons (N), so higher load values show a greater fixation of the device to the tissues. We applied an increasing force (N) and constant speed (2 mm/sec). The strength and the time varied for each test. As the time was not relevant to our study, only the force was measured.

In order to compare the maximum load in relation to the groups over the time (7, 14 and 30

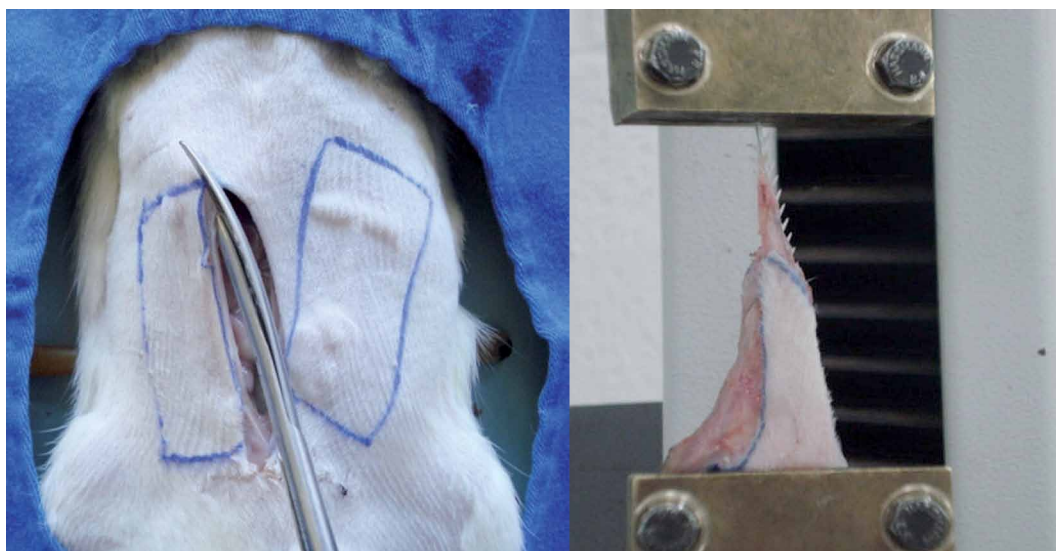
days), the Analysis of Variance (ANOVA) was used and the level of significance adopted was 5%.

RESULTS

There was a significant difference of the maximum load needed for detachment of the mini slings anchoring devices from the tissues.

After 7 days of the placement of the implant, it was observed that Ophira Mini Sling System® showed the highest maximum load fixation (6.21 ± 0.52 N) and the "T device", the worst fixation (1.50 ± 0.72 N). There was no significant differences among the other devices.

On 14th day after the implant, it was observed that Ophira Mini Sling System® and TFS showed the highest maximum load (11.77 ± 0.45 N and 11.63 ± 0.92 N, respectively), compared to the other devices, which presented similar results. After 30 days, it was observed that Ophira Mini Sling System® showed the highest maximum

Figure 3 - Experiment.

load (18.30 ± 0.55 N) and the “T device” showed the worst fixation (11.24 ± 0.23 N) (Tables 1 to 3 and Figure-4).

DISCUSSION

Since the introduction of the mini slings on the market, the failure rate that has been published by different authors differs markedly. Neuman published with TVT Secur® a prospective study showing a cure rate of 93.5% at 12-month follow-up [n: 100] (12). In other study Solà Dalenz et al. presented 100% of cure at 2-month follow-up with TVT Secur® [n: 16] (13). Gorlero et al. studied 15 patients, and reported a negative stress test in 86.7% with TVT Secur® at 6-month follow-up (14). In contrast, Meschia and Debodinance published an objective cure rate of 70.4% and 81% at 6-month follow-up [n: 110] and 15-month follow-up [n: 95] respectively (9,15).

Palma et al. have published preliminary results with arcus to arcus microsling. After 12 months, 88% of patients were dry, 5.5% improved and 5.5% incontinent [n: 20] (16).

The cure rates related to Mini Arc (American Medical Systems, USA) are different. Moore et al. reported a negative stress test in 90% of patients

treated at 12-week follow-up [n = 59] (17). Jiménez et al. published satisfactory results with a negative stress test in 90% of patients, with a mean follow-up of 101 days after treatment with MiniArc [n = 41] (18) and Debodinance and Delporte have found an objective cure rate of 75.7% at 2-month follow-up [n = 72] (19). Mini Arc was not included in this experiment because when the study was performed it was not available in the market yet.

One can assume that the rationale for the use of the mini slings is based on its capacity of fixation to the host tissue immediately after the implant, which is probably the main factor for achieving the continence and lowering the risk of vaginal exposure or extrusion.

To date, few cases of vaginal exposure of the mini slings have been described. One case of exposition was reported by Martan et al. (20) and Hazewinkel et al. (21), respectively with TVT Secur® and only two cases were reported by Debodinance et al. with the same mini sling.

Tissue reactions to different types of synthetic materials used to repair the defects of the pelvic floor are almost established and have not been dealt with in this study (22-25). It is known that, apart from the mechanical properties of the implanted material, local factors such as tissue

Table 1 - Loads for group and time (days).

Group	Time	Mean (N)	SD	Minimum (N)	Median (N)	Maximum (N)
TFS	7	5.02	0.74	3.99	4.88	5.88
	14	11.63	0.92	10.80	11.37	13.00
	30	15.66	1.19	14.99	15.10	17.77
TVT	7	4.45	0.49	3.99	4.18	5.10
	14	8.98	0.19	8.66	9.02	9.13
	30	14.00	0.52	13.25	14.10	14.66
T device	7	1.50	0.72	0.76	1.58	2.53
	14	7.25	0.99	5.75	7.76	8.12
	30	11.24	0.23	10.99	11.37	11.48
Mesh	7	3.80	0.72	3.04	3.50	4.66
	14	8.31	0.47	7.90	8.10	9.01
	30	13.36	0.64	12.99	13.01	14.48
Ophira	7	6.21	0.52	5.74	6.06	7.09
	14	11.77	0.45	10.99	11.90	12.10
	30	18.30	0.55	17.88	17.99	19.10
Prosurg	7	4.84	0.19	4.64	4.74	5.13
	14	9.74	0.44	9.12	9.87	10.23
	30	15.60	0.60	15.09	15.37	16.50

Table 2 - Results of the ANOVA with repeated measures (in ranks) to Maximum Load.

Source variation	p-value
Group	0.0001
Time	0.0001
Comparison of each group along the time	0.0088

tropism, infections and the surgical technique are directly related to the rates of extrusion (26,27).

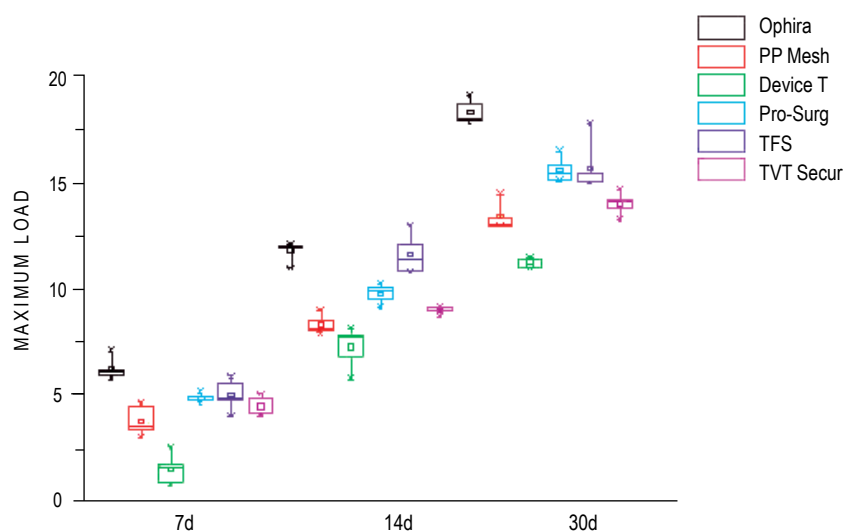
Since most of the modern slings have been built with materials with well-known biocompatibility, the surgeon's interest has been focused on

the design of the fixation devices and implant instruments like needles and trocars. Although a variety of experimental models can be proposed for the study of such dispositives, one which allows for uniaxial stress test seems to be suitable for biomechanical evaluation, since in the vaginal environment the slings are not prone to rotational or centripetal forces.

The trend to perform slings in an outpatient basis had been first proposed in the nineties, along with the retropubic minimally invasive midurethral slings, but till now it has never been fully adopted because of safety reasons. The mini slings can turn this concept to a real possibility, since most of the clinical data shows a high level of safety. Therefore, as the patient can come back

Table 3 - P-values for comparisons along time in each group (Contrast Test).

Times (days)	Ophira	Mesh	T-Device	Pro-surg	TFS	TVT
7 versus 14	0.0001	0.0002	0.0002	0.0002	0.0010	0.0014
7 versus 30	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
14 versus 30	0.0001	0.0001	0.0003	0.0001	0.0027	0.0001

Figure 4 - Maximum Load - Comparison of different fixation devices.

home in the same day of the procedure, the primary fixation of the sling becomes relevant.

The highest maximum load showed by Ophira Mini Sling System® probably is related to its multipoint fishbone-like polypropylene fixation device. But it is not possible to determine the minimal load which is sufficient to prevent sling misplacing in the clinical setting. TVT-Secur® and Zipper® fixation device slings have not been effective due to the fact that their stabilization on site depends mainly of the in growth of host fibrotic tissue. Previous experimental data showed that in the integration of monofilament polypropylene tapes, the formation of a mature conjunctive tissue around the mesh takes more than ninety days after the implant (28).

Polypropylene is a synthetic, inert, hydrophobic and non-absorbable material. Implants made of this have shown experimentally better tissue integration when compared to other synthetic materials. Other materials and coatings were studied for clinical use but have not demonstrated superiority to the polypropylene type I (28).

The response to foreign body is a physiological cascade triggered by the adhesion of proteins to the implant and, consequently, recruitment of inflammatory cells (29,30). The phases of tissue repair may be classified as: early or hemostasis and inflammation; proliferation phase and final phase, in which the maturation of collagen occurs (31). In the integration of synthetic mesh one can consider the occurrence of a similar process. Based on this, we

can infer that for any implant, the duration of each step may vary depending on various factors such as conditions of the host immune system, degree of bacterial contamination site and biocompatibility of implanted material. The complete resolution of the inflammatory response, represented by the reconstitution of the native tissue, may eventually not be possible due to the maintenance of aggression factors related to the physical and chemical properties of the implant. Thus, what is called integration is the end result of the interaction between the implant and the host, which is usually represented by varying degrees of fibrosis (32).

As the interface between the mesh and the host tissues is less extensive than in a conventional transobturator sling, a powerful fixation is necessary to stabilize the sling in the proper position in the early phase of the healing process. So, researchers should find ways to design fine but reliable biomaterials in order to improve results with minimal risk of recurrence of the incontinence or adverse events.

CONCLUSIONS

Ophira Mini Sling System® showed the best primary fixation at 7, 14 and 30 days. At day 14, Ophira® and TFS® were equally satisfactory. In addition, TVT-Secur® and Zipper® slings have not been effective due to the fact that they depend on tissue integration. These findings could influence clinical practice for outpatient procedures in which an effective primary fixation is needed.

CONFLICT OF INTEREST

None declared.

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EDITORIAL COMMENT

In this elegant study by Palma et al. authors tested the resistance of different anchoring systems in several commercially available models of mini slings by implanting each anchoring device under the subcutaneous tissue of rat abdominal wall and evaluating them under a tension meter. Ophira® mini sling seems to deliver the most adequate design for the purpose of maintaining the suburethral polypropylene mesh on site with a delicate, fine, multi-spiky fishbone-like format suggesting that the more contact the implant has with host tissue the better is its fixation. This contact must be, however, delivered in an intelligent manner as to provide immediate

adherent capacity to the anchoring system turning it less dependent on tissue interaction which tends to improve throughout time. Under this point of view, it seems logical that the "T" design proposed by Prosurg® would deliver less satisfying results.

Obviously, these results need to be confirmed in clinical setting, which implies a different scenario where dynamic and uneven strengths within the pelvic rim pull the sling device and might dislodge it.

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EDITORIAL COMMENT

Following the worldwide trend to adopt minimally invasive procedures, the single incision slings or the mini-slings have been developed. There is no doubt that the main issue of the third generation of the midurethral slings is how to ensure the primary fixation of the tape in order to maintain the sling in proper position while the healing process is completed. Precisely at this point devices differ. This initial biomechanical study has shown that the multipoint fish bone-like polypropylene fixation device is related to a greater initial tensile resistance compared to the others in an *in vivo* model, but in clinical practice the minimum tensile resistance to stabilize the tape has not been defined yet. On the other hand, it was also observed a significant increase in tensile resistance for all devices during

the first postoperative month. This is a very important information that should be considered when counseling the patients; although these procedures can be performed in an outpatient basis, the post-operative care should be the same as those for the others midurethral slings. Recent literature showed that single incision slings, addressed to obturator internous muscle, are associated with inferior patient-reported and objective cure rates on the short-term follow-up, when compared with others midurethral slings (1). This result suggests that the best local and / or form of anchoring the mesh have not been reached yet. In conclusion, the main issue of the single incision slings persists and more biomechanical and clinical studies are needed to clarify this point.

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Can the delivery method influence lower urinary tract symptoms triggered by the first pregnancy?

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ABSTRACT

Introduction and Objectives: The increase of the intensity of urinary symptoms in late pregnancy and postpartum has been well documented by several authors, but their causes remain uncertain, partly because of its probable multifactor origin. There are also controversies whether the etiology of lower urinary tract symptoms during pregnancy is the same as postpartum and whether the method of delivery could influence the risk of onset of urinary symptoms. This study aimed to evaluate the urinary symptoms triggered during pregnancy and its evolution in the late puerperium, correlating them with the delivery method.

Materials and Methods: A longitudinal study was conducted, which included 75 primigravidae women, classified according to method of delivery as: (VD) vaginal delivery with right mediolateral episiotomy (n = 28); (CS) elective caesarean section (n = 26); and (EC) emergency caesarean section (n = 21). Urinary symptoms were assessed in the last trimester of pregnancy and at 45 days (± 10) of puerperium with validated versions for Portuguese language of the following questionnaires: International Consultation on Incontinence Questionnaire - Urinary Incontinence Short Form (ICIQ-UI SF) and the International Consultation on Incontinence Questionnaire Overactive Bladder (ICIQ-OAB).

Results: It was observed that frequency, urgency, nocturia and urge incontinence, triggered during pregnancy, decreased significantly in the postpartum period, regardless of the delivery method ($p = 0.0001$). However, symptoms related to urinary loss due to stress persisted after vaginal delivery ($p = 0.0001$).

Conclusions: Urgency, frequency and nocturia triggered during pregnancy tend to disappear in the late postpartum period, regardless of the delivery method, but the symptoms related to urinary loss due to stress tend to persist in late postpartum period after vaginal delivery.

ARTICLE INFO

Key words:

Pregnancy; childbirth; postpartum; pelvic floor; urinary symptoms

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INTRODUCTION

Urinary incontinence (UI) is considered a clinical condition with multifactor etiology (1). Studies of UI during pregnancy have reported prevalence of 32% to 64% for all UI and 40% to 59% for stress UI, including mixed incontinence

(2), persisting after delivery in 6% to 29% of women (3). Estimating the post-partum prevalence of urinary incontinence is complex because the prevalence may depend upon the number of previous births, type of delivery, and history of previous in-

continence (2). Studies suggest that its incidence may increase with age (4,5), and multiparity (6-8), but the actual mechanisms remain unclear (2,5,9).

Vaginal birth has been recognized to be potentially traumatic to the pelvic floor (10-15). However, the pathophysiological effects of pregnancy and method of delivery on the lower urinary tract in general and the pelvic floor in particular remain uncertain, despite frequent references in obstetric literature (2,16).

Urinary continence is greatly committed to quality of life. Since 1997, the International Continence Society (ICS) recommends that the assessment of quality of life should be included in the studies, and it is consensual that the improved quality of life is important for the evaluation of the success of the proposed treatments as well as the impact and severity of the impairment (17,18).

This study aimed to evaluate the lower urinary tract symptoms (LUTS) triggered during pregnancy in primigravidae women, to assess their persistence in late puerperium and to correlate them with the different methods of delivery: vaginal, elective caesarean section and emergency caesarean section.

MATERIALS AND METHODS

We conducted a prospective, longitudinal, comparative clinical study which included 75 primigravidae women, aged between 14 and 39 years (mean 23.24 years), selected from health programs in Poços de Caldas (Minas Gerais, Brazil). All patients signed a consent form approved by the Ethics Committee in accordance with the Declaration of Helsinki (Clinical Trial Registration Number - Zip: 0207.0.213.146-05) and it was approved on 02/10/2006 (17:35:18).

Primigravidae women in the third trimester (average of 31.19 weeks of gestation), who presented some voiding complaints that had begun during the pregnancy, were included in the study. The patients had a mean body mass index (BMI) of 27.49 in the enrollment and 25.04 at reevaluation in the late puerperium.

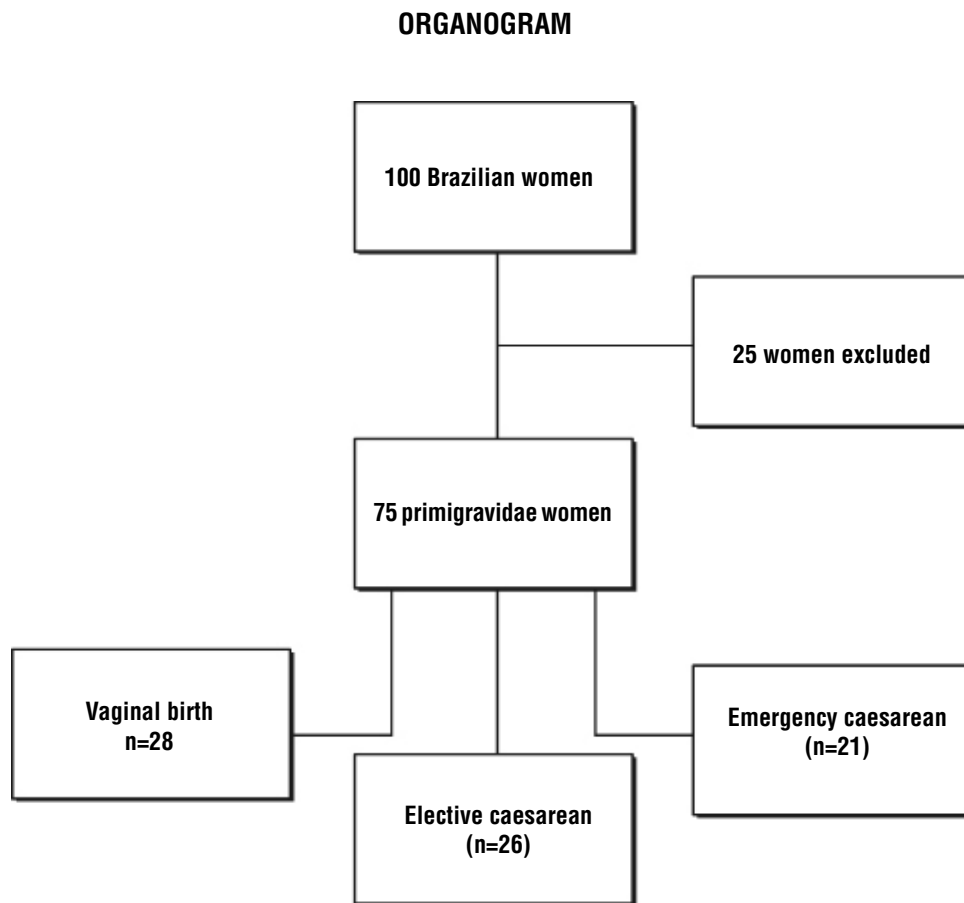
Twenty five pregnant women were excluded from the study due to high-risk pregnancy; urinary tract infection; urinary incontinence and/

or prior pelvic organ prolapse; myopathies and diseases with known modification of collagen; neurological abnormalities; metabolic disorders (diabetes) or chronic lung diseases; history of abdomino-pelvic surgery; premature labor; and those who did not attend the two evaluations provided or who underwent instrumental vaginal delivery with forceps (Figure-1).

Patients were evaluated during the last trimester of pregnancy and reassessed 45 days (\pm 10 days) after delivery, through culturally adapted and validated versions of the questionnaires: International Consultation on Incontinence Questionnaire - Urinary Incontinence Short Form (ICIQ-UI SF) and the International Consultation on Incontinence Overactive Bladder Questionnaire (ICIQ-OAB) (19,20).

The ICIQ-UI SF has four questions that assess the frequency, volume and impact of urinary symptoms in the quality of life, and its score is calculated by the sum of the first three questions. The score can vary from zero to twenty-one points, the greater the total value the higher the commitment. The questionnaire also assesses the causes and situations that trigger the loss of urine (19). The ICIQ-OAB consists of four questions that assess overactive bladder symptoms - frequency, urgency, nocturia, with or without urge incontinence - with scores varying from zero to sixteen points. If the symptoms are more severe, the score increases (20). Both questionnaires are part of the ICIQ project modular questionnaire (www.iciq.net). The ICIQ provides clarity over the selection of questionnaires by recommending only those with evidence of high quality and robust psychometric validation including validity, reliability and sensitivity to change. This assurance provides the user confidence in the results obtained, which is important in clinical practice and research where treatment decisions or trial outcomes depend on these evidences. Increasing awareness of the ICIQ aims to promote increased use of standardized questionnaires, thereby facilitating communication between clinicians and researchers enabling more widespread comparisons between different treatments and patient groups worldwide (21,22).

Subsequently, patients were classified into three groups according to the delivery method

Figure 1 – Organogram representing the study population.

based on the patient's obstetrician's indication: (VD) vaginal delivery with right mediolateral episiotomy ($n = 28$); (CS) elective caesarean section ($n = 26$); (EC) emergency caesarean section (caesarean section during labor) i.e., women who went into labor, but for lack of progress, were submitted to a caesarean section ($n = 21$).

In each group, the following variables were analyzed: (a) demographic data (age, declared skin color, education level, marital status, occupation and family income); (b) puerperal and pregnancy history (change in weight during pregnancy, newborn's weight, length of labor, return to previous weight).

The statistical methods used were Fisher Exact Test and Analysis of Variance (ANOVA) with Rank Transformation.

RESULTS

It was shown that frequency, urgency, nocturia and urge incontinence, which have been observed during pregnancy, decreased significantly in the postpartum period, regardless of the delivery method ($p = 0.0001$). However, symptoms related to urinary loss due to stress persisted after vaginal delivery ($p = 0.0001$), compared to the two types of caesarean section (Table-1).

The demographic information as well as pregnancy and postpartum history were also analyzed, as shown in the Tables 2 and 3. There was a lower educational level ($p = 0.0079$) and family income ($p = 0.0203$) in the group that underwent vaginal delivery compared to caesarean groups.

Table 1 - Puerperal and gestational urinary symptoms evaluated through ICIQ-OAB and ICIQ-UI SF.

	Vaginal birth (n = 28)	Elective caesarean (n = 26)	Emergency caesarean (n = 21)	P-value		
				Groups	Time	Interaction group versus time
ICIQ-OAB Gestational**						
Mean	5.68	6.87	4.86			
S.D	3.75	3.55	2.73			
Minimum	0.00	0.00	0.00			
Medium	5.00	7.00	5.00			
Max	14.00	13.00	9.00			
ICIQ-OAB Puerperal**				0.1375	0.0001	0.1818
Mean	2.40	1.87	1.24			
S.D	3.00	2.30	1.95			
Minimum	0.00	0.00	0.00			
Medium	1.00	1.00	0.00			
Max	10.00	8.00	6.00			
ICIQ-UI SF Gestational**						
Mean	5.00	7.91	3.00			
S.D	4.65	6.52	4.39			
Minimum	0.00	0.00	0.00			
Medium	5.00	8.50	0.00			
Max	18.00	20.00	17.00			
ICIQ-UI SF Puerperal**				0.0801	0.0001	0.0186
Mean	3.81	1.05	0.67			
S.D	5.39	3.00	1.71			
Minimum	0.00	0.00	0.00			
Medium	0.00	0.00	0.00			
Max	15.00	12.00	7.00			

** ANOVA with ranks

ICIQ-OAB: score variation possibility-0 - 16 points, being as higher the score as severe the UI.**ICIQ-UI SF:** score variation possibility 0 - 21 points, being as higher the score as severe the UI.**P-value:** the table represents comparison among the studied groups (vaginal birth, elective and emergency caesarean); the comparison between the evaluation time (gestational evaluation and puerperal reevaluation); and interaction between both (groups versus time).**S.D:** Standard Deviation

Table 2 - Demographic characteristics.

	Vaginal birth (n = 28)	Elective caesa- rean (n = 26)	Emergency cae- sarean (n = 21)	P-value
Age*(years)				
Mean	21.50	27.27	23.24	
S.D	5.51	5.99	5.71	0.0014
Skin color**				
White	78.57	76.92	76.19	
Black	7.14	19.23	4.76	0.3023
Other	14.29	3.85	19.05	
Educational level**				
Illiterate	0.00	0.00	9.52	
Elementary school	25.00	19.23	28.57	0.0079
High School	71.43	42.31	38.10	
Graduate School	3.57	38.46	23.81	
Marital Status**				
Single	42.86	19.23	42.86	
Married	53.57	80.77	57.14	0.1088
Other	3.57	0.00	0.00	
Occupation**				
Unemployed	64.29	34.62	38.10	
Employed	35.71	57.69	57.14	0.1129
Other	0.00	7.69	4.76	
Family income**				
1 to 2 minimum wages	46.43	34.62	38.10	
3 to 4 minimum wages	50.00	23.08	38.10	0.0203
Above 4 minimum wages	3.57	42.30	23.81	

* ANOVA with ranks

**Fischer's Exact Test

S.D: Standard Deviation

Table 3 - Gestational and puerperal history.

	Vaginal birth (n = 28)	Elective caesarean (n = 26)	Emergency caesarean (n = 21)	P-value
Weight increase during pregnancy** (KG)				
Mean	12.97	12.12	10.28	
S.D	5.89	4.21	5.89	0.1970
Newborn weight** (KG)				
Mean	3239.04	3337.65	3085.24	
S.D	475.51	332.19	435.75	0.1716
Labor duration** (Hours)				
Mean	11.05		9.86	0.2652
S.D	7.31	---	9.46	
Return to pre pregnancy weight** (weight %)				
Mean	10.65	11.77	8.24	
S.D	12.89	10.91	9.64	0.5559
Return to daily life activity* (%)				
Early puerperium	35.71	42.31	57.14	
Late Puerperium	64.29	57.69	42.86	0.3194

* Fischer's Exact Test

** ANOVA with ranks

S.D: Standard Deviation

The group that underwent vaginal delivery had a mean age significantly lower than the elective caesarean section group ($p = 0.0014$), and there was no statistical difference from the emergency caesarean group.

No significant differences were found among groups in the other examined criteria, like body mass index, newborn's weight, weight gain during pregnancy and labor time.

DISCUSSION

There is evidence in the literature that pelvic floor disorders are at least partially determined by changes resulting from pregnancy and childbirth, and can be responsible for the onset of LUTS (15,16).

During pregnancy, urinary complaints tend to widen with increasing gestational age (8,23,24).

Symptoms of overactive bladder - frequency, urgency, nocturia, with or without urge incontinence - are common in the third trimester and may be related to the pressure exerted by the fetal head on the bladder (17,24-26).

However, this study demonstrated that such symptoms tend to decrease in the late postpartum period, regardless of the delivery method ($p = 0.0001$). Other authors confirm these findings, concluding that overactive bladder symptoms are more transient, compared to symptoms related to urinary loss due to stress (27-32).

Van Brummen et al. (2007) compared LUTS three months and one year after both vaginal delivery and caesarean section. Their results showed a lower prevalence of overactive bladder symptoms in the group submitted to caesarean section, but without any significant difference after one year of follow-up (25). Scarpa et al. (2008) assessed the frequency of LUTS three years after childbirth in women previously interviewed in the third trimester and concluded that pregnancy, rather than delivery, was associated with the appearance of stress urinary incontinence (SUI) and nocturia, while the urge incontinence was significantly higher after delivery. Most women stated further that the presence of SUI triggered social problems (26).

The high prevalence of urinary complaints during pregnancy are expected in obstetrical practice. In contrast, there is a growing concern about the persistence of these symptoms, such as complaints associated with defecation symptoms, sex and presence of genital prolapse (2,3,5,6,16,19,28,29).

Urinary incontinence is usually experienced by women during the first pregnancy (29). Thomason et al. (2007) found 51% of incontinence after six and nine months of delivery in primiparous women with a history of urinary leakage, compared to 60% of women without this history who developed urinary incontinence in the first pregnancy (33). A study of Brazilian women revealed the presence of LUTS in 63% of the 500 women who had been interviewed (8).

Wesner et al. (2009) (34) and Arrue et al. (2010) (11) observed that women who developed incontinence during pregnancy had a higher prevalence of incontinence six months after delivery, with

a risk of 3.2 (confidence interval = 95%; 2.2 to 4.7) for spontaneous vaginal delivery and 2.9 (confidence interval = 95%; 2.3 to 3.4) for elective caesarean delivery (34). Another study in our department showed that urinary symptoms remained present six months after delivery; however, women did not correlate them with a worsening of quality of life (35).

Thus, the UI after delivery may be transient or not, but its persistence may be considered a predictor for reduced quality of life of women later on (36-38).

This study showed that stress urinary incontinence symptoms persisted after vaginal delivery ($p = 0.0001$), compared to two types of caesarean section - elective caesarean delivery and emergency caesarean.

Other research confirms a higher prevalence of SUI after vaginal delivery. Ekström et al. (2008) reported that the relative risk of SUI nine months after vaginal delivery is 8.9%, while for urge incontinence is 7.3% (confidence interval = 95%). These authors confirm that the presence of SUI during pregnancy until the third month after birth is a predictor for the maintenance of the symptoms for longer periods (32).

According to the study by Rortveit et al. (2003) the risk of urinary incontinence is higher among women who had caesarean delivery than among nulliparous women and is even higher among women who had vaginal deliveries. However, these results should not be used to justify an increased rate of recommendation of caesarean sections (10).

There is no doubt that vaginal delivery is associated with urinary incontinence after childbirth and that the presence of UI during pregnancy can predispose to UI in the postpartum period (13). However, Huebner et al. (2010) related that showed that the effects of pregnancy on the pelvic floor are different from those triggered after vaginal delivery (12). Brown et al. (2010) studied the risk factors for UI in pregnancy, and concluded that there are probably different mechanisms on the onset of symptoms at each stage of life of women and on their recurrences. Thus, it is important to consider the interaction of the effects of pregnancy and delivery (9).

The body weight gain during pregnancy, multiparity, vaginal delivery, forceps delivery, epi-

dural anesthesia, newborn's weight above 3.5 kilograms, and prolonged labor are factors that must be considered when investigating urogy-necologic complaints (30). However, in several studies no significant relationship among them was observed (2).

Burgio et al. (2003) associated the UI in first six months and then one year after delivery with the presence of incontinence during pregnancy, vaginal delivery and use of forceps. However, these authors found no significant relationship between age, race, education, episiotomy and number of vaginal deliveries (39). In the present study, we assessed the demographic and obstetric history. There were no significant influences of the variables: weight gain during pregnancy; constipation; newborn's weight and duration of labor as well as the evolution of lower urinary tract symptoms.

Although no significant differences were observed among groups, patients who joined the vaginal delivery group had lower BMI than the others, demonstrating that increasing maternal weight did not influence the results.

Our findings also show that the group submitted to elective caesarean section showed age, education level and family income higher than other groups. Possibly, these findings are related to the mother's and obstetrician's preference of a more selective group of women. This characteristic of the studied subjects reflects the Brazilian reality, since it is observed that the higher the socioeconomic level, the greater the prevalence of elective cesarean section.

To Bruschini (2005) (40) and Leijonhufvud et al. (2011) (14) vaginal delivery is strongly associated with late need of SUI correction surgery. The maternal mortality reduction and the increase of life expectancy have contributed to highlight late sequelae related to childbirth. This probably justifies the preference for caesarean section in the occidental world (16,40).

Although many studies have demonstrated the protective effect of caesarean section (7,10,14,15,34), McLennan et al. (2006) (38) suggest that information regarding the route of delivery should be offered as a routine in obstetric consultations, emphasizing the real indications, contraindications, complications and sequelae, which would

help educate women about the risks, helping them choosing the method of delivery (14,38).

By comparing the two types of caesarean section - elective and emergency, no significant differences between the two groups were found. However, Chin et al. (2006) found a higher frequency of urinary incontinence in women submitted to vaginal delivery and emergency caesarean delivery compared to elective caesarean section (41).

Van Brummen et al. (2007), assessing the bladder symptoms after three months of postpartum, found a significant reduction in urgency and urge incontinence after caesarian section; however, no significant alterations were found a year after delivery. On the other hand, SUI was significantly higher in the vaginal delivery group, both after three and twelve months. The authors confirmed that SUI during pregnancy was a predictive factor of its persistence after delivery. The presence of SUI during pregnancy increased the risk of SUI by 18 times one year after delivery (25).

Thus, the concern with the prevention and treatment of urinary incontinence is imminent. The implementation of awareness and prevention programs must be emphasized (15,19,42,43).

CONCLUSIONS

Urgency, frequency and nocturia triggered during pregnancy trend to disappear in the late postpartum period, regardless of the delivery method, but the symptoms related to urinary loss due to stress tend to persist in late postpartum period after vaginal delivery.

ABBREVIATIONS

BMI: body mass index;
 ICIQ-OAB: International Consultation on Incontinence Questionnaire Overactive Bladder;
 ICIQ-UI SF: International Consultation on Incontinence Questionnaire - Urinary Incontinence Short Form;
 ICS: International Continence Society;
 LUTS: lower urinary tract symptoms;
 SD: standard deviation;
 SUI: stress urinary incontinence;
 UI: urinary incontinence.

CONFLICT OF INTEREST

None declared.

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Impact of castration with or without alpha-tocopherol supplementation on the urethral sphincter of rats

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ABSTRACT

Objective: To analyze the impact of low levels of testosterone induced by orchiectomy and the effect of alpha-tocopherol supplementation on oxidative stress in the urethral sphincter.

Materials and Methods: Forty male Wistar rats weighing 250-300g were divided into four groups with 10 each: Sham group; Orchiectomy group: bilateral orchiectomy; Orchiectomy-pre-Tocopherol group: bilateral orchiectomy preceded by alpha-tocopherol supplementation for four weeks; Orchiectomy-full-Tocopherol group: bilateral orchiectomy with alpha-tocopherol supplementation for four weeks preceding the procedure and for eight weeks afterwards. At the protocol end, animals were euthanized and had the sphincter analyzed stereologically focusing on collagen and muscle fibers percentage. Oxidative stress levels were determined using 8-epi-PGF2.

Results: The 8-epi-PGF2 levels were statistically higher ($p < 0.0003$) in the Orchiectomy group compared to others groups while Sham and Orchiectomy-full-Tocopherol groups presented statistically similar values ($p = 0.52$). Collagen volumetric densities were significantly lower in Sham and Orchiectomy-full-Tocopherol groups ($p < 0.022$). Sham group presented statistically greater muscle fiber percent.

Conclusion: Castration caused oxidative stress in the urethral sphincter complex, with increased collagen deposition. Alpha-tocopherol had a protective effect and its supplementation for twelve weeks provided the greatest protection.

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INTRODUCTION

The urethral sphincter complex is subject to degeneration in the same way as muscles are. While little is known about the physiopathological basis of sphincter dysfunction at the molecular level (1), urinary incontinence physiopathology is frequently described in terms of the damage that occurs at the cell and/or tissue level. Experi-

mental studies on the levator ani muscle in rats have shown that orchiectomy leads to incapacity of the satellite cells to respond mitotically, with consequent muscle atrophy (2).

The skeletal musculature diminishes with age by means of reduction in the number of muscle fibers and atrophy among the remaining fibers, through mechanisms that have still not been completely identified (3,1). Among the

mechanisms responsible for such atrophy are proteolytic routes and neurological and hormonal changes, such as decrease in the serum levels of growth hormone and testosterone (4-6).

Diabetic animals presented reductions in sphincter pressures associated with increased oxidative stress on the anal sphincter musculature, with a return to normality after administering an antioxidant agent (7).

The present study evaluated the changes on the urethral sphincter apparatus resulting from low levels of sexual hormones and the action of alpha-tocopherol supplementation on the oxidative stress induced by orchiectomy.

MATERIALS AND METHODS

Forty male Wistar rats of reproductive age (three months old), weighing 250-300g were kept in a controlled environment (25 ± 2 °C) with exposure to light for 12 hours a day, water available ad libitum and Labina® animal chow (Purina®).

The study was done according to the guidelines of the Brazilian College for Animal Experimentation (COBEA) under approval of the Institutional Committee for Ethics in Animal Research.

Groups (10 animals each one) were formed as follows: Sham group - underwent opening and closing the scrotal sac; Orchiectomy group - underwent bilateral orchiectomy; Orchiectomy-pre-Tocopherol group - underwent bilateral orchiectomy preceded by alpha-tocopherol supplementation for four weeks; Orchiectomy-full-Tocopherol group - underwent bilateral orchiectomy with alpha-tocopherol supplementation for four weeks preceding the procedure and for eight weeks afterwards.

Alpha-tocopherol was administered dissolved in 0.1 mL of sesame oil (Galênica, SP) at a dosage of 1000 UI/kg/week intramuscularly into the animal's dorsum using a fine insulin-type needle.

At the end of the protocol, all the animals were anesthetized by means of ether inhalation, and venous blood was collected from the dorsal vein of the tail, in order to assay the 8-epi-

PGF2 and testosterone levels. They were then euthanized through pneumotorax and CO₂ narcosis and resection of the urethral sphincter was performed. The blood samples were stored at -80 °C until analysis, using tubes containing 0.5g/L of butyrate hydroxytoluene as a free radical chelating agent.

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

Total serum testosterone was assayed by means of competitive radioimmunoassaying (testosterone direct radioimmunoassay kit, Immunotech, Brazil; cat # 1119).

The concentration of 8-epi-PGF2 was measured using a commercial enzymatic immunoassaying kit (Cayman Chemical, USA). The 8-epi-PGF2 conjugated with antibody was detected by means of a reaction with 5,5-dithio-bis (2-nitrobenzoic acid)-DTNB (absorbed at 405 nm), which was hydrolyzed by the acetylcholinesterase of the conjugate. The detection limits were 6 to 500 pg/mL.

Stereology was the method chosen for morphometrically evaluating the muscle and collagen fibers of the urethral sphincter. The fiber analysis was performed using preparations from seven-micrometer thin sections (8).

The modified picrosirius red staining technique was used. The slides were analyzed via optical microscopy at a magnification of 40x. Ten fields per slide and ten slides per animal were evaluated. The volumetric density of the collagen fibers and muscle fibers was analyzed by overlaying the M-42 grid system on the computed morphological image of the slides. The volumetric density is the relative density taken up by fibers in the tissue under examination. The stereological method quantitatively determines the parameters of the anatomical structural base on the two-dimensional thin sections, in three dimensions (9).

The data obtained were analyzed using the SPSS 12.0 statistics software (SPSS Inc., USA) and were expressed as means \pm standard

deviation. To validate the results, analysis of variance (multivariate analysis) and the nonparametric Newman-Keuls test for multiple comparisons and the nonparametric Kruskal-Wallis test, with the Bonferroni correction test were used to assess the differences between the independent samples. The criterion for significance was taken to be two-sided $p < 0.05$.

RESULTS

The alpha-tocopherol concentrations were 0.61 ng/mg of body weight in Sham group; 0.69 ng/mg in Orchiectomy group; 1.73 ng/mg in Orchiectomy-pre-Tocopherol group; and 1.83 ng/mg in Orchiectomy-full-Tocopherol group. The comparative analysis showed that the values in both tocopherol-supplemented groups were statistically greater ($p < 0.05$).

The serum testosterone concentrations immediately before sacrifice in the orchiectomized groups with and without alpha-tocopherol replacement were less than 20 pg/mL (sensitivity limit for the method applied). This demonstrated that there was a significant hormone deficit at

the time of sacrificing these animals, except for Sham group.

Analysis of the 8-epi-PGF2 levels demonstrated statistically higher values ($p < 0.0003$) in Orchiectomy group (5.1 pg/mL), in relation to Sham group (2.3 pg/mL), Orchiectomy-pre-Tocopherol group (2.4 pg/mL) and Orchiectomy-full-Tocopherol group (2.6 pg/mL).

Figure-1 shows picosirius stained external urethral sphincter highlighting collagen fibers distributed among muscle fibers.

Table-1 and Figure-2 demonstrate the volumetric density of the collagen fibers in the external urethral sphincter, in the four studied groups. It was observed that Sham and Orchiectomy-full-Tocopherol groups presented statistically similar values ($p = 0.52$). Orchiectomy and Orchiectomy-pre-Tocopherol groups presented volumetric densities that were greater than those of Sham and Orchiectomy-full-Tocopherol groups ($p < 0.022$). When analyzing Orchiectomy and Orchiectomy-pre-Tocopherol groups, it was also observed that the first presented a greater volumetric concentration of collagen fibers compared to the second ($p = 0.024$).

Figure 1 - Picosirius stained external urethral sphincter. Arrows showing collagen fibers distributed between muscle fibers (40X). A: Sham group; B: Orchiectomy group; C: Orchiectomy-pre-Tocopherol group; D: Orchiectomy-full-Tocopherol group.

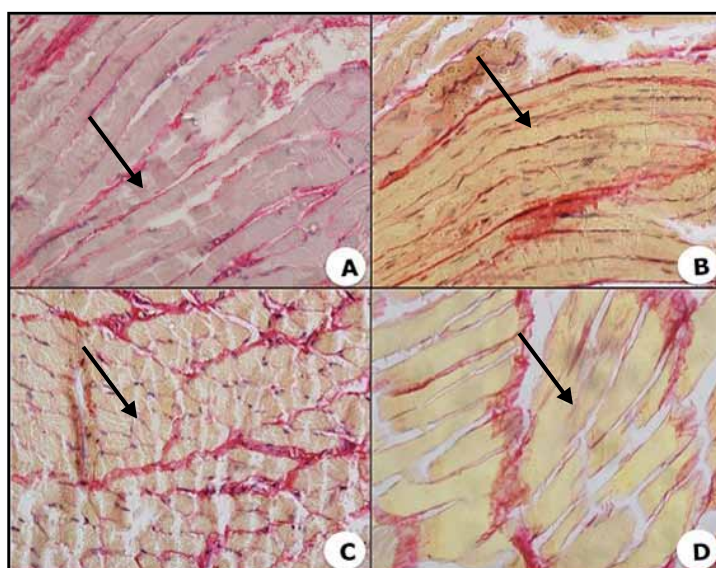


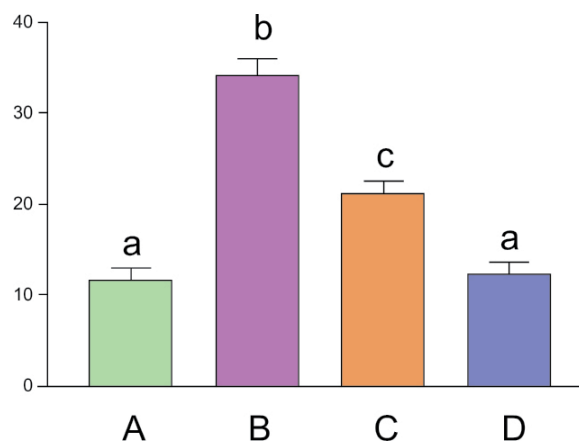
Table 1 - Descriptive analysis on collagen fibers of the external urethral sphincter.

Volumetric density of the collagen fibers of the external urinary sphincter of male rats (Vv) - %				
Values	Sham	Orchiectomy	Orchiectomy-pre-Tocopherol	Orchiectomy-full-Tocopherol
Minimum	9.05	29.32	17.85	9.29
25th percentile	10.35	31.98	20.22	11.60
Median	11.34	31.98	20.22	11.60
75th percentile	12.85	36.32	24.19	14.98
Maximum	13.10	39.10	26.52	16.52
Standard error	0.50	1.57	1.85	0.88
Mean (SD)	11.70 (± 1.43)	34.21(± 4.3)	21.12 (± 4.00)	12.23 (± 1.8)
95% CI	10.42 – 12.70	30.08 – 38.51	18.34 – 25.60	10.65 – 14.01

SD - standard deviation; **CI** - confidence interval; **N** = 10 in each group

Table-2 and Figure-3 demonstrate the volumetric density of the muscle fibers in the four groups. It can be observed that Sham group presented values that were statistically greater than those in the other groups ($p = 0.028$).

Figure 2 - External urethral sphincter collagen fibers density (n = 10 per group; different lowercase letters indicate statistically significant differences between the groups, $p < 0.05$). A: Sham group; B: Orchiectomy group; C: Orchiectomy-pre-Tocopherol group; D: Orchiectomy-full-Tocopherol group.



DISCUSSION

The process of aging and concurrent hormone decline is responsible for the decrease of the percent of muscle fibers of the rhabdosphincter, which are closely related to urinary sphincter culminating in urinary dysfunction (10-12). Urodynamic evaluation has demonstrated decreased maximum urethral pressure, urethral closing pressure and sphincter length in elderly men, compared with younger controls (10).

This study showed increased collagen fiber density in the animals with higher rates of oxidative stress. The animals in Orchiectomy and Orchiectomy-pre-tocopherol groups presented greater volumetric density for collagen fibers than Sham and Orchiectomy-full-tocopherol groups, ($p < 0.022$). Thus, it can be noted that alpha-tocopherol had a protective effect and avoided higher rates of oxidative stress, thereby resulting in lower deposition of collagen fibers in the sphincter tissue.

Comparing Orchiectomy and Orchiectomy-pre-tocopherol groups, a statistically significant difference in the volumetric density of

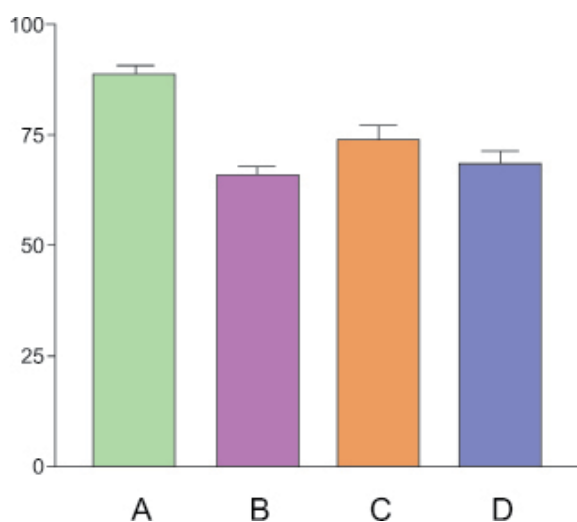
Table 2 - Descriptive analysis on muscle fibers of the external urethral sphincter.

Volumetric density of the muscle fibers of the external urinary sphincter of male rats (Vv) - %				
Values	Sham	Orchiectomy	Orchiectomy-pre-Tocopherol	Orchiectomy-full-Tocopherol
Minimum	83.48	59.20	67.86	65.28
25th percentile	84.22	61.48	68.21	67.92
Median	87.76	64.25	73.11	67.10
75th percentile	89.10	68.07	76.53	69.81
Maximum	91.05	71.89	79.16	71.00
Standard error	1.38	1.96	1.95	1.52
Mean (SD)	88.3(± 2.87)	65.79(± 5.88)	73.88(± 5.85)	68.77(± 2.50)
95% CI	85.14 - 90.17	60.14 - 70.92	68.50 - 78.49	66.15 - 70.15

SD – standard deviation; **CI** – confidence interval; **N** = 10 in each group

the collagen fibers was found ($p < 0.05$), from which it could be commented that supplementation with alpha-tocopherol before the orchiectomy procedure led to partially decreased effects on the collagen fibers.

Figure 3 - External urethral sphincter muscle fibers density (n = 10 per group; * $p < 0.05$ compared to the other groups). A: Sham group; B: Orchiectomy group; C: Orchiectomy-pre-Tocopherol group; D: Orchiectomy-full-Tocopherol group.



The animals in Sham group presented greater volumetric density of muscle fibers than other groups, thus allowing the hypothesis that castration led to decreased numbers of muscle fibers in the urethral sphincter complex and that supplementation with alpha-tocopherol was not effective for inhibiting the process.

The presence of notable decrease in the number of striated muscle cells in the urethral sphincter of elderly people have been reported due to apoptosis, possibly related to urinary dysfunction (12,13).

With decreasing volume of the striated sphincter musculature and blood vessels, increasing collagen deposition was found to occur in the region of the external urethral sphincter related to aging (14).

The presence of androgen receptors in motor neurons of the pudendum nerve that controlled the urethral sphincter of rats has been reported, thus suggesting that androgens have an influence on urinary continence control (11).

Orchiectomy in rats was found to increase lipid peroxidation in muscle tissue while, interestingly, testosterone supplementation in such

rats promoted reversal of this lipid peroxidation in the tissue (14,15).

There is a report in the literature showing protection against aging with the use of alpha-tocopherol in Sprague-Dawley rats (16). In that study, assaying for 8-epi-PGF2 was used to prove the presence of oxidative stress eight weeks after bilateral orchiectomy, since this is a marker for oxidative stress. This has been used in both animals and humans (17,18).

Antioxidant agents are capable of limiting or preventing the harmful effects of these radicals, although these protective mechanisms diminish during the process of aging (18,19).

Antioxidants have been demonstrated to be effective defenders from the well-known free radicals damage in a wide range of situations; in urology from those causing infertility to the systemic effect of antineoplastic drugs (20). Alpha-tocopherol is one of the most powerful and effective exogenous antioxidants, since it prevents peroxidation and avoids the effects of oxidative stress (21-23). We decided to use alpha-tocopherol because its pharmacokinetics have been well established, which helped determine the dose to be used in its oral or parenteral presentation (21).

Increased levels of 8-epi-PGF2 in cells of the urethral sphincter complex in rats subjected to orchiectomy without alpha-tocopherol replacement have been demonstrated in the present study, thereby translating in an increase in oxidative stress associated with hypoandrogenism. This difference was statistically significant when compared with the Sham group and the groups with alpha-tocopherol supplementation.

This study revisited the protective effect of alpha-tocopherol in reducing the oxidative stress levels and expanded its possible application to the level of the urethral sphincter complex, based on several previous studies that demonstrated the alpha-tocopherol beneficial action in relation to different urological diseases (23-25).

Nonetheless, further studies will be needed in order to achieve a better explanation for the influence of alpha-tocopherol on the concentration of sphincter collagen fibers.

Oxidative stress plays a considerable part in the degenerative process in the urethral

sphincter complex consequent to low testosterone levels. It was observed that supplementation with alpha-tocopherol following bilateral orchiectomy decreased the intensity of this process. It is suggested that low testosterone levels are associated with induction of free radical formation. These would at least partially be responsible for the degeneration of the sphincter muscle fibers through collagen fiber deposition.

Together with this hypothesis, it is also suggested that the use of antioxidants has a protective role in situations of hypogonadism, thereby avoiding or minimizing the effects of sphincter dysfunction arising from oxidative stress.

Considering the trend of progressively wider survival, world populace tends to increasingly get older, encouraging large population studies to prove possible clinical benefits of antioxidants as a protective management against aging and its related co-morbidities known to accelerate free radicals correlated degenerative process.

CONCLUSIONS

Bilateral orchiectomy in rats led to increased levels of oxidative stress, along with increased deposition of collagen fibers and decreased number of muscle fibers in the urinary sphincter complex of rats.

Supplementation with alpha-tocopherol was shown to be effective for decreasing the levels of 8-epi-PGF2 (a marker for oxidative stress) and it avoided increase in the volumetric density of collagen fibers. However, it did not influence the density of muscle fibers after the castration.

CONFLICT OF INTEREST

None declared.

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Mucinous Cystadenoma of the Appendix Mimicking Polycystic Kidney Disease

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A 70-year-old Japanese male with autosomal dominant polycystic kidney disease (ADPKD) and chronic renal failure presented with abdominal distention. His family history was remarkable for ADPKD in his mother, a brother and a sister. He had been receiving hemodialysis for 10 years and his general health had otherwise been maintained.

Computed tomography (CT) in the axial and coronal views showed both kidneys to have been almost completely replaced by multiple cysts and both occupied considerable space in the abdomen, consistent with ADPKD (Figure-1). It was also revealed that the largest cyst, which measured 160 mm in diameter, was located at the lower pole of the right kidney.

Renal transcatheter arterial embolization was performed for both kidneys to achieve volume reduction because abdominal distention usually results from progressive dilatation of renal cysts and is therefore one of the most common symptoms of ADPKD. Transcatheter arterial embolization is standard treatment for ADPKD at our hospital. Needle aspiration of the largest cyst was performed at the same time, and the cytological result was benign (Class II).

Two years later, the patient presented again with progressive abdominal distention and pain.

CT two years after embolization showed that only the largest cyst on the right side was refractory to treatment and had increased in size to 280 mm in diameter, while the other cysts had shrunk (Figure-2).

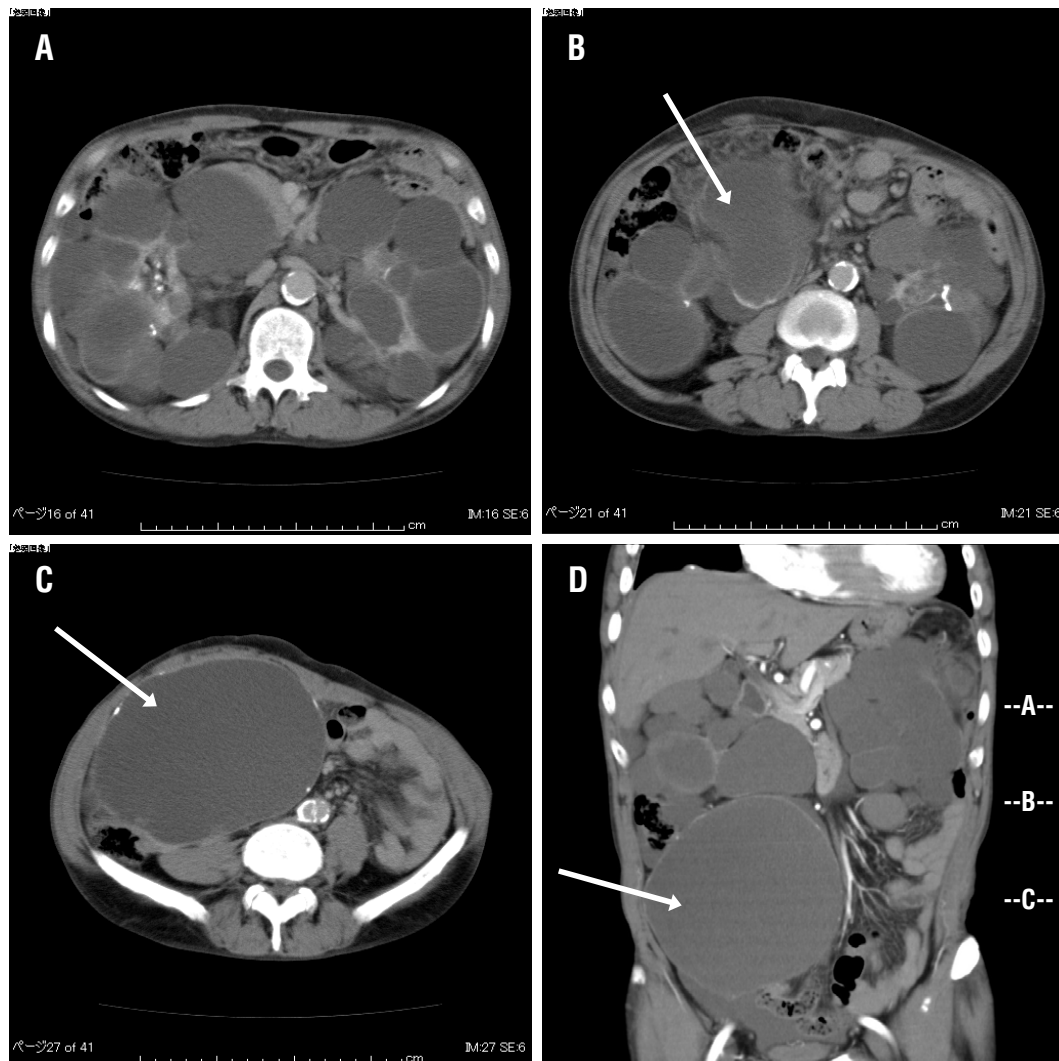
Laparotomy was subsequently performed. During exploration of the abdominal cavity, the largest cyst, which was believed to be one of the renal cysts, was identified as being completely separate from the aggregation of other cysts, instead being contiguous with the appendix. We performed an appendectomy together with resection of the largest cyst and right nephrectomy. Histological examination demonstrated mucinous cystadenoma of the appendix. The postoperative course was uneventful. The patient has been closely followed up for one year without relapse.

Mucinous cystadenoma is accounting for more than half of appendiceal mucocoeles, cystic neoplasms characterized by distension of the appendiceal lumen with mucus. As histological subtypes, appendiceal mucocoeles also include retention cysts, mucosal hyperplasia, and cystadenocarcinoma (1-4).

CT scan or other imaging studies can lead to a presumptive diagnosis of an appendiceal mucocoele. However, the differential diagnosis can be challenging and should include cysts of other origins, especially, as in our case, when CT scan or ultrasonography is controversial, but fails to demonstrate the continuity of the lesion with the cecum (5,6).

To our knowledge, this is one of the largest reported cases of mucinous cystadenoma of the appendix and also the first case associated with ADPKD. There is a case of appendiceal mucinous cystadenoma of the same size as this case, which caused bilateral hydronephrosis and

Figure 1 - Contrast-enhanced CT of the abdomen before embolization reveals bilateral polycystic kidneys, with the largest cyst (arrow) occupying the right lower quadrant of the abdomen. A-C) Axial views at different levels. D) Coronal view.



renal failure (7). There are several other reports of mucinous cystadenoma of the appendix misdiagnosed as a cyst of another origin, such as liver cyst, renal cyst and right adnexal cyst (8-12). This case demonstrates that mucinous cystadenoma of the appendix and other cystic lesions should be considered in the differential diagnosis of ADPKD.

ABBREVIATIONS

ADPKD: autosomal dominant polycystic kidney disease

CT: Computed tomography

Figure 2 - Contrast-enhanced CT of the abdomen two years after embolization demonstrates, in comparison with Figure 1, that only the largest cyst had increased in size while the others had regressed.



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Penile Fracture and Magnetic Resonance Imaging

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A thirty-three-year-old male presented to an outside emergency department with scrotal swelling and pain after intercourse. A scrotal ultrasound revealed hematoma, with no other abnormalities and the patient was discharged. He then presented to our institution where examination showed diffuse ecchymosis through the shaft of the penis, suprapubic region, and scrotum without a palpable cavernosal defect.

Magnetic resonance imaging (MRI) without contrast was obtained after the injection of 10 micrograms of intracavernosal alprostadil. The low signal tunica albuginea is easily demar-

cated compared to the high T2 and intermediate T1 signal of the corpora cavernosum (Figures 1-3) (1,2). Hematoma shows heterogeneous intermediate T1 and T2 signal (Figures 2 and 3) (1).

Penile fracture is rupture of the corpus cavernosum from blunt trauma to the erect penis (3,4). Typical presentation is a pop during intercourse, immediate detumescence with edema, hematoma and penile deformity (3,4). In atypical presentations, radiological studies may be useful to determine the diagnosis. MRI provides the ability to identify disruption of the corpus cavernosum due to excellent tissue contrast and

Figure 1 - Coronal T1 image shows disruption of the low intensity inferior left tunica albuginea and associated low signal corpus cavernosal hematoma at the base of the penis.

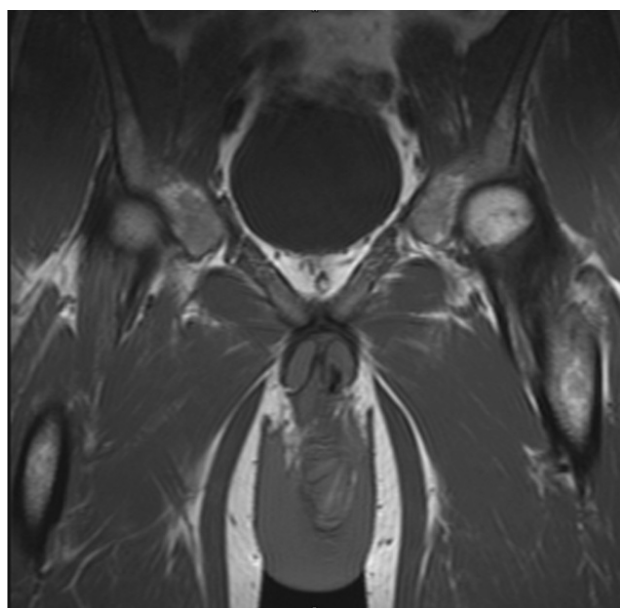
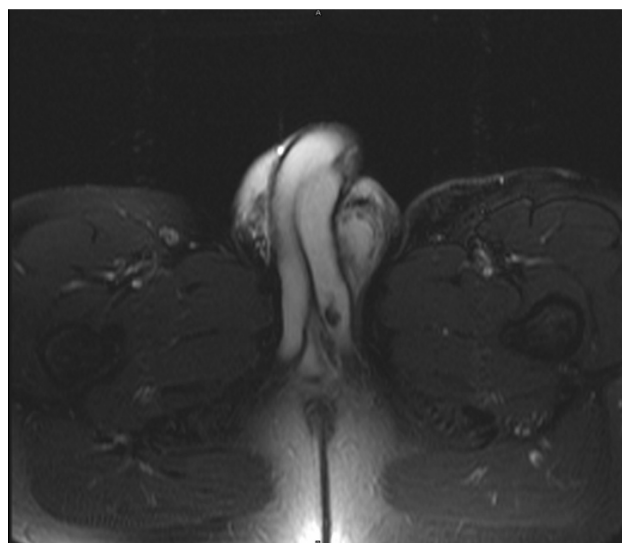


Figure 2 - The heterogeneous left scrotal hematoma and low signal left corpus cavernosum hematoma are more conspicuous on this T2 coronal image.



Figure 3 - Coronal T2 image shows disruption of the linear low signal left tunica albuginea at the base of the penis with associated heterogeneous hemorrhage medially.



visualization of soft tissue pathological processes (5). MRI is an adjunctive tool in the evaluation of atypical presentations of suspected penile fracture (3).

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Video-assisted left inguinal lymphadenectomy for penile cancer

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ABSTRACT

Background: Penile cancer is a rare disease, most commonly encountered in developing countries. It constitutes 0.4% of cancers in U.S. men and 2.1% in Brazil, with the highest prevalence in the North and Northeast regions. Inguinal lymph node metastasis of penile cancer occurs in 20 to 40% of patients and is an important predictor of cancer-specific mortality. The preferred diagnostic and therapeutic tool to assess the regional lymph nodes is a lymphadenectomy which can, in addition to establishing staging, offers curative potential.

Materials and Methods: A 44 years old man, previously to underwent a partial penectomy for penile cancer, whose pathology showed a moderately differentiated squamous cell carcinoma with neural and angiolymphatic invasion and negative surgical margins. The pathologic stage of the primary tumor was pT3NxMx. Following a one month course of oral antibiotics, the patient underwent a video-assisted bilateral inguinal lymphadenectomy. In the present video, we highlight the left video-assisted inguinal lymphadenectomy.

Results: Seventeen lymph nodes were dissected on the left side, two of them positive for cancer without extracapsular extension. On the right side, fourteen lymph nodes were dissected and one was positive for cancer with extracapsular extension, and the patient underwent based on these pathological findings a pelvic lymphadenectomy, which was similarly conducted using a video-assisted laparoscopic approach.

Conclusions: The conventional open lymphadenectomy has a morbidity that can approach 50% in the current series, despite on the refinements in technique. The video-assisted endoscopy is a recent technique aiming to decrease this inherent complication rate promoting a lymph node resection rate which may be equivalent to the open procedure. This video confirms its feasibility, reduced morbidity, and cancer control efficacy.

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EDITORIAL COMMENT

In this video, Britto et al. nicely depict that video-assisted laparoscopic inguinal lymph node dissection is a safe and effective diagnostic and therapeutic tool for penile cancer. This minimally invasive approach follows the same anatomical landmarks and surgical principles well established to impart a successful oncologic outcome and minimize morbidity within our penile cancer patient cohort. I am pleased to see the authors have selected a case ideally suited for such a minimally invasive approach namely a patient without palpable inguinal adenopathy and exhibiting high-risk features within the primary penile tumors (i.e. lymphovascular invasions, pT1G3, > 50% poorly differentiated tumor). The authors should be applauded for their technical expertise and high-quality depiction of a surgical approach which will likely be integrated in our established surgical armamentarium to evaluate and treat inguinal lymph nodes in appropriately selected

penile cancer patients. Herein lies the key, careful patient selection remains the primordial deterrent of whom should be offered such an approach at the present time. Video assisted laparoscopic inguinal lymph node dissection should not be offered to patients with bulky inguinal lymph node metastases (> 4 cm) and/or in the setting of neoadjuvant or salvage chemotherapy until prospective data can validate the oncologic equivalence of such a minimally invasive approach. These patients more than likely have a single therapeutic window of potential curability and failure to ensure complete locoregional tumor eradication by open superficial and deep inguinal lymph node dissection and likely pelvic lymph node dissection is doing a disservice to your patient although the intent of minimizing perioperative morbidity maybe noble. The art of surgery lies not in the discovery of novel surgical technologies or approaches but rather in applying them to appropriately selected cases in which the therapeutic outcomes can be optimized.

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**Books:**

- Sabiston DC: Textbook of Surgery. Philadelphia, WB Saunders. 1986; vol. 1, p. 25.

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