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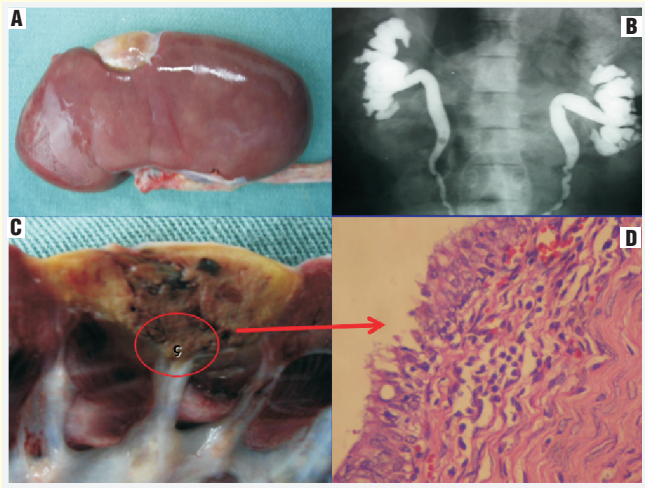

Figure 1: A) external aspect of the lesion; B) Ascending pyelogram without alterations; C) macroscopic aspect of the excretory system; D) Microscopy, 40X, urothelium.

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Editor Comment from the Video Section Editor

Dear readers,

I want to take this opportunity to wish all of you and your families very happy holidays and best wishes for the upcoming year. This year has been another very successful year for the International Brazilian Journal of Urology and the video section of our journal specifically. I would like to congratulate Dr. Sidney Glina editor-in-chief and his entire editorial team for the continued success of our journal as a highly regarded international peer reviewed journal in the scientific literature. This year we have selected again what are considered the top 3 videos published in the International Brazilian Journal of Urology. Nominations for best videos of the year have been made by editorial consultants of the video section in a completely blinded manner selecting what are considered the most original and best depicted surgical technique or approach. These selections were then tabulated and thereafter we attributed a 1st, 2nd, and 3rd prize for best videos of the year and here they are.

First prize: The group of Dr. Cesar Britto from Brazil are awarded the first prize "Retrocaval ureter repair" (http://www.brazjurol.com.br/vis.asp?code=2896) for their truly novel laparoendoscopic single site surgery to repairing a retrocaval ureter. This video very accurately depicts how single site minimally invasive surgery can be used to do such refined surgical procedures. The applications of single surgery are clearly evolving and as demonstrated in the present case, advanced laparoscopic skills can allow such cases to be undertaken with excellent functional and cosmetic results.

Second prize: The group of Cesar Britto is awarded as well the second prize "Hemi NX for horseshoe kidney" (http://www.brazjurol.com.br/vis.asp?code=2964) as a result of their excellent depiction of a pure laparoscopic technique in the management of large renal tumor in a horseshoe kidney. As most authors will realize, the surgical approach to a horseshoe kidney can be quite challenging in consequence to the aberrant anatomic location and vascular anatomy. The authors of this video have demonstrated how such challenging cases can be done using a pure laparoscopic approach with excellent treatment outcomes.

Third prize: The pediatric urology group from Dr Hubert Swana and colleagues from the University of South Florida have depicted a truly original transurethral neo-orifice (TUNO) approach to managing upper pole renal obstruction "TUNO abstract" (http://www.brazjurol.com.br/vis.asp?code=2897). The authors have delineated a very original technique to managing such issues in children using a "scalpless" technique i.e. natural orifice surgery is essentially what is being employed. As urologists, we often endoscopically manage bladder tumors, benign prostatic hyperplasia, and nephrolithiasis but in this case the indication of such endoscopic surgery is broadened while maintaining great results.

EDITOR'S COMMENT

I would like to conclude this newletter by once again thanking all of our submitters and readers of the International Brazilian Journal of Urology for their continued support and dedication to the video section. I believe the upcoming year will be a truly defining year for our section as we are working on developing an electronic platform for video submissions and likely will be launching other resources to promote accepted videos across several international media resources which would allow urologists across the world to comment on the novelty and benefits/drawbacks of specific surgical approaches depicted in these videos.

Very best wishes,

Philippe E. Spiess, MD

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Moffitt Cancer Center

REVIEW ARTICLE

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A review of continuous vs intermittent androgen deprivation therapy: Redefining the gold standard in the treatment of advanced prostate cancer. Myths, facts and new data on a "perpetual dispute"

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ABSTRACT

Objectives: To review the literature and present new data of continuous androgen deprivation therapy (ADT) vs intermittent androgen deprivation (IAD) as therapies for prostate cancer in terms of survival and quality of life and clarify practical issues in the use of IAD.
Materials and Methods: We conducted a systematic search on Medline and Embase databases using "prostatic neoplasm" and "intermittent androgen deprivation" as search terms. We reviewed meta-analyses, randomised controlled trials, reviews, clinical trials and practise guidelines written in English from 2000 and onwards until 01/04/2013. Ten randomized controlled trials were identified. Seven of them published extensive data and results randomizing 4675 patients to IAD versus CAD. Data from the other three randomized trials were limited.
Results: Over the last years studies confirmed that IAD is an effective alternative approach to hormonal deprivation providing simultaneously several potential benefits in terms of quality of life and cost effectiveness. Thus, in patients with non metastatic, advanced prostate cancer IAD could be used as standard treatment, while in metastatic prostate cancer IAD role still remains ambiguous.
Conclusions: Nowadays, revaluation of the gold standard of ADT in advanced prostate cancer appears essential. Recent data established that IAD should no longer be considered as investigational, since its effectiveness has been proven, especially in patients suffering from non-metastatic advanced prostate cancer.

ARTICLE INFO

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Prostatic Neoplasms; Therapeutics; Quality of Life; Androgens

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INTRODUCTION

Rationale

Prostate cancer continues to be the leading malignancy afflicting males in the Western world and the second leading cause of cancer death after lung cancer (1,2). Androgen deprivation therapy (ADT) is the mainstay of therapy for men with advanced prostate cancer. According to European Association of Urology (EAU) guidelines, GnRH analogues have become the standard of care in ADT (3). They offer a non surgical castration, having lower risk of cardiotoxicity compared to DES (4,5) and having the potential for reversi-

bility enabling the use of intermittent androgen deprivation (IAD).

Continuous AD (CAD) is based on the assumption that malignant prostate cells require androgen stimulation for growth and proliferation. Deprivation of the androgens will impede the growth of malignant prostate cells. Testosterone is the primary circulating androgen representing more than 90% of androgenic activity. Testosterone's production is controlled by a regulatory feedback between the hypothalamus-pituitary axis and the testes. GnRH is secreted in pulses from the hypothalamus and stimulates the release of luteinizing hormone (LH) from the pituitary gland. LH subsequently stimulates secretion of testosterone predominantly by the testes by binding to receptors. Testosterone (T) exerts a negative feedback on GnRH through androgen receptors in the hypothalamus and the pituitary gland. In prostate cell testosterone is converted into 5-a-dihydrotestosterone (DHT) via an enzyme called 5-a reductase. GnRH analogues provoke an initial surge in LH, T and DHT also known as flare phenomenon but overtime these hormones are suppressed through the negative feedback on hypothalamus.

Despite its undeniable effectiveness as a treatment, ADT is associated with multiple side effects including loss of libido, hot flushes, erectile dysfunction, cognitive dysfunction, decreased energy, osteoporosis, increased fracture risk, fatigue, a metabolic syndrome characterized by abdominal obesity and insulin resistance, gynecomastia, anemia and depression (6). The recognition and the evaluation of these side effects aroused the need for less patient exposure to ADT.

In 1986, in the pre prostate-specific antigen (PSA) era, Klotz et al. were the first to report the clinical use of IAD for advanced prostate cancer in 20 patients with symptomatic metastatic disease treated with DES. They withdrew DES once the patients demonstrated a good clinical response and treatment was reinitiated when patients became symptomatic again. That led to reduction of side effects, improved the quality of life of patients and demonstrated that treatment could be discontinued (7). The theoretical background of IAD was developed and proposed by Bruchovsky et al. In their preclinical studies they found that the re-exposure of prostate cancer cell to androgen could restore and increase the apoptotic potential of the androgen dependant cell that survived the AD (8,9). Using the Shionongi IAD tumor mouse model, they reported a prolongation of time to castration resistance up to three times with intermittent therapy compared with the continuous treatment models (10-12). Hence, apart from reducing side effects and ameliorating quality of life (QoL), IAD appears to be very appealing in the current era of cost-effective medicine, as it represents significant savings. Therefore IAD seems promising in many fronts: better QoL with reduced side effects, decreased treatment expenses and possible delayed onset of castration-resistant disease.

Indications

Several Phase II (13,14) and randomized Phase III (15-24) trials have been conducted to investigate IAD as an alternative treatment for advanced or metastatic prostate cancer, providing data that IAD is a treatment option in such patients. In 2012 EAU stated in its guidelines that "IAD is currently widely offered to patients with PCa in various clinical settings, and its status should no longer be regarded as investigational (LE: 2)" (25).

MATERIALS AND METHODS

Search Strategy

We performed a wide systematic literature research in the Medline and Embase databases. Prostate neoplasm and intermittent androgen deprivation were the search terms we used for specific study designs: meta-analysis, randomised controlled trials, reviews, clinical trials and practise guidelines. Our research was limited to studies published in English language from 2000 and onwards until 01/04/2013. Reference lists of the included articles were secondly hand-searched for studies that were not identified by the database search.

Study Selection Criteria

- Types of participants: the selected trials enrolled patients suffering from advanced (biochemical recurrence after definite therapy) or metastatic prostate cancer.

- Type of studies: we included randomized controlled trials comparing the use of intermittent to continuous androgen deprivation as therapy in advanced prostate cancer.
- Type of outcome measures: primary outcomes; overall survival, cancer-specific survival, time to progression and secondary outcomes; adverse effects, quality of life.

Assessment of selected studies and data collection process

Title and abstract from all the retrieved studies were evaluated. For every study that fulfilled the inclusion criteria, the complete article were retrieved, assessed and included or excluded according to the pre-mentioned criteria. Data were either collected directly from the text or calculated from the published information. A total of 121 references were identified and assessed. From them, 44 were selected and full-article was retrieved for evaluation. Seven randomized multicenter phase III trials were identified and included (18-24) in the review. These seven studies enrolled and randomized 4675 patients to continuous versus intermittent androgen deprivation therapy, publishing extensive data and results. Hence, another 3 randomized trials (15-17) were retrieved from the reference list of the retrieved articles but they lack of sufficient published data.

RESULTS

Phase II studies

In the early 1990's, the availability of reversible agents made it possible to utilize IAD, switching from treatment to non treatment periods. Furthermore, serum PSA allowed a more accurate monitoring of the disease's progression than clinical condition. As a result, PSA thresholds were used as trigger points for withdrawing and reinitiating therapy. In 1995 Goldenberg et al. were the first to define the trigger points for IAD using serum PSA measurements. In their study, therapy was withdrawn when serum PSA had reached a nadir below 4 ng/mL and reintroduced when serum PSA increased to value between 10 ng/mL and 20 ng/mL (13).

Several clinical Phase II trials have been published since then. These trials were single-institution with heterogeneous population of patients and with different stages of the disease. In 2007, Shaw et al. published a meta-analysis including 1446 patients participating in 10 phase II trials. This meta-analysis estimated the 5 year overall survival to be 90% in patients with localized disease, 86% in patients with biochemical recurrence and 68% in patients with metastatic disease. Three factors were identified as independently prognostic factors through these trials: the initial PSA, the PSA nadir after AD and the duration of the off-treatment period (14). Thus, anti-androgen monotherapy proved to be inferior as a treatment in metastatic disease. In this meta-analysis, the percentage of the off-treatment period was 39% while a low PSA nadir after AD appeared to be a good predictor of the overall survival, of the disease free survival and of the off-treatment period (14). In a recent systematic review, Abrahamsson concluded that IAD is at least as effective as combined androgen deprivation and has improved tolerability over CAD (26). Phase II trials concluded that IAD shows good acceptance and feasibility and that QoL is consistently improved during off-treatment periods, although most of the studies have not used validated QoL instruments. Nevertheless, the main question yet to be answered is the effect of IAD on overall survival. This question along with others such as the QoL output, which patients will benefit most and whether PSA can be used as a surrogate biomarker could only be answered in Phase III clinical trials.

Phase III studies

Mixed populations

Several randomized phase III trials have been conducted and their results have been reported or published. Studies reporting survival data concluded that there is no statistically important difference between the two groups in overall and cancer-specific survival (15,16). The majority of these trials were underpowered, enrolling a relatively small population of patients.

In the FinnProstate VII trial (19), 554 patients with locally advanced or metastatic cancer were enrolled in the study. The induction period of treatment was 6 months. Patients in whom PSA nadir value after the induction therapy was less than 10ng/mL, or by 50% or more reduced from the initial pre-treatment value, were randomized in two groups, one treated with continuous AD and the other with intermittent AD. In the IAD arm the PSA level to stop treatment was below 10ng/mL while the PSA trigger to reinitiate treatment was 20ng/mL. The duration of each treatment cycle in the IAD arm was at least 6 months. The median follow-up was 65 months. 392 (71%) of the patients died, 186 (68%) in the IAD arm and 206 (74%) in the continuous arm. Primary endpoints were progression free survival, PCa specific survival and overall survival. No statistically important differences were reported in any of the three specified endpoints between the two treatment arms. Median overall survival was 45.2 months in the IAD and 45.7 months in the CAD group (HR: 1.15; p = 0.17), median PCa specific survival was 45.2 months in tha IAD and 44.3 months in CAD arm (HR: 1.17; p = 0.29) while median time to progression was 34.5 months in the IAD and 30.2 months in the continuous group (HR: 1.08; p = 0.43).

Calais da Silva et al., (20) in the Southern European Uroncological Group (SEUG) study enrolled 766 patients with locally advanced or metastatic cancer who received a 3-month induction treatment with GnRH analogue and cyproterone acetate. Patients with PSA nadir value below 4ng/mL or a 80% reduction in the initial pre-treatment PSA were randomized in 2 groups (626 patients). In the IAD arm, in men with PSA nadir below 4ng/mL, PSA trigger for reinitiating treatment was 10ng/mL for symptomatic patients and 20ng/mL for asymptomatic patients. In men with 80% reduction of the pretreatment PSA, therapy reinitiated when PSA value rose to 20% of the nadir measurement. In the continuous arm complete androgen deprivation was deployed. There was no difference in overall survival [HR 0.99; CI 0.80 to 1.23] and disease progression [HR 0.81; p = 0.11] between the two groups. In the IAD arm, cancer deaths were higher whereas in the CAD arm cardiovascular deaths increased. Furthermore, cost was by far reduced in the IAD arm. Time to castration-resistance was non-significantly different between the two groups. Authors concluded that intermittent therapy should be considered for use in routine practice and that an on-treatment phase of only 3 months seems to be efficient. However, the majority of these trials enrolled heterogeneous populations such as patients with different disease stages.

Homogenous populations

Langenhuijsen et al. in a TULP trial (21) enrolled 193 patients suffering from metastatic prostate cancer. 96 were treated with continuous AD and 97 with IAD. They announced that patients treated with IAD with a PSA nadir < 0.2ng/mL had a statistically significant 2-year risk of progression compared to those treated with continuous AD (53% in the IAD arm, 31% in the continuous arm; p = 0.03).

The TAP22 trial (22) was a multicenter randomized study that enrolled 383 patients suffering from metastatic prostate cancer with PSA value > 20ng/mL. Patients were randomized after a 6 month induction ADT if the PSA value decreased below 4ng/mL. 173 patients were randomized and treated either with continuous or with intermittent AD. PSA trigger point to stop ADT was below 4ng/mL while therapy was reinitiated when PSA increased over 10ng/mL. Primary endpoint was overall survival, while secondary were progression-free survival, health related quality of life and safety criteria. No statistically significant differences arose between the treatment arms in terms of overall survival (p = 0.75) and progression-free survival (p = 0.74).

Two large randomized with homogeneous population and long-term follow-up trials have been performed. The National Cancer Institute of Canada NCT3653 (23) and the Southwest Oncology Group (SWOG) 9346 (24) trials were both designed as non-inferiority studies aiming to address the impact of ADT on overall and cause specific survival.

The NCT3653 study (23) was designed to investigate whether IAD treatment was inferior to CAD with respect to survival in patients with rising PSA after definite radiotherapy with no evidence of metastatic disease. The trial enrolled 1386 non metastatic patients with localized prostate cancer and PSA level higher than 3ng/mL more than a year after radiotherapy. In the IAD arm, patients were

treated with 8-month cycle of therapy of an LH-RH analogue combined with a non steroidal antiandrogen. At the end of the cycle treatment was withdrawn if the PSA level was below 4ng/mL. During the non-treatment period PSA was monitored every two months. The PSA trigger point for reinitiating therapy was determined at 10ng/mL. Median follow-up period was 6.9 years and primary endpoint was overall survival while secondary included QoL, duration of off-treatment and time to castration resistance. Overall median survival on the IAD group was 8.8 years with 268 deaths compared with 9.1 years and 256 deaths in the continuous group [HR: 1.02; 95% CI: 0.86 to 1.21]. Patients in the IAD arm were treated 27% of the time and 79% of them reached the trial entry threshold of testosterone recovery. In conclusion, NCT 3665 trial demonstrated an oncological equivalent efficiency of IAD compared to CAD in terms of overall and disease specific survival. In the intermittent arm prostate-cancer deaths were 9% increased but this difference was statistically non significant. As in SEUG trial, the increased prostate cancer deaths in the IAD group were scaled by increased non-prostate cancer deaths in the continuous arm. Although time to androgen independence was longer in the IAD group, the authors attributed this difference to a bias in the trial design. Only 35% of the patients in the IAD arm returned to the pre-treatment levels of serum testosterone within two years after completing the first treatment period.

The SWOG trial (24) was designed with similar structure aiming to determine if survival with IAD is not inferior to CAD with respect to survival, in patients with hormone sensitive metastatic disease, using a one sided test with an upper bound hazard ratio of 1.2. The trial enrolled and randomized 1535 patients with metastatic disease, either lymph node, visceral or bone metastases, and a PSA greater than 5ng/mL. Patients were treated with a 7 month induction therapy with goserelin and bicalutamide. If the PSA was lower than 4ng/mL patients were randomized in two groups. Treatment was reinitiated when PSA exceed 20ng/mL and withdrawn again after 7 months if PSA was less than 4ng/mL. If PSA exceeded 4ng/mL patients received CAD until progression. Primary endpoint was overall survival and secondary was QoL comparing three treatment specific symptoms (impotence, libido, energy) and physical-emotional function. Overall median survival in the CAD group was 5.8 years while 29% of the patients survived for at least 10 years. On the other hand, overall median survival in the IAD group was 5.1 years while 23% of the patients survived for at least 10 years. The authors concluded that although their results were statistically inconclusive, intermittent AD may compromise survival in men with metastatic prostate cancer, based on their pre-specified definition of survival comparability [HR: 1.09; 95% CI: 0.95 to 1.24].They could not rule out a 20% greater risk of death with intermittent rather than continuous therapy. In an additional sub-analysis, IAD proved to be non inferior in patients with extensive disease (any or a combination of long bones, ribs, skull or viscera) [HR for death with IAD: 1.02; 95% CI: 0.85 to 1.23] while in patients with minimal disease (metastasis confined to axial skeleton and pelvis or to lymph nodes) CAD proved to be statistically significantly superior [HR for death with IAD: 1.19; 95% CI: 0.98 to 1.43] (Table-1).

Testosterone: Castration levels in ADT and recovery levels in IAD

During ADT, breakthroughs in serum testosterone levels seem to have negative clinical consequences. In the last few years, a reassessment of the serum testosterone levels for clinical castration is consented and the testosterone threshold of castration is shifted from 50ng/dL to 20ng/dL (27,28).

Nevertheless, prospective trials analyzing the impact of serum testosterone levels are not available yet. Morote et al., in a retrospective trial attempted to determine the optimum testosterone levels in patients treated with ADT, in terms of survival (29). He enrolled 73 patients with non metastatic disease treated with a 3-month GnRH analogue who had at least 3 measurements of serum testosterone in follow-up > 1 year. Authors defined testosterone threshold at 20ng/mL and define androgen independent progression (AIP) as three consecutive rises from PSA nadir. They announced that testosterone breakthroughs are directly linked to PSA progression and that 32ng/dL is the testosterone threshold for a clinical impact. Patients whom all three testosterone measurements were

Table 1 - Population, cycling characteristics and oncologic results in randomized phase III trials of IAD.

Parameter	De Leval et al. (18)	Finn Prostate VII (19)	SEUG (20)	TULP (21)	TAP22 (22)	NCT3665 (23)	SWOG (24)
No patients	68	554	766	193	173	1386	1535
Tumor stage	Locally Advanced-Metastatic-After Rp	Locally advanced-metatstatic	Locally advanced-metastatic	Metastatic	Metastatic	Non metastatic-after RT	Metastatic
Inclusion PSA, ng/mL	Any value	Any value	4-100	Any Value	>20	> 3	> 5
Induction period, mo	6	6	3	6	6	8	7
PSA trigger point to stop IAD, ng/mL	< 4	< 10	< 4	< 4	< 4	< 4	< 4
PSA trigger point to restart IAD, ng/mL	> 10	> 20	> 10 for symptomatic, >20 for asymptomatic	> 10 non Metastatic > 20 for Metastatic	> 10	> 10	> 20
Follow-up, median, mo	30.8	65	50	31	44	84	108
Time to progression, mo	IAD: 28 CAD: 21	IAD: 34.5 CAD: 30.2 HR: 1.08; p = 0.43	HR: 0.81 in favor of CAD p = 0.11	IAD: 18.0 CAD: 24.1	IAD: 20.7 CAD: 15.1 p = 0.74	-	IAD: 16.6 CAD: 11.5 p = 0.17
Pca specific survival	-	IAD: 43% dead; 45.2 mo CAD: 47% dead; 44.3 mo HR: 1.17; p = 0.29	IAD: 23.6% dead CAD: 20.8% dead HR: 0.88	-	-	IAD:17.4% dead CAD:13.5% dead HR: 1.23; p = 0.13	IAD: 64% dead CAD:56% dead
Overall survival	-	IAD: 45.2 mo CAD: 45.7 mo HR: 1.15; p = 0.17	IAD: 54.1% dead CAD: 54.2% dead HR: 0.99; p = 0.84	-	IAD: 56.9% dead; 42.2 mo CAD: 54.2% dead; 52.0 mo (p = 0.75)	IAD: 38.8% dead 8.8 yr CAD: 36.8% dead; 9.1 yr HR: 1.02	IAD: 5.1 years CAD 5.8 years HR: 1.09

under 32ng/mL had a median AIP-free survival of 137 months compared to 88 months for those with any breakthrough above 32ng/mL.

Hence, apart from AIP-free survival, Perachino et al. (28) correlated testosterone levels of ADP to the risk of death. Their retrospective study involved 129 patients with metastatic bone-only disease. Patients were treated with a three-month depot goserelin. PSA and testosterone levels were measured every three months. A Cox regression model was utilized to identify independent predictors of cancer free survival. Authors announced that Gleason score ($P < 0.01$), 6-month PSA level ($P < 0.01$) and 6-month serum testosterone level ($P < 0.05$; HR: 1.32) were independent predictors of cancer free survival, correlated directly with the risk of death. They found that the initial pre-treatment testosterone level did not predict survival while baseline PSA ($P< 0.01$) and 6 month serum testosterone level ($P = 0.0286$) correlated directly with overall survival.

These recent data appear to be in total contrast with the rationale of IAD therapy. In IAD therapy recovery of testosterone in the off treatment periods is the primary cause of less adverse effects and improved QoL in patients. Testosterone rises in off treatment periods and yet according to the trials this rise has no adverse effect on overall survival. This still remains a great unsolved mystery and only hypothesis can be made regarding the answer. The level of testosterone recovery varies and appears to be affected by different factors such as baseline testosterone, duration of ADT treatment, patient age and ethnicity (30). Moreover testosterone recovery lags with successive cycles of therapy. Along with the testosterone recovery, duration of the off treatment period varies among the phase III trials ranging from 50% to 82% (15,20,23). Off treatment duration, along with testosterone recovery, is reduced with successful cycles of therapy, probably reflecting a gradual acquisition of androgen independence phenotype in the cancer cells.

Adverse effects and quality of life

Continuous ADT is associated with several early and long term adverse effects. Hot flushes and sexual dysfunction appears to be the most prevalent and common early side effects of ADT (31). Although the benefit in the QoL is not as profound as the expected, in some phase III trials QoL is ameliorated during the off treatment periods of IAD (15,16,21-23). In the TAP22 (22) study, patients treated with IAD experienced significantly fewer adverse effects (84.4%) than patients treated with continuous AD (93.6%) ($p = 0.042$). In the NCT3653 trial (23), QoL was ameliorated in the intermittent groups in terms of erectile function, libido ($P < 0.001$), hot flashes ($P < 0.001$), fatigue ($P = 0.07$), urinary symptoms ($P = 0.006$) and physical condition. In the SEUG trial (20), in the IAD arm there were fewer side effects such as hot flashes, gynecomastia and headaches reported and patients showed better sexual function and increased sexual activity ($P < 0.01$). The FinnProstate VII phase III trial (32) announced a study that was specifically focused on the affect of IAD on the adverse effects and on the QoL. Decreased incident of hot flushes ($p = 0.44$) was reported in the IAD arm but the result was statistically non-significant. An unexpected statistically significant result arose with respect to erectile dysfunction and depression, which were more common in the IAD group of treatment ($p < 0.05$). In the SWOG trial (24), IAD treatment was associated with better erectile function ($P < 0.001$) and mental health ($P = 0.003$) at month 3 but not thereafter. In conclusion, according to the reviewed data, IAD therapy is pursued by a decreased incidence of early adverse effects such as hot flushes and sexual dysfunction.

ADP has also been correlated with long-term adverse effects such as metabolic syndrome and bone mineral density decline. GnRH analogues increase abdominal weight and decrease muscle size (33) and insulin sensitivity (34). These metabolic changes seem to have an impact on cardiovascular health. Recently FDA mentioned an increased risk of diabetes, heart attack, sudden death and stroke with GnRH analogues (35). In the SEUG (20) trial an increased risk of dying from cardiovascular disease in the continuous arm was reported (cardiovascular deaths: 52 [16.7%] in the continuous arm, 41 [13.1%] in the IAD group). Hence, in the FinnProstate VII trial (32), no significant differences arose between the treatment arms in terms of cardiovascular adverse effects ($P = 0.59$) and cardiovascular-related mortality ($P = 0.38$). Thus, due to limited existing data, this issue remains ambiguous and blinded randomized trials are required. Bone

mineral density declines with ADT increasing the risk of fractures and osteoporosis. In continuous ADT biophosphonates should be considered in patients with fracture or BMD T scores of -2.5 or less (36). Spry et al. (37) reported that in 72 patients treated with IAD, BMD decrease was lagged during the off treatment intervals. Moreover, testosterone recovery levels were strongly correlated with BMD changes.

Furthermore, as far as QoL is concerned, only the SEUG (20) and the FinnProstate VII (32) trial reported some significant differences, mainly in terms of sexual function and activity limitation. A possible explanation for this might be that the off treatment periods are too short to alter significantly the QoL. Another cause might be that the questionnaires are not adapted to indicate so limited differences. It is also essential to mention that in all trials, except from the FinnProstate VII, QoL was assessed at fixed points regardless of the treatment phase, leading to the evaluation of patients both in on and off treatment phase in the IAD arm. This approach of evaluation may compromise the results in the in QoL differences between the two arms (Table-2).

IAD monitoring, trigger points and prognostic factors

The optimal thresholds of withdrawing and reinitiating AD are empirical. In almost all Phase III studies PSA levels rather than testosterone levels have been used as trigger points of resuming or withdrawing the AD. Most of the patients required 6 to 9 months to achieve PSA nadir. A prolonged induction cycle (more than a year) is considered as inappropriate for IAD, as testosterone recovery is unlikely. Most of Phase III trials required a PSA nadir value less than 4ng/mL after induction cycle before withdrawing AD. PSA threshold of resuming therapy varies among trials and was empirically set between 10 and 20ng/mL, depending on the disease stage. In patients treated with IAD, PSA and testosterone levels should be monitored at least every 3 months.

In patients treated with ADT, PSA nadir is a strong predictor of early progression (15,20,21). In IAD, PSA nadir is also predictive (21). According to Sciarra et al., in men treated with IAD a failure to achieve PSA nadir lower than 0.4ng/mL, after the first cycle, is associated with an increased risk of clinical progression and development of castration resistant disease (38). Thus, the duration of the off treatment period has also been proven to be predictive for the time to progression. According to two recent studies shorter off treatment period correlates to a 3.8 risk for death and a 2.9 risk for disease progression (38,39).

International Guidelines

EAU was the first international urological association that included IAD as an alternative therapy for advanced prostate cancer in its 2012 guidelines stating that IAD should no longer be considered as investigational (25). EAU stated in the guidelines that the possible benefit of CAD in disease progression is balanced by the increased toxicity and adverse effects, leading to absence of difference in overall survival. According to EAU, although the overall amelioration in the QoL is less than the expected, IAD is better tolerated having benefits for the sexual functioning.

In contrast to EAU, the American Urological Association (AUA) has not included IAD in its guidelines yet (40), while American Society of Clinical Oncology (ASCO) states that the existing data is still insufficient for the use of IAD outside clinical trials (41). The National Comprehensive Cancer Network (NCCN) guidelines state that IAD may reduce adverse effects without difference in overall survival in comparison to CAD. Nevertheless, IAD's long term efficacy has not been proven yet (42) (Table-3).

General recommendations and future perspectives

Based on the data reviewed, men with biochemical recurrence after definite therapy are the most appropriate candidates for IAD, since the majority of them have no bone metastasis. Based on the SWOG trial, patients suffering from hormone sensitive metastatic disease should be treated with continuous AD (24). As mentioned, PSA nadir value is a strong predictor of progression and can be used for the evaluation of the response to the treatment along with the clinical response. Thus, patients with undetected PSA value and after induction treatment with AD should be considered as suitable for IAD. A

Table 2 - Adverse effects and Quality of life in randomized phase III trials of IAD.

Parameters	De Leval et al. (18)	SEUG (20)	TULP (21)	TAP22 (22)	NCT3665 (23)	SWOG (24)	FinnProstate VII (32)
Hot Flushes, %	-	IAD: 19 CAD: 30	IAD: 50 CAD: 59	IAD: 60.4 CAD: 63.8	-	-	IAD: 47.1 CAD: 50.4
Sexual Dysfunction, %	-	Sexually active at mo 15 IAD: 28 CAD:10	IAD: 9 CAD: 10	-	-	-	IAD: 15.7 CAD: 7.9
Long-term Consequences, %	-	Cardiovascular deaths: IAD: 13.1 CAD: 16.7		-	-	-	Cardiovascular deaths: IAD: 12.8 CAD: 15.4
Quality of Life	-	IAD favored in terms of sexual function	No statistically significant differences	No statistically significant differences	No statistically significant differences	IAD favored in terms of mental health (p=0.003), erectile function (p < 0.001) and libido score (at 3 and 9 mo)	IAD favored in terms of sexual function, activity limitation and physical capacity

Table 3 - International guidelines for IAD.

Guideline	EAU 2013 (5)	AUA 2007 (35)	ASCO 2007 (36)	NCCN 2012 (37)
Recommendation	IAD is currently widely offered to patients with PCa in various clinical settings, and its status should no longer be regarded as investigational	IAD is not included yet	Existing data is still insufficient for the use of IAD outside clinical trials	IAD may reduce adverse effects without difference in survival in comparison to continuous ADT, but long term efficacy has not proven yet

failure in achieving a low PSA nadir should exclude patients from IAD. Apart from failure in low PSA nadir, men with bulky tumors, extended nodal and bone metastasis, severe pain, PSA above 100ng/mL and rapid PSA progression should not be considered as appropriate candidates for IAD (43,44).

According to the reviewed data, along with EAU guidelines, an induction cycle of 8 to 9 months is recommended. Patients with biochemical response should be treated with IAD. The threshold of accepted biochemical support is empirically defined as PSA nadir value below 4ng/mL. Close scrutiny is essential in the off treatment periods, since the treatment is withdrawn. Every 3 to 6 months patients should be screened with clinical examination, PSA levels and testosterone levels. PSA trigger points for reinitiating treatment are 10ng/mL for asymptomatic patients and 15ng/mL for symptomatic ones. The on treatment period should be at least 6 months and subsequent cycles continue until clinical evidence of development of castration resistant disease.

Furthermore, well organized large scale randomized Phase III trials are required in order to illuminate several crucial aspects in IAD treatment that remain obscure. Defining the clear response to the treatment, clarifying the PSA kinetics and setting the optimum PSA trigger points are essential in order to achieve better treatment results with IAD. Hence, elucidating more specific patients' selection criteria is substantial in order to maximize the benefits of IAD as treatment. Illustrating the effect and the influence that pre-treatment factors such as Gleason score, disease extension and pre--treatment PSA might have to the treatment results is also extremely important. Several agents have been studied aiming to prolong the duration of the off treatment period in IAD, including finasteride (45), pazopanib (46) COX-2 inhibitors (47) and thalidomide (48). The results of these primary studies are encouraging especially for the use of thalidomide and finasteride but larger scale, randomized and double-blinded trials are required to achieve definite answers.

CONCLUSIONS

IAD appears as a suitable therapy for many patients suffering from advanced and recurrent prostate cancer. Based on the results of Phase III trials, IAD is oncological non inferior to CAD in terms of overall and disease free survival in patients with biochemical recurrence. Thus, in patients with metastatic disease the proper treatment remains ambiguous and consensus is not reached yet. Hence, continuous AD seems to be a more secure treatment up until today. On the other hand, considering the additional benefits of IAD in the QoL and the cost reduction, IAD appears to be a very appealing alternative treatment for advanced and recurrent prostate cancer. Nevertheless, more multicenter well-designed randomized trials are necessary in the future, in order to clarify several vague aspects in the treatment of advanced prostate cancer.

ABBREVIATIONS

ADT = Androgen Deprivation Therapy
IAD = Intermittent androgen deprivation
DES = Diethylstilbestrol
GnRH = Gonadotropin-releasing hormone
EAU = European Association of Urology

PCa = Prostate cancer
LH = Luteinizing Hormone
T = Testosterone
DET = Dihydrotestosterone
PSA = Prostate specific antigen
QoL = Quality of Life
CAD = Continuous Androgen deprivation
SEUG = Southern European Uroncological Group
HR = Hazard Ratio
CI = Confidence Interval
SWOG = Southwest Oncology Group
AUA = American Urological Association
ASCO = American Society of Clincal Oncology
NCCN = National Comprehensive Cancer Network

REFERENCES

1. Center MM, Jemal A, Lortet-Tieulent J, Ward E, Ferlay J, Brawley O, et al.: International variation in prostate cancer incidence and mortality rates. Eur Urol. 2012; 61: 1079-92.
2. Baade PD, Youlden DR, Krnjacki LJ: International epidemiology of prostate cancer: geographical distribution and secular trends. Mol Nutr Food Res. 2009; 53: 171-84.
3. EAU 2013 online guidelines: Prostate cancer: page 82. Available at http://www.uroweb.org/gls/pdf/09_Prostate_Cancer_LR.pdf
4. Byar DP, Corle DK: Hormone therapy for prostate cancer: results of the Veterans Administration Cooperative Urological Research Groupstudies. NCI Monogr. 1988; 7: 165-70.
5. de Voogt HJ, Smith PH, Pavone-Macaluso M, de Pauw M, Suciu S: Cardiovascular side effects of diethylstilbestrol, cyproterone acetate, medroxyprogesterone acetate and estramustine phosphate used for the treatment of advanced prostatic cancer: results from European Organization for Research onTreatment of Cancer trials 30761 and 30762. J Urol. 1986; 135: 303-7.
6. Higano CS: Side effects of androgen deprivation therapy: monitoring and minimizing toxicity. Urology. 2003; 61: 32-8.
7. Klotz LH, Herr HW, Morse MJ, Whitmore WF Jr.: Intermittent endocrine therapy for advanced prostate cancer. Cancer. 1986; 58: 2546-50. Erratum in: Cancer 1987; 59: 43.
8. Bruchovsky N, Rennie PS, Coldman AJ, Goldenberg SL, To M, Lawson D: Effects of androgen withdrawal on the stem cell composition of the Shionogi carcinoma. Cancer Res. 1990; 50: 2275-82.
9. Rennie PS, Bruchovsky N, Akakura K, Goldenberg SL, Otal N, Akakura S, et al.: Effect of tumour progression on the androgenic regulation of the androgen receptor, TRPM-2 and YPT1 genes in the Shionogi carcinoma. J Steroid Biochem Mol Biol. 1994; 50: 31-40.
10. Akakura K, Bruchovsky N, Goldenberg SL, Rennie PS, Buckley AR, Sullivan LD: Effects of intermittent androgen suppression on androgen-dependent tumors. Apoptosis and serum prostate-specific antigen. Cancer. 1993; 71: 2782-90.
11. Bruchovsky N, Goldenberg SL, Akakura K, Rennie PS: Luteinizing hormone-releasing hormone agonists in prostate cancer. Elimination of flare reaction by pretreatment withcyproterone acetate and low-dose diethylstilbestrol. Cancer. 1993; 72: 1685-91.
12. Eggener SE, Stern JA, Jain PM, Oram S, Ai J, Cai X, et al.: Enhancement of intermittent androgen ablation by "off-cycle" maintenance with finasteride in LNCaP prostate cancerxenograft model. Prostate. 2006; 66: 495-502.
13. Goldenberg SL, Bruchovsky N, Gleave ME, Sullivan LD, Akakura K: Intermittent androgen suppression in the treatment of prostate cancer: a preliminary report. Urology. 1995; 45: 839-44; discussion 844-5.
14. Shaw GL, Wilson P, Cuzick J, Prowse DM, Goldenberg SL, Spry NA, et al.: International study into the use of intermittent hormone therapy in the treatment of carcinoma of the prostate: a meta-analysisof 1446 patients. BJU Int. 2007; 99: 1056-65.
15. Miller K, Steiner U, Lingnau A, Keilholz U, Witzsch U, Haider A, et al.: Randomised prospective study of intermittent versus continuous androgen suppression in advanced prostate cancer. J Clin Oncol 2007; 25: 18S [abstract 5105].
16. Tunn U, Eckhart O, Kienle E, Hillger H: Intermittent androgen deprivation in patients with psa-relapse after radical prostatectomy—first results of a randomised prospective phase-iii clinical trial (AUO study AP06/95). Eur Urol Suppl 2003; 2: 24.
17. Verhagen PC, Wissenburg LD, Wildhagen MF: Quality of life effects of intermittent and continuous hormonal therapy by cyproterone acetate (cpa) for metastatic prostate cancer. Eur Urol Suppl. 2008; 7: 206.
18. de Leval J, Boca P, Yousef E, Nicolas H, Jeukenne M, Seidel L, et al.: Intermittent versus continuous total androgen blockade in the treatment of patients with advanced hormone-naive prostate cancer: results of a prospective randomized multicenter trial. Clin Prostate Cancer. 2002; 1: 163-71.
19. Salonen AJ, Taari K, Ala-Opas M, Viitanen J, Lundstedt S, Tammela TL; et al.: The FinnProstate Study VII: intermittent versus continuous androgen deprivation in patients with advanced prostate cancer. J Urol. 2012; 187: 2074-81.
20. Calais da Silva FE, Bono AV, Whelan P, Brausi M, Marques Queimadelos A, Martin JA, Kirkali Z, et al.: Intermittent androgen deprivation for locally advanced and metastatic prostate cancer: results from a randomised phase 3 study of the South European Uroncological Group. Eur Urol. 2009; 55: 1269-77.

21. Langenhuijsen JF, Badhauser D, Schaaf B, Kiemeney LA, Witjes JA, Mulders PF: Continuous vs. intermittent androgen deprivation therapy for metastatic prostate cancer. Urol Oncol. 2013; 31: 549-56.
22. Mottet N, Van Damme J, Loulidi S, Russel C, Leitenberger A, Wolff JM; et al.: Intermittent hormonal therapy in the treatment of metastatic prostate cancer: a randomized trial. BJU Int. 2012; 110: 1262-9.
23. Crook JM, O'Callaghan CJ, Duncan G, Dearnaley DP, Higano CS, Horwitz EM, et al.: Intermittent androgen suppression for rising PSA level after radiotherapy. N Engl J Med. 2012; 367: 895-903. Erratum in: N Engl J Med. 2012; 367: 2262.
24. Hussain M, Tangen CM, Berry DL, Higano CS, Crawford ED, Liu G, et al.: Intermittent versus continuous androgen deprivation in prostate cancer. N Engl J Med. 2013; 368: 1314-25.
25. EAU 2013 online guidelines: Prostate cancer: page 90. Available at in http://www.uroweb.org/gls/pdf/09_Prostate_Cancer_LR.pdf
26. Abrahamsson PA: Potential benefits of intermittent androgen suppression therapy in the treatment of prostate cancer: a systematic review of the literature. Eur Urol. 2010; 57: 49-59.
27. Oefelein MG, Feng A, Scolieri MJ, Ricchiutti D, Resnick MI: Reassessment of the definition of castrate levels of testosterone: implications for clinical decision making. Urology. 2000; 56: 1021-4.
28. Perachino M, Cavalli V, Bravi F: Testosterone levels in patients with metastatic prostate cancer treated with luteinizing hormone-releasing hormone therapy:prognostic significance? BJU Int. 2010; 105: 648-51.
29. Morote J, Orsola A, Planas J, Trilla E, Raventós CX, Cecchini L, et al.: Redefining clinically significant castration levels in patients with prostate cancer receiving continuous androgendeprivation therapy J Urol. 2007; 178: 1290-5.
30. Gulley JL, Figg WD, Steinberg SM, Carter J, Sartor O, Higano CS, et al.: A prospective analysis of the time to normalization of serum androgens following 6 months of androgen deprivation therapyin patients on a randomized phase III clinical trial using limited hormonal therapy. J Urol. 2005; 173: 1567-71. Erratum in: J Urol. 2005; 174: 796. Sartor, Oliver [added]; Higano, Celestia S [added]; Petrylak, Daniel P [added] Chatta, Gerkamal [added].
31. Gruca D, Bacher P, Tunn U: Safety and tolerability of intermittent androgen deprivation therapy: a literature review. Int J Urol. 2012; 19: 614-25.
32. Salonen AJ, Taari K, Ala-Opas M, Viitanen J, Lundstedt S, Tammela TL; et al.: Advanced prostate cancer treated with intermittent or continuous androgen deprivation in the randomised FinnProstateStudy VII: quality of life and adverse effects. Eur Urol. 2013; 63: 111-20.
33. Smith MR, Finkelstein JS, McGovern FJ, Zietman AL, Fallon MA, Schoenfeld DA, et al.: Changes in body composition during androgen deprivation therapy for prostate cancer. J Clin Endocrinol Metab. 2002; 87: 599-603.
34. Smith MR, Lee H, Nathan DM: Insulin sensitivity during combined androgen blockade for prostate cancer. J Clin Endocrinol Metab. 2006; 91: 1305-8.
35. US Food and Drug Administration (FDA). FDA Drug Safety Communication 10-20-2010. Available at http://www.fda.gov/Drugs/DrugSafety/ucm229986.htm. Accessed January 17, 2012.
36. Sharifi N, Gulley JL, Dahut WL: An update on androgen deprivation therapy for prostate cancer. Endocr Relat Cancer. 2010; 17: R305-15.
37. Spry NA, Galvão DA, Davies R, La Bianca S, Joseph D, Davidson A, et al.: Long-term effects of intermittent androgen suppression on testosterone recovery and bone mineral density: results of a 33-month observational study. BJU Int. 2009; 104: 806-12.
38. Sciarra A, Cattarino S, Gentilucci A, Alfarone A, Innocenzi M, Gentile V, et al.: Predictors for response to intermittent androgen deprivation (IAD) in prostate cancer cases with biochemical progression after surgery. Urol Oncol. 2013; 31: 607-14.
39. Yu EY, Gulati R, Telesca D, Jiang P, Tam S, Russell KJ, et al.: Duration of first off-treatment interval is prognostic for time to castration resistance and death in men with biochemical relapse of prostate cancer treated on a prospective trial of intermittent androgen deprivation. J Clin Oncol. 2010; 28: 2668-73.
40. Prostate cancer. Guideline for the management of clinically localized prostate cancer: 2007 update. American Urological Association Web site. Available at http://www.auanet.org/content/guideline-and-quality-care/clinical-guidelines/main-reports/proscan07/content.pdf
41. Loblaw DA, Virgo KS, Nam R, Somerfield MR, Ben-Josef E, Mendelson DS, et al.: Initial hormonal management of androgen-sensitive metastatic, recurrent, or progressive prostate cancer: 2006 update of an American Society of Clinical Oncology practice guideline. J Clin Oncol. 2007; 25: 1596-605.
42. Mohler JL, Armstrong AJ, Bahnson RR, Boston B, Busby JE, D'Amico AV, et al.: Prostate cancer, Version 3.2012: featured updates to the NCCN guidelines. J Natl Compr Canc Netw. 2012; 10: 1081-7.
43. Salonen AJ, Viitanen J, Lundstedt S, Ala-Opas M, Taari K, Tammela TL; et al.: Finnish multicenter study comparing intermittent to continuous androgen deprivation for advanced prostate cancer:interim analysis of prognostic markers affecting initial response to androgen deprivation. J Urol. 2008; 180: 915-9; discussion 919-20.

44. Prapotnich D, Fizazi K, Escudier B, Mombet A, Cathala N, Vallancien G: A 10-year clinical experience with intermittent hormonal therapy for prostate cancer. Eur Urol. 2003; 43: 233-39; discussion 239-40.
45. Scholz MC, Jennrich RI, Strum SB, Johnson HJ, Guess BW, Lam RY: Intermittent use of testosterone inactivating pharmaceuticals using finasteride prolongs the time off period. J Urol. 2006; 175: 1673-8.
46. Ward JE, Karrison T, Chatta G, Hussain M, Shevrin D, Szmulewitz RZ, et al.: A randomized, phase II study of pazopanib in castrate-sensitive prostate cancer: a University of Chicago Phase IIConsortium/Department of Defense Prostate Cancer Clinical Trials Consortium study. Prostate Cancer Prostatic Dis. 2012; 15: 87-92.
47. Di Silverio F, Sciarra A, Gentile V: Etoricoxib and intermittent androgen deprivation therapy in patients with biochemical progression after radical prostatectomy. Urology. 2008; 71: 947-51.
48. Figg WD, Hussain MH, Gulley JL, Arlen PM, Aragon-Ching JB, Petrylak DP, et al.: A double-blind randomized crossover study of oral thalidomide versus placebo for androgen dependent prostate cancer treated with intermittent androgen ablation. J Urol. 2009; 181: 1104-13; discussion 1113.

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EDITORIAL COMMENT

In this edition of the Int Braz J Urol, the authors present a systematic review of the existing literature comparing continuous versus intermittent hormonal deprivation therapy, having identified nine prospective clinical trials on the subject. Although the existing data on the safety of intermittent androgen deprivation therapy in prostate cancer is growing, there still isn't a precise indication for its use (1,2). We know from the published data that it is a safe approach in men with biochemical recurrence and minimal metastatic disease, but there are concerns about its use in men with symptomatic, more advanced disease, as exemplified by the recent publication of the Hussain (SWOG) trial.

One concern in the trials in which serum testosterone was measured along with PSA in the follow-up of patients receiving intermittent androgen deprivation therapy is that testosterone levels take a long time to level up after the suspension of the androgen suppression, many times more than one year, and that would signify that in reality patients in "intermittent" hormone suppression actually are being suppressed for many months after stopping antiandrogenic therapy.

We believe that intermittent suppression is really no longer experimental, but testosterone levels, as well as PSA, bone density status, and clinical parameters must be measured as closely in the follow-up of these men as in the follow--up of those men receiving continuous androgen deprivation.

REFERENCES

1. Mottet N, Van Damme J, Loulidi S, Russel C, Leitenberger A, Wolff JM, et al.: Intermittent hormonal therapy in the treatment of metastatic prostate cancer: a randomized trial. BJU Int. 2012; 110: 1262-9.
2. Niraula S, Le LW, Tannock IF: Treatment of prostate cancer with intermittent versus continuous androgen deprivation: a systematic review of randomized trials. J Clin Oncol. 2013; 31: 2029-36.

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ORIGINAL ARTICLE

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Transperineal versus transrectal prostate biopsy for predicting the final laterality of prostate cancer: are they reliable enough to select patients for focal therapy? Results from a multicenter international study

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ABSTRACT

Objectives: To compare the concordance of prostate cancer (PCa) laterality between the extended transperineal (TP) or transrectal (TR) prostate biopsy (BP) and radical prostatectomy (RP) specimens. To identify predictors of laterality agreement between BP and RP.

Materials and Methods: Data from 533 consecutive patients with PCa (278 TP and 255 TR-diagnosed) treated with RP were analyzed. A 12-core technique was used for both TP and TR biopsies. Additional cores were obtained when necessary.

Results: Overall, the percentage of agreement of PCa laterality between BP and RP was 60% (K = 0.27, p < 0.001). However, the RP confirmation of unilaterality at BP was obtained in just 33% of the cases. Considering the concordance on bilaterality as the "target" of our analysis, the sensitivity and specificity were 54.3% and 98.2%, respectively, with TP and 47.5% and 92.5%, respectively with TR. Focusing on patients with unilaterality at biopsy, none of the evaluated preoperative variables (biopsy technique, age, total positive biopsy cores, PSA, prostate volume, Gleason score on biopsy) were able to predict RP bilaterality in the multivariate analyses.

Conclusions: Most of the patients with unilateral involvement at BP harbored bilateral PCa after RP. TR and TP biopsy showed no difference in their capacity to predict the concordance of tumor laterality at RP. None of the preoperative evaluated variables can predict the tumor laterality at RP. Using BP unilaterality to include patients in focal therapy (FT) protocols may hinder the oncologic efficacy of FT.

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INTRODUCTION

Recent pathologic studies from contemporary radical prostatectomy (RP) series have cited frequencies of unifocal prostate cancer (PCa) ranging from 10% to 44% and unilateral PCa ranging from 10% to 40% (1). In this context, focal therapy (both focal ablation and hemiablation) has progressively gained interest as a new method to control clinically localized PCa and has been defined as 'a type of treatment that aims to eradicate known cancer within the prostate and, at the same time, spare uninvolved prostatic tissue, with the aim of preserving genitourinary function' (2). Consequently, the accurate preoperative assessment of PCa is crucial and the exact spatial location of the tumor is essential to deliver treatment.

Transrectal ultrasound (TRUS)-guided biopsy of the prostate, performed through the transrectal (TR) or the transperineal (TP) approach is the current standard for the diagnosis of PCa and to define the localization of the disease. Several studies have investigated the degree of concordance in tumor laterality between prostate biopsy (BP) and RP (3-11) and its implication for focal therapy (3,4,6,8-11). However, in the majority of these studies the BP was performed through the transrectal approach; no data have been published on the transperineal approach, while the use of the perineal template has been recently described, although the procedure is more invasive and has health-care resource implications (2,12,13). Multiparametric MRI is receiving a great interest in the last years having the performance characteristics required to localize significant areas of prostate cancer, but the routine use of this technique can not be proposed for clinical (expertise of the radiologist) and health-care (cost and diffusion of MRI) implications (12).

The aim of the current study was to compare the concordance in the tumor laterality between the contemporary extended pattern prostate biopsy performed via a transperineal (TP) or transrectal (TR) approach and RP specimens in patients with PCa. Secondarily, we sought to identify predictors of laterality agreement between the BP and RP.

MATERIALS AND METHODS

Data from 533 consecutive patients with PCa diagnosed after an extended biopsy (278 via TP approach and 255 via TR approach) who underwent RP were retrospectively analyzed. Seven different urology departments participated in the study. Three of these departments performed the TR biopsies (Denver, CO, USA; Rome, Italy; Teramo, Italy), and the others performed the TP biopsies (Rome; Milan; Turin; Modena, Italy).

TRUS guidance was used in all cases. The biopsy procedure was performed with an 18G biopsy needle in both TR and TP cases under local anesthesia. A 12-core technique was used in the TP biopsies (6 cores per lobe), covering the peripheral zone of both lobes from the lateral to the paramedian area and from the base to the apex. A 12-core technique was also followed for the TR biopsies, using the same scheme (two cores from the lateral peripheral zone, two from the paramedian-lateral zone and two from the paramedian apical and basal zone per lobe).

For prostate volumes > 50 mL, two more peripheral cores (one from the lateral zone and one from the paramedian-lateral) were added. Additional cores from the anterior part or from the transition zone of the prostate were taken, depending on the clinical and ultrasound characteristics.

RP was performed according to the surgical principles of each center. Each center had a dedicated uropathologist examining the specimens (both of BP and RP). At all the participating centers, RP specimens were en-bloc formalin fixed and inked to delineate the surgical margins; a step-sectioned transversally at 2-4mm intervals technique was used to examine the specimen; an apical shaved--section, 2-4mm thick, was truncated perpendicular to the prostatic urethra and sub-sequentially sectioned as slices parallel to the prostatic urethra. Bladder neck was examined sampling portions of tissue at the junction of the prostatic capsule and bladder neck or by sampling the most proximal portion of the submitted specimen corresponding to the anatomical bladder neck. The 2002-TNM classification was used.

The pathologic evaluation of the BP cores reported the ratio of total positive cores/total cores, laterality and the Gleason sum and score. The pathologic evaluation of the RP specimens reported the laterality, Gleason sum and score, pathologic stage and margin status.

Statistical analysis

The comparison of the patients' baseline characteristics between the TR and TP groups was performed in agreement with the statistical distribution of the variables (chi squared for nominal variables, Mann-Whitney for ordinal variables, t-test after log--transformation for interval scales such as PSA and prostate volume).

The concordance between the tumor laterality as assessed by BP and by RP was measured using the typical diagnostic indexes: sensitivity, specificity and accuracy. The chi-squared test was used to compare the diagnostic performance of the two groups (TR and TP).

A multiple logistic-regression with bilaterality at RP as the binary dependent variable and the biopsy approach (TP vs. TR), age, total positive specimens, PSA, Gleason score on biopsy and prostate volume as the independent categorical or continuous covariates was performed on cases with unilaterality at biopsy. The interactions between group-total positive specimens, group-prostate volume and group--pathological stage were also analyzed.

RESULTS

The demographic and baseline characteristics are summarized in Table-1. As shown, the two groups of patients did not differ in terms of age, PSA levels, total biopsy cores, biopsy total Gleason score or prostate volume.

Overall, the percentage of correct classifications of the tumor laterality by biopsy with respect to prostatectomy was 60%. The Kappa measure of agreement was equal to 0.27. This value was statistically significant ($p < 0.001$, thus rejecting the null hypothesis of lack of agreement). Specifically, we found that the overall prostatectomy confirmation of unilaterality at biopsy occurred only in 103 out of 311 cases (33%). Thus, the total agreement of 60% and the significant Kappa measurement were primarily due to the cases classified as bilateral by biopsy and confirmed to be bilateral after RP (217/222 = 97.7%). Considering the concordance of bilaterality (between the BP and RP) as the "target" of our analysis, the overall biopsy sensitivity was low (51.1%), while the specificity was quite high (95.4%). When the BP suggested bilaterality, this was almost always true, while the BP indication of unilaterality was confirmed at RP in only 1 out of 3 cases.

We observed similar patterns when comparing TP and TR. The percentage of correct concordance in laterality was 62.9% (Kappa = 0.31, $p < 0.001$) with TP and 56.9% (Kappa = 0.23, $p < 0.001$) with TR. The sensitivity and specificity with respect to bilaterality were 54.3% and 98.2%, respectively, with TP and 47.5% and 92.5%, respectively, with TR (Table-2). Even if TP showed slightly higher diagnostic performance, TP was not significantly better than TR in terms of sensitivity ($p = 0.165$), specificity ($p = 0.157$) and accuracy ($p = 0.152$).

The occurrence of a concordance between biopsy and prostatectomy was not dependent on the number of total BP cores (OR = 0.99, $p = 0.717$).

Focusing our analysis on cases classified as unilateral at biopsy, we sought to identify potential predictors of misclassification. Overall, none of the evaluated preoperative variables (biopsy technique, age, total positive biopsy cores, PSA, prostate volume, Gleason score on biopsy) was able to predict the bilaterality of the final pathology on the multivariate analyses (Table-3). Moreover, age, total positive biopsy cores, PSA, prostate volume and Gleason score on biopsy were not independent predictors of bilateral PCa within the TP and TR groups when analyzed separately.

DISCUSSION

In recent years, due to the stage migration of PCa, there has been an increased interest in alternative strategies that offer the possibility of delaying, obviating or minimizing the impact of radical treatments (such as RP or radiotherapy), while maintaining the same oncologic long-term results. One such strategy is active surveillance with selective delayed intervention. Despite the advantages (avoiding overtreatment and complications in patients with low-risk PCa) and disadvantages (risk of progression and psychological and healthcare burdens) that have recently been addressed, definitive results from ongoing randomized clinical trials are required to assess whether active surveillance should be routinely implemented in clinical practice (14).

Table 1 - Baseline characteristics of the two groups (TP and TR) of patients.

	TP (n = 278)				TR (n = 255)				
	Mean	SD	95% CI inf	95% CI sup	Mean	SD	95% CI inf	95% CI sup	t-test(df), p-value
Age	64.6	5.8	63.9	65.3	64.0	6.2	63.2	64.7	1.192, p = 0.234
Total PSA (ng/ml)*	8.6	5.3	8.0	9.3	8.6	4.1	8.1	9.1	0.184, p = 0.854
Total biopsy cores	12.6	5.3	11.9	13.2	12.2	3.5	11.7	12.9	1.524, p = 0.342
Prostate volume (mL)*	38.9	29.7	35.3	42.4	42.3	16.7	40.3	44.5	1.738, p = 0.084
Biopsy Total Gleason score	6.6	0.9	6.5	6.7	6.4	0.7	6.3	6.5	1.826, p = 0.068
RP Total Gleason score	6.9	1.0	6.8	7.1	6.7	0.9	6.6	6.8	2.590, p = 0.010
pT stage									
T_2: n = 329 (61.7%)	183 (65.9%)				146 (57.3%)				
T_3: n = 193 (36.2%)	93 (33.3%)				100 (39.2%)				Chi-Square (2) = 7.82, p = 0.020
T_4: n = 11 (2.1%)	2 (0.7%)				9 (3.5%)				

Table 2 - Agreement between biopsy and prostatectomy in terms of tumor laterality.

Pathologic evaluation		Prostatectomy (n=533)	
	PCa laterality	Unilateral (n) (% concordance)	Bilateral (n) (% concordance)
TP (n = 278)	Unilateral (n = 156)	54 (34.6)	102 (65.4)
	Bilateral (n = 122)	1 (0.8)	121 (99.2)
TR (n = 255)	Unilateral (n = 155)	49 (31.6)	106 (68.4)
	Bilateral (n = 100)	4 (4)	96 (96)

Table 3 - Multivariate analysis.

	Odds Ratio	95% C.I.for OR		p-value
		Lower	Upper	
TR vs. TP	1.33	0.59	3.04	0.492
Age (years)	1.02	0.96	1.08	0.517
Total positive biopsy cores (n)	1.05	0.96	1.16	0.273
Total PSA (log)	1.01	0.58	1.78	0.964
Prostate volume (log)	1.04	0.47	2.29	0.926
Biopsy Gleason score 7 vs. <=6	0.63	0.30	1.32	0.219
Biopsy Gleason score >7 vs. <=6	0.82	0.19	3.61	0.797

Focal treatment may be an acceptable alternative for low-risk PCa, representing a compromise between the ambiguity of surveillance and the potential reduction of the quality of life of a radical treatment; aiming to destroy only the areas of PCa, focal therapy (FT) could deliver cancer control while simultaneously avoiding damage to the surrounding structures (15). This may reduce incontinence, impotence, and rectal toxicity.

Whether current hypotheses regarding the efficacy of FT suggest that it may be effective so long as it treats the "index lesion", even if other lesions remain untreated in the gland, the optimal patient selection criteria for FT are not known and are therefore not standardized. Ongoing FT trials for PCa include the laterality, Gleason score and tumor volume as criteria for patient selection (1,12,16). The role of ultrasound and multiparametric MRI to define the exact disease localization is still debatable (12).

Currently, TRUS-guided transrectal BP remains the widespread approach to evaluate patient suitability for FT protocols in terms of tumor topography, volume and grading. The accuracy of transrectal BP for predicting PCa laterality has been widely evaluated (3-9,11). All these studies but one (4) agree that almost two thirds of the patients with unilateral PCa at BP harbor bilateral PCa at RP and consequently BP is considered as inadequate for the purposes of candidate selection for FT.

Only Polaschik et al. (4) suggested that BP unilaterality may be used to select men with low- to low-moderate-risk PCa for hemiablation FT. In their study, although less than one third of the patients had confirmed unilateral disease at the final pathological evaluation, the strongest predictor of pathologic unilaterality was prostate biopsy unilaterality (odds ratio [OR] = 3.88; 95% confidence interval [CI], 2.14-7.05; P < 0.0005).

Furthermore, in these studies, none of the biopsy or clinical features, including the PSA, PSA ratio, clinical stage, gland volume, number of positive biopsy cores, high grade prostatic intraepithelial neoplasm, Gleason score, perineural invasion, percentage of positive cores) could clearly and independently predict the presence of unilateral PCa in univariate and multivariate logistic regression models.

Increasing the number of BP cores is not traduced in improving the accuracy of TRUS biopsy in predicting laterality. Abdollah et al (8) conducted a retrospective study on 203 patients who underwent an initial TRUS prostate saturation biopsy (24 cores) followed by RP. They concluded that initial saturation BP is not sufficiently accurate as a method of predicting tumor laterality in RP specimens and that the use of saturation biopsy to guide hemi-ablation therapy of PCa may lead to mistreatment in a considerable proportion of patients. Again, none of the routinely available clinical and pathological characteristics appeared to improve the ability of unilateral PCa on biopsy to predict unilateral PCa in the RP specimen. The same conclusions were also reached by Falzarano et al (9) in a study based on 72 patients

who underwent RP after saturation biopsy. Only 4 of 39 patients (10%) with a unilateral positive TRUS saturation biopsy had unilateral cancer at the final pathological evaluation. Our study confirmed the poor role of extended TRUS biopsy to predict PCa laterality at final pathology, with a concordance rate of 31.6% for unilateral disease between BP and RP. The overall statistical significance of the Kappa measurement of agreement that we obtained was rather reached due to the large sample size because a value of 0.27 conventionally indicates low agreement (17).

Is the extended TP biopsy more accurate for mapping PCa?

To our knowledge, only an abstract by Hoshi et al (18) prospectively evaluated 147 consecutive men who subsequently underwent RP. The PPV of transperineal saturation BP (the probability that the tumor is unilateral on the final pathological exam when it was unilateral on preoperative biopsy) was only 30% and consequently this approach was not sufficiently accurate to detect unilateral PCa and it should not be used to decide which patients should be offered FT. However, a full-text manuscript did not follow this abstract.

Template transperineal prostate mapping biopsies might provide more exact information about the spatial tumor distribution of PCa and might accurately identify unilateral cancer for the purpose of FT (13,19,20). When used with a 5mm sampling frame, this approach can rule in and rule out PCa foci of 0.5cc and 0.2cc volumes, with 90% certainty (19). There exists unanimous agreement that the current gold standard for characterizing men who are considering FT is transperineal BP using a template-guided approach, although it is not yet widely available and imposes a high burden on healthcare services because of its requirement for anesthesia, pathology processing and slower reporting time (2). Moreover, correlation with whole-mount pathological specimens is necessary to fully analyze the accuracy of this technique, but to date, no study is available.

Our study did not demonstrate any significant advantage of the transperineal approach over the TR in the prediction of the laterality concordance of cancer. Even if TP showed slightly higher diagnostic performance, TP was not significantly better than TR in terms of sensitivity, specificity and accuracy. When unilateral cancer was diagnosed after transperineal biopsy, the unilaterality was confirmed in only 34.6% of the patients, suggesting that, as with the transrectal approach, two thirds of the patients had bilateral cancer that was only detected at final pathology.

Finally, none of the evaluated preoperative variables (biopsy technique, age, total positive biopsy cores, PSA, prostate volume and Gleason score on biopsy) were able to predict bilaterality at the final pathology in the multivariate analyses. Moreover, age, total positive biopsy cores, PSA, prostate volume and Gleason score on biopsy were not independent predictors of bilateral PCa within the TP and TR groups when analyzed separately.

The present study is the first to report the accuracy of TP extended biopsy in predicting the laterality of PCa, and it is the first to compare TR versus TP in the same setting, with a standardized biopsy scheme. The absence of data on the clinical stage and the pathologic tumor volume as well as the absence of comparison on the complication rates and costs of the two bioptic approaches are the main shortcomings of our study.

CONCLUSIONS

TRUS-guided biopsy is not accurate for predicting PCa laterality when compared to the RP pathology result. The majority of patients with unilateral involvement at biopsy harbor bilateral PCa; the transperineal approach does not show any advantages over the transrectal in the capacity to predict the concordance of tumor laterality at radical prostatectomy. None of the preoperative evaluated variables can predict the final laterality. Thus, using unilaterality as a parameter for treating patients with FT may lead to mistreatment. Future research should aim to improve imaging techniques (such as multiparametric MRI) to obtain an accurate map of the prostate and understanding the biological implications of the different PCa foci for focusing treatment on the significant (“index”) lesions.

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CONFLICT OF INTEREST

None declared.

REFERENCES

1. Karavitakis M, Winkler M, Abel P, Livni N, Beckley I, Ahmed HU: Histological characteristics of the index lesion in whole-mount radical prostatectomy specimens: implications for focaltherapy. Prostate Cancer Prostatic Dis. 2011; 14: 46-52.
2. de la Rosette J, Ahmed H, Barentsz J, Johansen TB, Brausi M, Emberton M, et al.: Focal therapy in prostate cancer-report from a consensus panel. J Endourol. 2010; 24: 775-80.
3. Tareen B, Godoy G, Sankin A, Temkin S, Lepor H, Taneja SS: Can contemporary transrectal prostate biopsy accurately select candidates for hemi-ablative focal therapy of prostatecancer? BJU Int. 2009; 104: 195-9.
4. Polascik TJ, Mayes JM, Schroeck FR, Sun L, Madden JF, Moul JW, et al.: Patient selection for hemiablative focal therapy of prostate cancer: variables predictive of tumor unilaterality based uponradical prostatectomy. Cancer. 2009; 115: 2104-10.
5. Isbarn H, Karakiewicz PI, Vogel S, Jeldres C, Lughezzani G, Briganti A, et al.: Unilateral prostate cancer cannot be accurately predicted in low-risk patients. Int J Radiat Oncol Biol Phys. 2010 ; 77: 784-7.
6. Quann P, Jarrard DF, Huang W: Current prostate biopsy protocols cannot reliably identify patients for focal therapy: correlation of low-risk prostate canceron biopsy with radical prostatectomy findings. Int J Clin Exp Pathol. 2010 ; 3: 401-7.
7. Mayes JM, Mouraviev V, Sun L, Tsivian M, Madden JF, Polascik TJ: Can the conventional sextant prostate biopsy accurately predict unilateral prostate cancer in low-risk, localized, prostatecancer? Urol Oncol. 2011; 29: 166-70.
8. Abdollah F, Scattoni V, Raber M, Roscigno M, Briganti A, et al.: The role of transrectal saturation biopsy in tumour localization: pathological correlation after retropubic radicalprostatectomy and implication for focal ablative therapy. BJU Int. 2011; 108: 366-71.
9. Falzarano SM, Zhou M, Hernandez AV, Moussa AS, Jones JS, Magi-Galluzzi C: Can saturation biopsy predict prostate cancer localization in radical prostatectomy specimens: a correlative study andimplications for focal therapy. Urology. 2010; 76: 682-7.
10. Sinnott M, Falzarano SM, Hernandez AV, Jones JS, Klein EA, Zhou M, et al.: Discrepancy in prostate cancer localization between biopsy and prostatectomy specimens in patients with unilateral positivebiopsy: implications for focal therapy. Prostate. 2012; 72: 1179-86.
11. Gallina A, Maccagnano C, Suardi N, Capitanio U, Abdollah F, Raber M, et al.: Unilateral positive biopsies in low risk prostate cancer patients diagnosed with extended transrectal ultrasound-guidedbiopsy schemes do not predict unilateral prostate cancer at radical prostatectomy. BJU Int. 2012; 110: E64-8.
12. Valerio M, Ahmed HU, Emberton M, Lawrentschuk N, Lazzeri M, Montironi R, et al.: The Role of Focal Therapy in the Management of Localised Prostate Cancer: A Systematic Review. Eur Urol. 2013 6. pii: S0302-2838(13) 00557-5.
13. Crawford ED, Rove KO, Barqawi AB, Maroni PD, Werahera PN, Baer CA, et al.: Clinical-pathologic correlation between transperineal mapping biopsies of the prostate and three-dimensionalreconstruction of prostatectomy specimens. Prostate. 2013; 73: 778-87.
14. Lawrentschuk N, Klotz L: Active surveillance for low-risk prostate cancer: an update. Nat Rev Urol. 2011; 8: 312-20.
15. Lecornet E, Ahmed HU, Moore CM, Emberton M: Conceptual basis for focal therapy in prostate cancer. J Endourol. 2010; 24: 811-8.
16. Taneja SS, Mason M: Candidate selection for prostate cancer focal therapy. J Endourol. 2010; 24: 835-41.
17. Hale CA, Fleiss JL: Interval estimation under two study designs for kappa with binary classifications. Biometrics. 1993; 49: 523-34.
18. Hoshi S, Yamamuro T, Ogata Y, Numahata K, Sugano O. Transperineal saturation biopsy did not identify unilateral prostate cancer potentially amenable to focal treatment. J Urol 2010; 183 (4): e55 #Abstract 135.
19. Crawford ED, Wilson SS, Torkko KC, Hirano D, Stewart JS, Brammell C, et al.: Clinical staging of prostate cancer: a computer-simulated study of transperineal prostate biopsy. BJU Int. 2005; 96: 999-1004.
20. Barzell WE, Melamed MR: Appropriate patient selection in the focal treatment of prostate cancer: the role of transperineal 3-dimensional pathologic mapping of the prostate--a 4-year experience. Urology. 2007; 70(6 Suppl): 27-35.

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The S.T.O.N.E. Score: A new assessment tool to predict stone free rates in ureteroscopy from pre-operative radiological features

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ABSTRACT

Objective: To develop a user friendly system (S.T.O.N.E. Score) to quantify and describe stone characteristics provided by computed axial tomography scan to predict ureteroscopy outcomes and to evaluate the characteristics that are thought to affect stone free rates.

Materials and Methods: The S.T.O.N.E. score consists of 5 stone characteristics: (S)ize, (T)opography (location of stone), (O)bstruction, (N)umber of stones present, and (E)valuation of Hounsfield Units. Each component is scored on a 1-3 point scale. The S.T.O.N.E. Score was applied to 200 rigid and flexible ureteroscopies performed at our institution. A logistic model was applied to evaluate our data for stone free rates (SFR).

Results: SFR were found to be correlated to S.T.O.N.E. Score. As S.T.O.N.E. Score increased, the SFR decreased with a logical regression trend ($p < 0.001$). The logistic model found was $SFR=1/(1+e^{(-z)})$, where $z=7.02-0.57*Score$ with an area under the curve of 0.764. A S.T.O.N.E. Score ≤ 9 points obtains stone free rates > 90% and typically falls off by 10% per point thereafter.

Conclusions: The S.T.O.N.E. Score is a novel assessment tool to predict SFR in patients who require URS for the surgical therapy of ureteral and renal stone disease. The features of S.T.O.N.E. are relevant in predicting SFR with URS. Size, location, and degree of hydronephrosis were statistically significant factors in multivariate analysis. The S.T.O.N.E. Score establishes the framework for future analysis of the treatment of urolithiasis.

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Ureteroscopy; Urolithiasis; Lithotripsy, Laser; Logistic Models; Nomograms

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INTRODUCTION

The prevalence of stone disease is increasing not only in the United States, but worldwide (1,2). The total cost of treating stones in the US currently exceeds five billion dollars annually (2). With this increase in incidence and the cost of healthcare continuing to rise, new approaches to stone disease may be necessary.

Extra-corporeal shockwave lithotripsy (ESWL) is the present mainstay of treatment for intra-renal and proximal ureteral stones ≤ 1cm in diameter due to its relatively low complication rate and high success rate (3,4). Current guidelines from American Association of Urology (AUA) recommend ESWL as the first treatment option for proximal ureteral calculi. Modern stone therapy should ensure high effectiveness combined with

low complication rate. URS has become common in the treatment of proximal and intrarenal stones < 20mm as a result of technological improvements in visualization and laser technology (5,6).

Multiple studies have examined the predictive factors associated with ESWL outcomes as well as nomograms to predict stone free rates (SFR) (7,8). To our knowledge there is a lack of user friendly assessment tools to determine the complexity of ureteral and kidney stones and determine SFR after URS. Factors that influence SFR are pivotal to elect best treatment modality for stone disease. We propose a simple method to estimate the SFR for URS to treat urolithiasis.

MATERIAL AND METHODS

A retrospective review of patients who underwent URS was approved by the institutional review board. Known factors associated with stone free rates were used to establish a simplified method to estimate SFR. Inclusion criteria consisted of consecutive patients with ureteral and renal stones with preoperative non-contrast computed axial tomography (CT-KUB). Patients with anatomical abnormalities such as duplicated ureters, horseshoe kidney, ureteral strictures were excluded from analysis.

Stone free was defined as absent of stone fragments or fragments ≤ 2 mm post URS after rigorous endoscopic inspection and real time fluoroscopy with the capability of high magnification imaging (9). If combined endoscopic visualization and fluoroscopy was sub-optimal, a CT scan was obtained to confirm stone free status. If a patient was considered stone-free intraoperatively and did not have a postoperative CT scan, a chart reviewed was performed. These patients were considered clinically stone-free if no admissions or visits to the emergency were found during follow-up.

The S.T.O.N.E. Score is a proposed system to predict the stone free status of a patient from preoperative characteristics available on CT-KUB: (S)ize of the stone, (T)opography or location, degree of (O)bstruction of the urinary system, (N)umber of stones, and (E)valuation of Hounsfield units. Higher scores indicate higher complexity and assumingly lower stone free rates. Each feature from the CT was graded on a 1-3 point scale as described in Table-1. In cases with multiple calculi, the stone with the highest grade for each feature was recorded. All scores were assigned after a consensus of two observers.

Stone (S)ize was the initial variable in S.T.O.N.E. Score. Size was measured as the maximum diameter of the stone in any plane. Our scoring system was based on the AUA's stratification to estimate ureteral stone passage by size (3). One point was given for stones < 5mm, 2 points were given to stones ≥ 5mm and < 10mm, and 3 points were given to stones ≥ 10mm.

(T)opography or location was another factor included that affects stone free rates in URS. While distal stones tend to be easily treated with SFR of 90%, stones in the proximal ureter and within the kidney may be challenging for URS (4).

Table 1 - S.T.O.N.E. Score.

Feature	1 pt.	2 pt.	3 pt.
(S)ize	< 5mm	5-10mm	> 10mm
(T)opography	Distal to Mid-Ureter	Proximal Ureter through Mid and Upper Pole	Lower Pole
(O)bstruction	Preoperative Stent or No Hydronephrosis	Grade 1-2	Grade 3-4
(N)umber of stones	1 stone	2 stones	≥ 3stones
(E)valuation of HU	< 750HU	750-1000HU	> 1000HU

Mid and upper pole renal stones were considered less challenging than the lower pole stones, since several factors may challenge the surgeon i.e. infundibular angle and diameter. Therefore, we scored 1 point for distal and mid ureter stones, 2 points for proximal ureter, mid pole, and upper pole stones, and 3 points to lower pole stones.

(O)bstruction was scored as the degree of hydronephrosis in the collecting system and the presence of a stent. The greater the degree of hydronephrosis, the higher is the obstruction and the lower the SFR after URS. We implemented a modified version of the Society for Fetal Urology Hydronephrosis Grading System (10). Grade 1 hydronephrosis was defined as local dilation of the ureter. Grade 2 included ureteral and renal pelvis dilatation. Grade 3 also included calix dilatation. Grade 4 exhibited parenchymal thinning. The benefits of pre-stenting prior to ureteroscopy have been previously shown (11). The reasons for pre stenting in our population of patients included: severe preoperative pain, infection managed and treated prior ureteroscopy, and from outside referrals to our safety net hospital. All patients had their stents for at least 2 weeks prior to ureteroscopy. Patients with pre-stenting or no hydronephrosis received 1 point, Grade 1-2 hydronephrosis received 2 points, and Grade 3-4 received 3 points.

The (N)umber of stones is well known to influence treatment. Stones greater than 2mm were counted into the score. Patients with 1 stone were assigned 1 point, patients with 2 stones were assigned 2 points, and patients with ≥ 3 stones were assigned 3 points.

Finally, (E)valuation of the Hounsfield Units (HU) was calculated as the average Hounsfield Units instead of standard deviation or maximum attenuation (12). Hounsfield Units < 750 received 1 point, between 750 and 1000 HU received 2 points, and 3 points for ≥ 1000HU. These minimum and maximum values were chosen due to known SFR correlations (13,14). Ureteroscopy and stone treatment was performed with the patient in the lithotomy position. A 7.5 Fr semi-rigid ureteroscope (Olympus Corporation, Tokyo, Japan) was used for stones in the distal and mid ureter. For proximal ureteral and renal stones, an access sheath (Cook Medical, Bloomington, IN) and a 9 Fr flexible (Olympus Corporation, Tokyo, Japan) ureteroscope was used. Prior to 2009 a fiber optic flexible ureteroscopy (Karl Storz Flex--X™, Inc., Tuttlingen, Germany) was utilized. The stone was assessed and laser lithotripsy was performed when necessary using a Holmium Laser (Donnier Medilas, Kennesaw, GA) set at 6-10W with a 270 or 400µm laser fiber (Gyrus ACMI, Southborough, MA). Stone fragments were then removed by a stone retrieval basket (Boston Scientific, Natick, MA). Following complete stone removal, stone free status was evaluated with the methods previously described.

Statistical analyses used to construct the S.T.O.N.E. Score were performed using the R version 2.11 software (the R foundation for Statistical Computing, Vienna, Austria). The goodness of fit using the Area Under the Curve (AUC) for the S.T.O.N.E. Score was performed using the ROCR statistical package (15). Data are presented as average ± standard deviation or frequency (percentage of total). A p-value < 0.050 was considered significant. The corresponding author had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

RESULTS

A total of 200 URS procedures from August 2006 to January 2012 were assessed by the S.T.O.N.E. Score. Patients age was 44.1 ± 13.9, were equally represented by gender (male:female of 87:113) and obesity (BMI > 30kg/m^2). Often, cases were unilateral stones, were not favored to either side of the urinary tract (right:left:bilateral of 106:91:3), and had a mean stone size of 9.3 ± 5.9.

The overall SFR in the entire cohort was 82%. A total of 28 patients were diagnosed with residual stones intra-operatively and another 8 patients were found to have residual stones on postoperative CT. Postoperative imaging (CT) demonstrated 67 patients were stone free. The remaining 97 patients were considered stone free following ureteroscopy and did not seek further treatment in Urology or the Emergency Department after a mean follow-up time of 20 months.

The multivariate regression demonstrated that as stones became larger, more proximal to the lower pole, caused greater hydronephrosis, increased in number, and had higher Hounsfield units, the probability of becoming stone free decreased. According to the multivariate model, stone size, location, and hydronephrosis grades had similar impact (weighting) on the stone free status (Table-2). Stone size and location were significant factors affecting stone free rates ($p < 0.05$). The severity of hydronephrosis was nearly significant ($p = 0.07$). The accuracy of the multivariate model by the area under the curve was 0.837.

The S.T.O.N.E. Score was established as a simplified assessment tool to predict stone free rates (Table-3). The formula derived from the logistic model for stone free rate was as follows: SFR=1/(1+exp(-z)), where z=7.02-0.57 * Score. As the S.T.O.N.E. Score increases, the stone free rate decreases ($p < 0.001$) (ESM 1). The accuracy of the S.T.O.N.E. Score was comparable to the multivariate model (AUC = 0.764) (Figure-1).

Table 2 - Multivariate Logistic Regression Model.

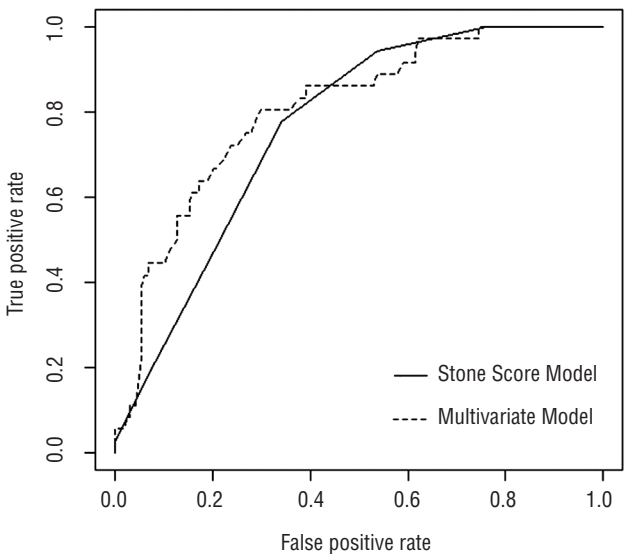
Variable	Estimate	95% CI	P-value
S	1.1	-1.8 - -0.4	0.002
T	1.3	-2.1 - -0.6	< 0.001
O	0.5	-1.1 - 0.1	0.077
N	0.2	-0.7 - 0.3	0.472
E	0.0	-0.5 - 0.5	0.926

AUC = 0.806

Table 3 - S.T.O.N.E. score compared to URS outcomes.

S.T.O.N.E. Score	N	200 Patients	S.T.O.N.E. Score	Rounded Score
5	8	100%	99%	100%
6	18	100%	97%	100%
7	30	100%	95%	100%
8	30	93%	92%	90%
9	27	81%	87%	90%
10	25	64%	78%	80%
11	34	74%	67%	70%
12	19	68%	54%	50%
13	6	67%	40%	40%
14	3	33%	28%	30%
15	0	-	18%	20%

Figure 1 - Receiver operator curves for the S.T.O.N.E. Score (AUC = 0.764) and the Multivariate Regression Model (AUC = 0.806).



DISCUSSION

The development of statistical models provides physicians with new insight into patient planning and counseling. Furthermore, models typically standardize terminology and improve communication in reports. In stone disease, features affecting the success of ESWL such as skin-to-stone distance, optimal location, density (Hounsfield Units), and size have all been adequately reported (7,14,16). On the other hand, there is presently a paucity of nomograms or score systems to predict stone free rates for URS.

The S.T.O.N.E. Score is a user friendly model to predict SFR post URS with laser lithotripsy. It further establishes a standardized terminology for reporting urolithiasis characteristics. We attempt to identify the five most important preoperative features that could be related to surgical outcomes in URS: (S)ize, (T)opography/location, (O) bstruction, (N)umber of stones, and (E)valuation of Hounsfield Units (12,17).

The S.T.O.N.E. Score remains consistent with 77% of the present literature as stone size is usually described by the maximum diameter (18). A recent study revealed stones > 20 and < 40mm treated by URS reported a stone free rate of 100% with mean 1.4 number of procedures (19). The general consensus in the literature cites a negative correlation between stone size and SFR and is included in the S.T.O.N.E. Score (5,6).

Stone location is an important factor in the success of URS, especially lower pole stones (5,6,18). Although visualization of these stones may be possible with flexible endoscopes, the acute pelvic infundibular angle may prevent access to the stone, especially with less deflection of the ureteroscope when the basket or laser fiber is inside of the working channel. Generally, SFR for lower pole stones have typically been reported around 80% (5,6,18). Other intra-renal and proximal ureteral stones have been reported with a stone free rate of approximately 90% (18). More distal stones from the mid ureter to the ureterovesical junction stones have been reported with a stone free rate > 95% (18). These stones have been easily managed with rigid ureteroscopy. This data along with our own observations have led to the stratification levels established for the S.T.O.N.E. Score.

Impacted stones and hydronephrosis are other mechanisms that diminish stone clearance. Although impacted stones are difficult to be evaluated on CT, the presence and degree of hydronephrosis may be an indirect indicator. Additionally, prolonged obstruction increases the amplitude and frequency of ureteral peristalsis contractions resulting in histological changes including smooth muscle hypertrophy and collagen deposition (20). These histological changes may affect ureteroscopy instrumentation limiting the ability to manipulate stones proximal at the level of obstruction. Stenting has shown to improve SFR compared to non-stented patients (12). Rubenstein et al. saw a stone free rate of 78 and 54% for patients with and without pre--stenting respectively. We have accounted for the pre-stenting effect by incorporating this into the (O)bstruction score. The (O)bstruction score was further graded by a modified Society for Fetal Urology score in order to quantify the severity of hydronephrosis. There are few grading systems objectively quantifying hydronephrosis. Our system is simple to implement and correlates with stone free rates. Although there has been little data investigating the effect of hydronephrosis

in URS, our data shows this is a significant factor and is included in the S.T.O.N.E. Score.

The number of stones has been shown to be significant in other studies (21). A number of methods have been developed to describe stone burden by incorporating both the size and number of stones (21,22). The description of total stone burden applies two dimensional measurements using rectangular and elliptical approximations (18). Furthermore, a three dimension description of stone burden would be the most accurate system, but the difficulty in implementation and software availability has inhibited its use at the present time. For simplicity, we chose to incorporate the number of stones to describe stone burden in our nomogram.

Stone hardness and Hounsfield Units on CT have often been overlooked in URS. Hounsfield Units has commonly been reported to be a significant factor in shock-wave lithotripsy (23). Chung et al. reported a statistical difference between successful and unsuccessful ESWL (675.29 versus 1075.00, respectively). This trend has been observed in URS but without statistical significance. A study found that stones successfully treated by URS had a mean of 858 HU while stones that were unsuccessfully treated had a mean of 1115 HU (6). Stone composition increasing density and hardness may prolong OR time.

The limitations of this study include a retrospective single institution analysis. Furthermore, a standardized definition and methodology of evaluating stone free status is lacking currently in the literature. The clinical significance of residual stone size is presently unknown with stone free definitions most commonly ranging from the complete absent of stones to residual fragments < 4mm (24). Our definition of stone free corroborates with a study that has shown that stones > 2mm are related to a recurrent stone event (25). A number of radiological methods for evaluating stone free status include CT, KUB (fluoroscopy), and Ultrasound (24). Although CT may be the best imaging modality to evaluate presence and or burden of stone post treatment, certainly, it is not free of limitations. Furthermore, the presence of ureteral stents may obscure the presence of small stone fragments following URS (26). This pragmatic method of limiting postoperative imaging to questionable and complicated cases is commonly accepted and minimizes radiation and costs to the patient (9,27). Aggressive endoscopic inspection combined with high magnification fluoroscopy has shown high sensitivity and specificity in evaluating 0-4mm stones (9,27). Nonetheless, the S.T.O.N.E. Score correlates with SFR and can be applied in daily practice without complex instrumentation or time consuming calculations. Future work is needed to validate the S.T.O.N.E. Score.

CONCLUSIONS

The S.T.O.N.E. Score is a novel assessment tool to predict SFR in patients undergoing URS. Features of S.T.O.N.E. (stone size, location, and degree of hydronephrosis) were relevant in predicting SFR with URS. The S.T.O.N.E. Score establishes the framework for future analysis for the treatment of urolithiasis.

ABBREVIATIONS

AUC = Area Under the Curve
ESWL= Extracorporeal shockwave lithotripsy
SFR= Stone Free Rate
URS= Ureteroscopy

CONFLICT OF INTEREST

Wilson Molina - Boston Scientific Course Proctor and Supported Fellowship

Fernando J. Kim - Olympus Proctor

Joshua Spendlove, Alexandre Pompeo, Stefan Sillau and David Sehrt - None declared.

REFERENCES

1. Nowfar S, Palazzi-Churas K, Chang DC, Sur RL: The relationship of obesity and gender prevalence changes in United States inpatient nephrolithiasis. Urology. 2011; 78: 1029-33.
2. Turney BW, Reynard JM, Noble JG, Keoghane SR: Trends in urological stone disease. BJU Int. 2012; 109: 1082-7.
3. Preminger GM, Tiselius HG, Assimos DG, Alken P, Buck C, Gallucci M, et al.: 2007 guideline for the management of ureteral calculi. J Urol. 2007; 178: 2418-34.

4. Tiselius HG, Ackermann D, Alken P, Buck C, Conort P, Gallucci M; et al.: Guidelines on urolithiasis. Eur Urol. 2001; 40: 362-71.
5. Bagley DH: Expanding role of ureteroscopy and laser lithotripsy for treatment of proximal ureteral and intrarenal calculi. Curr Opin Urol. 2002; 12: 277-80.
6. Hussain M, Acher P, Penev B, Cynk M: Redefining the limits of flexible ureterorenoscopy. J Endourol. 2011; 25: 45-9.
7. Kim FJ, Rice KR: Prediction of shockwave failure in patients with urinary tract stones. Curr Opin Urol. 2006; 16: 88-92.
8. Wang M, Shi Q, Wang X, Yang K, Yang R: Prediction of outcome of extracorporeal shock wave lithotripsy in the management of ureteric calculi. Urol Res. 2011; 39: 51-7.
9. Schoenthaler M, Wilhelm K, Katzenwadel A, Ardelt P, Wetterauer U, Traxer O, et al.: Retrograde intrarenal surgery in treatment of nephrolithiasis: is a 100% stone-free rate achievable? J Endourol. 2012; 26: 489-93.
10. Fernbach SK, Maizels M, Conway JJ: Ultrasound grading of hydronephrosis: introduction to the system used by the Society for Fetal Urology. Pediatr Radiol. 1993; 23: 478-80.
11. Rubenstein RA, Zhao LC, Loeb S, Shore DM, Nadler RB: Prestenting improves ureteroscopic stone-free rates. J Endourol. 2007; 21: 1277-80.
12. Kacker R, Zhao L, Macejko A, Thaxton CS, Stern J, Liu JJ, Nadler RB: Radiographic parameters on noncontrast computerized tomography predictive of shock wave lithotripsy success. J Urol. 2008; 179: 1866-71.
13. Gupta NP, Ansari MS, Kesarvani P, Kapoor A, Mukhopadhyay S: Role of computed tomography with no contrast medium enhancement in predicting the outcome of extracorporeal shock wave lithotripsy for urinary calculi. BJU Int. 2005; 95: 1285-8.
14. Wen CC, Nakada SY: Treatment selection and outcomes: renal calculi. Urol Clin North Am. 2007; 34: 409-19.
15. Sing T, Sander O, Beerenwinkel N, Lengauer T: ROCR: visualizing classifier performance in R. Bioinformatics. 2005; 21: 3940-1.
16. Joseph P, Mandal AK, Singh SK, Mandal P, Sankhwar SN, Sharma SK: Computerized tomography attenuation value of renal calculus: can it predict successful fragmentation of the calculus byextracorporeal shock wave lithotripsy? A preliminary study. J Urol. 2002; 167: 1968-71.
17. Macejko A, Okotie OT, Zhao LC, Liu J, Perry K, Nadler RB: Computed tomography-determined stone-free rates for ureteroscopy of upper-tract stones. J Endourol. 2009; 23: 379-82.
18. Patel SR, Nakada SY: Quantification of preoperative stone burden for ureteroscopy and shock wave lithotripsy: current state and futurerecommendations. Urology. 2011; 78: 282-5.
19. Takazawa R, Kitayama S, Tsujii T: Successful outcome of flexible ureteroscopy with holmium laser lithotripsy for renal stones 2 cm or greater. Int J Urol. 2012; 19: 264-7.
20. Kinn AC, Lykkeskov-Andersen H: Impact on ureteral peristalsis in a stented ureter. An experimental study in the pig. Urol Res. 2002; 30: 213-8.
21. Herrera-Gonzalez G, Netsch C, Oberhagemann K, Bach T, Gross AJ: Effectiveness of single flexible ureteroscopy for multiple renal calculi. J Endourol. 2011; 25: 431-5.
22. Breda A, Ogunyemi O, Leppert JT, Schulam PG: Flexible ureteroscopy and laser lithotripsy for multiple unilateral intrarenal stones. Eur Urol. 2009; 55: 1190-6.
23. Park YI, Yu JH, Sung LH, Noh CH, Chung JY: Evaluation of possible predictive variables for the outcome of shock wave lithotripsy of renal stones. Korean J Urol. 2010; 51: 713-8.
24. Deters LA, Jumper CM, Steinberg PL, Pais VM Jr.: Evaluating the definition of "stone free status" in contemporary urologic literature. Clin Nephrol. 2011; 76: 354-7.
25. Raman JD, Bagrodia A, Gupta A, Bensalah K, Cadeddu JA, Lotan Y, et al.: Natural history of residual fragments following percutaneous nephrostolithotomy. J Urol. 2009; 181: 1163-8.
26. Tang VC, Attwell-Heap A: Computed tomography versus ureteroscopy in identification of renal tract stone with ureteral stent in situ. Ann R Coll Surg Engl. 2011; 93: 639-41.
27. Portis AJ, Laliberte MA, Drake S, Holtz C, Rosenberg MS, Bretzke CA: Intraoperative fragment detection during percutaneous nephrolithotomy: evaluation of high magnification rotationalfluoroscopy combined with aggressive nephroscopy. J Urol. 2006; 175: 162-5; discussion 165-6. Erratum in: J Urol. 2006; 175(3 Pt 1): 1176.

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Effectiveness of tamsulosin in prevention of post-operative urinary retention: a randomized double-blind placebo-controlled study

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ABSTRACT

Purpose: Urinary retention is one of the most common complications contributing to surgical procedures. Recent studies have shown the benefits of alpha-adrenergic blockers in preventing post-operative urinary retention (POUR). The aim of this prospective study was to compare the prophylactic effect of tamsulosin with placebo on postoperative urinary retention.

Materials and Methods: In this randomized placebo controlled, clinical trial, 232 male patients aged 18 to 50 years old admitted to Razi University Hospital for varicocelectomy, inguinal herniorrhaphy, and scrotal surgery were randomly assigned to receive either three doses of 0.4mg tamsulosin (n = 118) or placebo (n = 114), 14 and 2 hours before, and 10 hours after surgery. Patients were closely monitored for the development of urinary retention 24 hours after surgical intervention. The primary endpoint was to investigate the effect of tamsulosin in prevention of post-operative urinary retention during the first 24 hours after surgical intervention. Collected data were analyzed using SPSS software version 18 and the $P < 0.05$ was considered statistically significant.

Results: One hundred and eighteen patients were included in tamsulosin arm and 114 in placebo arm. POUR in patients who received tamsulosin was significantly lower than placebo, as 5.9% of the patients treated with tamsulosin and 21.1% placebo group, reported urinary retention following surgery ($P = 0.001$). No serious adverse effects were seen in both groups.

Conclusions: This study suggests that short perioperative treatment with tamsulosin can reduce the incidence of urinary retention and the need for catheterization after varicocelectomy, inguinal herniorrhaphy, and scrotal surgery.

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Key words:
Urinary Retention; tamsulosin [Supplementary Concept]; Herniorrhaphy

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INTRODUCTION

Post-operative urinary retention (POUR) is defined as the inability to void after surgery when the bladder is full (1,2) POUR is common and represents between 5% to 70% of all surgeries (1), especially after herniorrhaphy (3,4) and anorectal surgery (5-7). Typically, this phenomenon is painful and can result in increased cost of hospitalization, prolonged length of hospital stay, bladder overdistension, and urinary tract infection (UTI) which can occur primarily or secondarily to

catheterization (1,8). Urethral catheterization, a mainstay of initial management for patients with POUR, is associated with some complications and increase in cost of care (1,2,8). Therefore, pharmacological therapy is considered as an interesting approach for patients developing urinary retention following surgery (2).

It seems that high sympathetic activity increases the risk of urinary retention (1). Therefore, inhibition of alpha-adrenergic receptors located on the bladder neck and proximal urethra may prevent POUR and improve voiding (1,9). Several drugs including alpha-blockers and parasympaticomimetics had been under investigation for their effectiveness in preventing POUR (8). Recent evidence has shown that the use of alpha-blockers facilitate voiding by decreasing the resistance of the proximal urethra and bladder neck and improving the urine flow (9,10).

Several studies evaluating the effect of alpha-blockers in preventing POUR have suggested an improvement in urinary function after hysterectomy (11), inguinal herniorrhaphy (12), colorectal surgery (1,2), and genital prolapsed repair (13). Recently, a prospective randomized study by Mohammadi-Fallah et al. (10) showed that the POUR rate after inguinal herniorrhaphy among patients who received tamsulosin was significantly lower than those who received placebo (2.5% versus 15%). Tamsulosin is a safe selective alpha 1-adrenergic receptor blocker characterized by its favorable side effect profile (14). The prophylactic effect of tamsulosin in reducing POUR has not been investigated in a large randomized double-blind study; therefore the present study was conducted to investigate the efficacy of tamsulosin compared with placebo in preventing POUR.

MATERIAL AND METHODS

Study Design

This prospective randomized double-blind, placebo-controlled trial was performed between August 2011 and July 2012 in the Department Urology of Razi University Hospital in Rasht, Iran. The study was conducted in accordance with the Declaration of Helsinki, and was approved by the local Institutional Review Board and the ethics committee of Guilan University of Medical Sciences (GUMS). It was registered online at Iranian registry of clinical trials http://www.irct.ir (identifier- IRCT201109084582N5).

Subjects

Male patients aged 18 to 50 years who were admitted in our center for elective inguinal herniorrhaphy, varicocelectomy or scrotal surgeries under spinal anesthesia were included in our study. Patients who had urinary symptoms before surgery, a known history of neurological, urological or significant systemic disease (such as diabetes mellitus), previous history of urinary retention, previous urological or abdominal surgery, history of using medications that could interfere with natural voiding function such as benzodiazepines, cholinergic drug prior to surgery, or current treatment with alpha or beta agonists were not included. Exclusion criteria were: IV fluid administration of more than 1500cc during surgery, or use of other anesthetic drugs except for lidocaine for spinal anesthesia. Patients were further excluded if their surgical procedures last more than 90 minutes.

After obtaining written informed consent from all participants, patients were randomized to receive either three doses of 0.4mg of tamsulosin or placebo using random block design. Treatment allocation sequence was carried out based on a block size of four generated with a computer random--number generator (using excel program). Group allocation was concealed in sealed opaque envelop by a third party (independent researcher) prior to the treatment. The study medication was administered by a nurse who was not informed which medication was used. Patients, study staff and investigators were blinded to treatment assignment.

Interventions

Half-life of tamsulosin is 9-15 hours, so we administered three doses with 12 hours intervals. The medications were administered 14 and 2 hours before and 10 hours after surgical operation. Patients were asked to empty their bladder prior to surgery. Surgery was performed under spinal anesthesia using 2cc lidocaine. The patients were then closely followed up (monitored) by blinded research associates for the presence of urinary

retention, any voiding difficulty and side effects during 24 hour after surgery, and the occurrence of POUR was compared between both groups. Patients were allowed to void once they felt they had a full bladder. NSAIDs were prescribed for postoperative analgesia. Opioid analgesics were not administered to any patient postoperatively.

Measurement

The diagnosis of urinary retention was established when the patient had a painful and palpable mass in his suprapubic area, and was unable to void during the first 24 hours after surgery. The diagnosis was confirmed by emptying of more than 400mL of urine by catheterization. A 14-French nelaton catheter was placed to decompress the bladder of patients who could not urinate 12 hours after surgery.

Data Collections

Upon admission, data including age and type of surgery were collected from all patient's files. Perioperative fluid administration and operative time were also collected 24 hours after surgical intervention.

Endpoints

Our primary endpoint was to investigate the effect of tamsulosin in prevention of POUR during the first 24 hours after surgical intervention.

Statistical analysis

According to previous studies, reporting 15% incidence of urinary retention in patients who undergo scrotal surgery, varicocelectomy and Herniorrhaphy with spinal anesthesia, we calculated that the study could be done with 111 subjects in each study arm. This number of subjects would give 80% of power at the 0.05 level to show 75 percent reduction in the rate of urinary retention by Chi-square test.

Collected data were analyzed using SPSS software version 18. Descriptive data were reported as mean $\pm$ SD or median (interquartile range) as appropriate. Normality was assessed by Kolmogorov-Smirnov test and a Chi-square test applied to compare the efficacy of treatments between two groups. Univariate analysis of factors related to incidence of POUR was compared using the independent sample t-test for continuous variables and the Chi-square test for categorical variables. A backward stepwise logistic regression model yielding odds ratio (OR) and 95% confidence interval (CI) was performed on POUR to analyze the treatment effect when adjusting for other related covariates. The model included baseline variables (e.g. age, type of surgery, operative time, treatment groups and serum volume) and variables showing an univariate association ($P < 0.1$) with POUR (e.g. age, treatment groups and operative time).The goodness of fit of the regression model was evaluated by the Hosmer-Lemeshow test. All statistical tests were two tailed, and the $P < 0.05$ was considered statistically significance.

RESULTS

Two hundred and thirty two patients who were randomly assigned to tamsulosine group (n = 118) or placebo group (n = 114) were included in the analysis.

Demographic data and clinical features of both treatment groups are presented in Table-1. Varicocelectomy was the most frequent surgery in both groups. All patients were male, and the mean age was the same in both Tamsulosin and placebo arms (27.59 $\pm$ 7.29 vs. 27.72 $\pm$ 7.2 years). There were also no significant differences between two groups in terms of surgery type, perioperative fluid volume, and operative time.

In tamsulosin group, there was a significantly lower proportion of patients with POUR compared with the placebo group (5.9% vs. 21.1%; P = 0.001).

Univariate analysis showed a significantly lower incidence of POUR in patients who received tamsulosin (P = 0.001). Longer duration of surgical intervention (P = 0.039) and lower age (P = 0.017) were also associated with POUR. At logistic regression analysis, the odds of POUR in the tamsulosin group was about 0.24 times lower (OR = 0.24, 95% CI = 0.09 - 0.6, P = 0.002) than in the placebo group after adjustment for potential risk factors including age and operative time. Longer

Table 1 - Demographic characteristics and clinical features of patients in both treatment groups.

	Tamsulosin group (N = 118)	Placebo group (N = 114)
Type of surgery		
Herniorrhaphy	14 (11.9%)	11 (9.6%)
Varicocelectomy	58 (49.2%)	57 (50%)
Scrotal surgery	46 (39%)	46 (40.4%)
Mean age ± SD (year)	27.59 ± 7.29	27.72 ± 7.2
Mean operative time ± SD (min.)	50.37 ± 13.35	53.50 ± 11.92
Mean perioperative fluid administration ± SD (mL)	1094.92 ± 200.78	1096.67 ± 214.08

P value was not significant for all the values.

operative time (OR = 1.03, 95% CI = 1 - 1.07, P = 0.027) and younger age (OR = 0.93, 95% CI = 0.87 - 0.99, P = 0.029) were other parameters that significantly influenced the rate of POUR (Tables 2 and 3).

Two patients in tamsulosin arm showed side effects at 24 hours follow-up. Both patients experienced vomiting and dizziness. Side effects were mild to moderate, and did not lead to exclusion of patients from the study.

DISCUSSION

POUR is one of the most common complications of anesthesia and surgery. It occurs more frequently after lower abdominal and pelvic, gynecologic and anorectal surgeries (10). Overall incidence of POUR ranges from 5% to 70% (1). Development of POUR is associated with age, gender, history of underlying urologic and non-urologic disease, perioperative fluid intake, type of anesthesia and surgery and duration of surgery (4,10). POUR causes to major discomfort and pain after surgery and catheterization for resolving it, may lead to urethral injury or stricture or urinary tract infection and increase cost and work load and hospitalization period. Occasionally patients may suffer persistent POUR that complicates at the postoperative period. There are several mechanisms involving development of POUR. Multiple facets of surgery, anesthesia and perioperative management may interrupt the voiding reflex. Anesthesia interferes with sensation of bladder fullness. Other factor include the balance between sympathic and parasympatic disturb during perioperative period, systemic sympatic discharge due to anesthesia and pain after surgery and local sympatic motor activity due to bladder distention, inhibition of detrusor contraction and intensity of the bladder.

Outlet closure is done via increasing alpha--mediated tone in bladder outlet (8). Perineal and lower abdominal pain can inhibit the perineal relaxation that is necessary for voiding. Detrusor contractures can also be inhibited by a reflex involving afferent fibers of the pudental nerve. Immobilization and have to void in supine position contribute to post-operative voiding dysfunction (8).

Three methods have been used to diagnose POUR: 1) history and physical examination (lower abdominal pain and discomfort and palpation or percussion of bladder in suprapubic area); 2) bladder catheterization; 3) ultrasonographic assessment of bladder postoperatively (4).

We used these criteria to confirm POUR in our study: patients discomfort or palpable bladder or inability to void more than 12 hours after induction of anesthesia (1,15).

We included patients 18-50 years old, because in older age there is a decrease in contractility of detrusor and increase in incidence of some

Table 2 - Associated factors of POUR within 24 hours after surgical intervention.

	All	POUR		P-value
		No	Yes	
Mean age ± SD (year)	27.65 ± 7.59	28.02 ± 7.55	25.25± 7.55	0.017
Type of surgery				0.919
Herniorrhaphy	25 (10.8)	21 (84%)	4 (16%)	
Varicocelectomy	115 (49.6)	100 (87%)	15 (13%)	
Scrotal surgery	92 (39.7)	80 (87%)	12 (13%)	
Mean operative time ± SD (min.)	51.91 ± 12.73	51.16 ± 12.48	56.77 ± 13.51	0.039
Mean perioperative fluid administration ± SD (mL)	1095.78 ± 206.98	1097.01 ± 200.47	1087.74 ± 248.71	0.376
Treatment groups				
Placebo	114 (49.1)	90 (78.9%)	24 (21.1%)	0.001
Tamsulosin	118 (50.9)	111 (94.1%)	7 (5.9%)	

Table 3 - Adjusted odd ratio for treatment group and potential risk factors of POUR.

	Odd- ratio	95% confidence Interval	P-value
Age	0.93	0.87- 0.99	0.029
Operative time (min.)	1.03	1- 1.07	0.027
Treatment groups			
Placebo	1	0.09- 0.6	0.002
Tamsulosin	0.24		

diseases such as benign hyperplasia of prostate that present with urinary symptoms and may interfere with patient randomization and study results (development of urinary retention).

In Petros et al. study comprising patients who were submitted herniorrhaphy, age below 53, spinal anesthesia and preoperative fluid administration less than 1200cc significantly decreased risk of POUR (3).

Some studies have reported higher incidence of POUR in men compared with women (6) but in other studies, there isn't significant difference between men and women (3). In our study only men participated due to type of surgeries. There are various medical prophylactic methods for prevention of POUR, such as parasympathomimetic and α-adrenergic blockers. Restriction of preoperative fluid intake, induction of local instead of regional or general anesthesia, use of short acting anesthesia agent, early ambulation of patients after surgery, and use of warm compress in suprapubic area can prevent POUR (1,8).

Excessive perioperative fluid intake lead to bladder over distention, that increases risk of POUR. So, restriction of perioperative fluid intake may prevent POUR (16).

Type of anesthesia is another important factor in the development of POUR. Risk of POUR in local anesthesia is lower than regional and general anesthesia (1,8,17).

The purpose of pharmacologic prevention of POUR is the increase of detrusor contractility or bladder neck and proximal urethral relaxation.

Parasympathomimetic agents such as bethanechol theoretically increase bladder smooth muscle contractility, but their clinical utility is under question owing to poor efficacy and adverse side effects (18).

Alpha-adrenergic blockers decrease bladder outlet resistance and facilitate micturation. Several studies found that prophilatic administration of phenoxybenzamine significantly decreases the incidence of postoperative urinary retention (19-21).

Gönüllü et al. used prazosin (another α-blocker) for prevention of post-herniorrhaphy urinary retention and concluded that prazosin decreased the incidence of POUR from 25% to 10.8% ($P<0.05$) (12).

Tamsulosin is a superselective long acting alpha-1a blocker with acceptable side effects. Mohammadi-Fallah et al. assessed preventive effect of tamsulosin on post-herniorraphy urinary retention. In this randomized study, 40 patients received 0.4mg tamsulosin 6 hours before and 6-12 hours after surgery and 40 patients received placebo in the same manner. They concluded perioperative administration of tamsulosin reduced the risk of POUR from 15% to 2.5% ($p = 0.04$). They mentioned that type of anesthesia, the duration of surgery and severity of preoperative urinary symptoms had no significant effect on the incidence of POUR (10).

In our study in tamsulosin group, 7 from 118 patients developed POUR (5.9%) while in placebo group 24 from 114 patients developed POUR (21.1%). So tamsulosin reduced development of POUR significantly ($P = 0.001$). Although we have excluded patients with duration of surgery more than 90 min., but even in patients in this range longer duration there was associated a higher risk of POUR ($p = 0.039$). Also, in our study, lower age was associated with higher risk of POUR ($p = 0.017$). Although clinical importance of this finding may be insignificant because all of patients were below 50 years old. In our study, the type of surgery (varicocelectomy, herniorrhaphy and scrotal surgery) didn't affect the risk of POUR ($P = 0.919$). We have excluded patients with excessive fluid intake perioperatively (> 1500cc) and long duration of surgery (> 90 min.) since their effects on development of POUR were confirmed in several studies.

In the majority of medical centers these kinds of surgeries are performed very frequently as an outpatient procedure, and admission of all patients in our department was one of the most limitations of our study. Diagnosis of POUR by history and physical examination instead of sonography is another study limitation.

CONCLUSIONS

This study suggests that perioperative tamsulosin administration reduces the incidence of postoperative urinary retention and the need for catheterization after varicocelectomy, herniorrhaphy and scrotal surgery.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Baldini G, Bagry H, Aprikian A, Carli F: Postoperative urinary retention: anesthetic and perioperative considerations. Anesthesiology. 2009; 110: 1139-57.
2. Buckley BS, Lapitan MC: Drugs for treatment of urinary retention after surgery in adults. Cochrane Database Syst Rev. 2010; 10: CD008023.
3. Petros JG, Rimm EB, Robillard RJ, Argy O: Factors influencing postoperative urinary retention in patients undergoing elective inguinal herniorrhaphy. Am J Surg. 1991; 161: 431-3; discussion 434.
4. Chen J, Matzkin H, Lazauskas T, Lelcuk S, Braf Z: Posthernioplasty urinary retention: a noninvasive work-up for prediction. Urol Int. 1993; 51: 243-5.

5. Barone JG, Cummings KB: Etiology of acute urinary retention following benign anorectal surgery. Am Surg. 1994; 60: 210-1.
6. Zaheer S, Reilly WT, Pemberton JH, Ilstrup D: Urinary retention after operations for benign anorectal diseases. Dis Colon Rectum. 1998; 41: 696-704.
7. Toyonaga T, Matsushima M, Sogawa N, Jiang SF, Matsumura N, Shimojima Y, et al.: Postoperative urinary retention after surgery for benign anorectal disease: potential risk factors and strategy forprevention. Int J Colorectal Dis. 2006; 21: 676-82.
8. Darrah DM, Griebling TL, Silverstein JH: Postoperative urinary retention. Anesthesiol Clin. 2009; 27: 465-84.
9. Fitzpatrick JM, Kirby RS: Management of acute urinary retention. BJU Int. 2006; 97(Suppl 2): 16-20; discussion 21-2.
10. Mohammadi-Fallah M, Hamedanchi S, Tayyebi-Azar A: Preventive effect of tamsulosin on postoperative urinary retention. Korean J Urol. 2012; 53: 419-23.
11. Livne PM, Kaplan B, Ovadia Y, Servadio C: Prevention of post-hysterectomy urinary retention by alpha-adrenergic blocker. Acta Obstet Gynecol Scand. 1983; 62: 337-40.
12. Gönüllü NN, Dülger M, Utkan NZ, Cantürk NZ, Alponat A: Prevention of postherniorrhaphy urinary retention with prazosin. Am Surg. 1999; 65: 55-8.
13. Lose G, Lindholm P: Prophylactic phenoxybenzamine in the prevention of postoperative retention of urine after vaginal repair: a prospectiverandomized double-blind trial. Int J Gynaecol Obstet. 1985; 23: 315-20.
14. Lucas MG, Stephenson TP, Nargund V: Tamsulosin in the management of patients in acute urinary retention from benign prostatic hyperplasia. BJU Int. 2005; 95: 354-7.
15. Faas CL, Acosta FJ, Campbell MD, O'Hagan CE, Newton SE, Zagalaniczny K: The effects of spinal anesthesia vs epidural anesthesia on 3 potential postoperative complications: pain, urinary retention, and mobility following inguinal herniorrhaphy. AANA J. 2002; 70: 441-7.
16. Bross S, Schumacher S, Scheepe JR, Zendler S, Braun PM, Alken P, et al.: Effects of acute urinary bladder overdistension on bladder response during sacral neurostimulation. Eur Urol. 1999; 36: 354-9.
17. Finley RK Jr, Miller SF, Jones LM: Elimination of urinary retention following inguinal herniorrhaphy. Am Surg. 1991; 57: 486-8; discussion 488-9.
18. Hindley RG, Brierly RD, Thomas PJ: Prostaglandin E2 and bethanechol in combination for treating detrusor underactivity. BJU Int. 2004; 93: 89-92.
19. Jensen P, Mikkelsen T, Kehlet H: Postherniorrhaphy urinary retention--effect of local, regional, and general anesthesia: a review. Reg Anesth Pain Med. 2002; 27: 612-7.
20. Goldman G, Leviav A, Mazor A, Kashtan H, Aladgem D, Greenstein A, et al.: Alpha-adrenergic blocker for postherniоplasty urinary retention. Prevention and treatment. Arch Surg. 1988; 123: 35-6.
21. Savona-Ventura C, Grech ES, Saliba I: Pharmacological measures to prevent post-operative urinary retention; a prospective randomized study. Eur J Obstet Gynecol Reprod Biol. 1991; 41: 225-9.

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Polyacrylamide Hydrogel (Bulkamid®) in Female Patients of 80 or More Years with Urinary Incontinence

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ABSTRACT

Introduction: To assess the effectiveness of polyacrylamide hydrogel (Bulkamid ®) in injection therapy for urinary incontinence in women of 80 or more years.
Materials and Methods: Twenty consecutive women mean age 84.5 (range 80-87) with stress or mixed urinary incontinence were enrolled in this prospective study. All subjects were evaluated at baseline and re-evaluated 7 days, 6,12,18 and 24 months after treatment. A detailed clinical evaluation, physical examination, daily pad count, urodynamic investigation and evaluation of urethral mobility by trans-labial ultrasound were performed.
Results: A statistically significant decrease in the number of pads was observed in the follow-up (p = 0.0002 after 24 months). Physical examination showed a statistically significant lack or reduced lost of urine with stress test (p = 0.0163 after 24 months). Urodynamic findings showed an increase of Valsalva leak point pressure, maximum urethral closure pressure and functional length. Maximum flow and post void residual were respectively observed to be significantly reduced and increased only after 7 days from injection therapy. Quality of life (QoL) assessed with the Incontinence Impact questionnaire short form (IIQ-7) showed a statistically significant improvement (p = 0.0001 after 24 months). Patient satisfaction assessed with the Visual Analogue Scale and Patient Global Impression of Improvement questionnaire respectively produced evaluation of "satisfied" and "much improved" even after 24 months.
Conclusions: Polyacrylamide hydrogel (Bulkamid®) is an effective treatment with low morbility in patients of 80 or more years.

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INTRODUCTION

The first report on the use of urethral injection therapy was in 1904 (1). Since then many substances have been used in the treatment of stress urinary incontinence in women looking for "agents" that were durable, non-migratory, hypoallergenic, biocompatible and which does not evoke granuloma formation (2): polytetrafluorethylene, bovine collagen, autologous fat, silicone, carbon spheres, calcium hydroxylapatite, porcine dermal implant, ethylene vinyl alcohol copolymer, hyaluronic acid and polyacrylamide hydrogel.

Polyacrylamide hydrogel (Bulkamid®) was introduced in Europe as a bulking agent in 2006 (3): it is a polymer gel consisting of 2.5% cross-linked polyacrylamide and 97.3% water for injection (4). It seems to have the necessary characteristics required of a bulking agent and appears to be effective and safe in women with stress or mixed incontinence (5).

Minimal invasiveness, low complication rate, use of local anaesthesia and non-hospitalization of patient make this a particularly recommended therapy for older women.

Therefore we decided to evaluate the efficacy of Bulkamid® on women of 80 or more years in a follow-up of 24 months.

MATERIALS AND METHODS

From January 2010 to September 2010, 20 consecutive women of 80 or more years (mean age 84.5, range 80-87) with stress or mixed urinary incontinence were enrolled in this prospective study.

A detailed clinical evaluation including a complete history, physical examination , daily pad count, urodynamic investigation and evaluation of urethral mobility by trans-labial ultrasound were performed.

Urodynamic investigation was performed including spontaneous uroflowmetry with post void residual (PVR) measurement, urethral pressure profile at rest, water cystometry (filling rate 30mL/min.; catheter used: 6 Fr double lumen; patients' position: sitting) with pressure/flow study and abdominal Valsalva leak point pressure (AVLPP).

All patients underwent transurethral injection in lithotomic position after local anesthesia (EMLA cream and after 10 minutes, lidocaine 5%: 8mL). Bulkamid® (2mL) was injected into the submucosa of the mid urethra using a 23 G needle and a special cystoscope (Bulkamid Urethral Bulking System®). The drug was injected at 6,3 and 9 o'clock. All subjects were evaluated at baseline and re-evaluated 7 days, 6,12,18 and 24 months after treatment.

Patients with urge incontinence, urinary tract infection, neurological disease, bladder lithiasis, prolapse of anterior vaginal wall and/or prolapse of the apical segment of vagina ≥ stage II on POP-Q system or pelvic tumours were excluded.

End points: reduction in number of pad per 24 hours was considered the primary efficacy endpoint in this study. Secondary end-points included changes in urodynamic findings evaluated (maximum flow (Qmax), PVR, AVLPP, functional length (FL) and maximum urethral closure pressure (MUCP).

Stress test assessment was performed with patients coughing in lithotomy position and bladder filled with 300mL of saline solution.

Patients were divided into four groups according to the extent of leakage: (-) no leakage; (+) low leakage (a few drops from the urethral meatus); (++) a discrete urine leakage (weak stream from the urethral meatus); (+++) a considerable urine leakage (strong jet from the urethral meatus).

Quality of life (QoL) was assessed with the Incontinence Impact Questionnaire short form (IIQ-7). The questionnaire consists of 7 items relating to the impact of UI on the woman's life with 5 possible options ranging from "not at all" (score = 0) to "almost always (score = 4) with a total score ranging from 0 (no impact of UI on the life of the subject) to 28 (maximum disturbance of UI on the life of the subject). Patient satisfaction was assessed with: a) Visual Analogue Scale (VAS) with the least satisfaction expressed as 0 and most satisfaction expressed as 10. Results between 0-3 were considered as not satisfied, 4-7 moderately satisfied and 8-10 as satisfied. b) Patient Global Impression of Improvement Questionnaire (PGI-I). The PGI-I is a validated generic tool for assessment of the overall improvement or deterioration that patients experience following the treatment. It is a 7-point scale from "very much improved" (score = 1) to very much worse (score = 7).

Number of pads, urodynamic findings, stress test and quality of life were performed before and at every periodic control. VAS and PGI-I were performed only after treatment at every periodic control.

All patients signed an informed consent before starting treatment.

Statistical analyses: Statistical analysis was performed using the MedCalc® software package (version 9.4.2.0.). Data are expressed as means ± SD and median. Comparisons were carried out using Wilcoxon test.

For stress test, statistical analysis was performed by Chi square test.

A p value of < 0.05 was considered significant.

RESULTS

Patients' characteristics are described in Table-1.

Average execution time of injection was 7 minutes (range 5-10).

Two patients required a further treatment. 18 women completed 24 months of follow-up. Urinary retention was present in two patients but only immediately after urethral injection.

Table 1 - Patients' characteristics.

Nº of patients at baseline	20
Nº of patients at 24 months	18
Mean age	84.5 (80-87)
Stress urinary incontinence	12
Mixed urinary incontinence	8
Previous anti-incontinence surgery	6
Body Mass Index	27 (20-34)
Hypermobility	7
Detrusor overactivity	7
Prolapse	4

In one patient mild urethral bleeding was observed. *De novo* urge incontinence was present in two patients only for a short period.

The number of pads showed a statistically significant reduction from the first control after 7 days, to the end of follow-up (Table-2).

Stress test showed both a statistically significant reduction of incontinent patients and a reduction in the amount of urine lost in all the controls carried out (Table-3).

Table-4 describes urodynamic results.

After 7 days Qmax showed a reduction with a subsequent moderate increase. PVR increased only after 7 days without a statistical significance and then decreased.

AVLPP showed a statistically significant increase in the controls performed after 7 days and after 6 months, whilst in successive controls values were still higher than pre-operative results, but there was no statistical significance. FL showed a statistically significant increase after 7 days and after 6 months.

MUCP showed a statistically significant increase for all 24 months considered.

Responses to items in the IIQ-7 showed a considerable improvement in the women's quality of life with a statistical significance that remained until the end of the follow-up (Table-5).

Table 2 - Number of pads used by the patients before and after treatment with Bulkamid®.

	Number of Pads					
	Before Treatment	After 7 days	After 6 months	After 12 months	After 18 months	After 24 months
mean ± st. dev.	5.5 ± 2.5	0.7 ± 1.6	1.1 ± 2.0	1.5 ± 2.6	1.6 ± 2.7	1.9 ± 2.8
median	5	0	0	0	0.5	1
*p-value		< 0.0001	< 0.0001	0.0001	0.0001	0.0002

*Wilcoxon test (paired samples).

Table 3 - Degree of urinary incontinence assessed by stress test before and after treatment with Bulkamid®.

	Stress Test					
	Before treatment	After 7 days	After 6 months	After 12 months	After 18 months	After 24 months
Degree	Patients	Patients	Patients	Patients	Patients	Patients
-	3	8	7	5	4	4
+	1	9	8	8	8	8
++	10	2	3	3	4	3
+++	6	1	1	3	2	3
*p-value		0.0005	0.0024	0.0135	0.0179	0.0163

*Chi Square test

Table 4 - Urodynamic findings before and after treatment with Bulkamid®.

	Before treatment	After 7 days	After 6 months	After 12 months	After 18 months	After 24 months
QMAX						
mean ± st. dev.	24 ± 11.7	19.6 ± 12.4	22.2 ± 13.7	22 ± 11.6	21.6 ± 12.1	22 ± 12.3
median	20	16	17	18	16.5	18
*p-value		0.0141	0.1089	0.0348	0.0448	0.1594
PVR						
mean ± st. dev.	15.7 ± 14.4	17 ± 20	6.6 ± 9.4	7.4 ± 9.6	7.2 ± 13	5 ± 8.8
median	15	10	0	0	0	0
*p-value		0.7197	0.0266	0.0353	0.0398	0.0034
AVLPP						
mean ± st. dev.	48.6 ± 17.3	75.5 ± 17.7	68.2 ± 23	58.3 ± 19	59.4 ± 17	59 ± 17.6
median	49	72.5	70	65	57.5	55.5
*p-value		0.0002	0.0591	0.1870	0.4954	0.6436
LF						
mean ± st. dev.	29.2 ± 5	36 ± 4.8	33.4 ± 3.5	31.6 ± 4.2	31.2 ± 4.7	30.7 ± 4.4
median	29	35.5	34	31	30.5	30
*p-value		0.0012	< 0.0001	0.5376	1	1
MUCP						
mean ± st. dev.	42.4 ± 18	59.7 ± 18.8	52 ± 17.3	50 ± 18.7	51 ± 16	50 ± 16.6
median	40	54.5	50	48	46.5	45
*p-value		< 0.0001	0.0066	0.0204	0.0056	0.0305

*Wilcoxon test (paired samples).

Table-6 presents patient evaluations of the results by VAS and PGI-I. After 7 days VAS showed very satisfactory results which remained even after 24 months, albeit with slightly lower values. Similar results were also obtained with PGI-I with most patients reporting a great improvement after 24 months.

The eight patients with mixed urinary incontinence in seven cases had a detrusor overactivity in the urodynamic examination. Three of these seven

Table 5 - Quality of life (QoL) assessed by Incontinence Impact Questionnaire short form (IIQ-7).

	IIQ-7					
	Before treatment	After 7 days	After 6 months	After 12 months	After 18 months	After 24 months
mean ± st. dev.	19.5 ± 7	3.1 ± 5.7	3.4 ± 5.5	5.3 ± 8.7	5.5 ± 5.7	6 ± 9.3
median	22	0	0	0	0	0
*p-value		< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0001

*Wilcoxon test (paired samples).

Table 6 - Patients' satisfaction after treatment with Bulkamid®, assessed by Visual Analogue Scale (VAS) and Patient Global Impression of Improvement questionnaire (PGI-I).

	After 7 days	After 6 months	After 12 months	After 18 months	After 24 months
VAS (mean)	9.0	8.5	8.2	8.2	8.1
PGI-I					
Degree of improvement	1.7	1.7	2.1	2.2	2.2

patients (45%) continued to have urgency after injection therapy with worse clinical results (an average of 5.2 pads a day after 24 months). The six patients who previously underwent suburethral slings showed no clinical and urodynamic differences in the results compared to other patients. Furthermore no difference was found in the results between patients with or without urethral hypermobility as well as in relation to the body mass index.

DISCUSSION

The results of this study seem to confirm the validity of Bulkamid® in the treatment of stress urinary incontinence, even in women of 80 or more years who often suffer from a more severe level of urinary incontinence and who have a higher percentage of mix incontinence compared to younger women.

The reduced number of pads used by patients that stays low even 24 months after treatment is most probably the most important result of this study and which leads to a positive evaluation in the responses to items in the questionnaire about QoL and in the results of VAS and PGI-I. Regarding the stress test and urodynamic findings, the good results obtained with the former and the improvement in the urodynamic parameters used in the evaluation of urinary incontinence (AVLPP, LF, MUCP) should be underlined. These results appear to be in agreement with finding of other authors (6-8).

Results of Qmax and PVR highlight the low obstructing capacity of Bulkamid® with Qmax values significantly reduced only at the 7th day control with values then returning approximately to normal. PVR shows a slight increase only at the 7 day control, then reduces. These results can explain the low incidence of post-treatment urine retention, reported also in other studies (3,5,9).

The presence of urgency and/or detrusor overactivity is very important in elderly women. In our experience 45 percent of patients with detrusor overactivity in the urodynamic study remain with urgency, with clinical results lower than patients without DO.

In previous articles some authors underlined that injection therapy was contraindicated in patients with DO (10-12). More recently, Chappel et al. (9) claimed that the success of injection therapy was reduced in patients with DO, but that its use should not be excluded because surgical procedures such as colposuspension and urethral slings more significantly cause *de novo* DO. Furthermore, positive results were achieved both in patients with stress incontinence and mixed incontinence (5).

In this study, the presence of previous anti-incontinence surgery (suburethral slings) did not influenced the success of injection therapy. These data are in agreement with those reported by others authors (9,13-15).

Body mass index did not affect the results probably because few patients were overweight.

Moreover, Bulkamid® was effective regardless of the presence or absence of urethral hypermobility confirming results described by other authors (16).

The study was partially conditioned by the fact that the patients we evaluated were aged 80 or more years. Almost from the start of the study we observed that the patients found it difficult to compile the micturition diary, normally used in this type of study, in particular those patients with severe incontinence who were not able correctly to quantify their loss of urine. For this reason we decided not to make use of this tool.

Compilation of the quality of life questionnaire was performed under supervision of a physi-

cian not involved in the study to facilitate the interpretation of the questions without influencing patients' assessments.

Indeed, the difficulties encountered in understanding the QoL questions represented a possible bias (9).

We also observed how these women were often resigned to their condition and in some cases they only came to request pads. It was rare for them to ask to be cured of incontinence, whilst more often they simply wanted to improve their condition, to be able to lead a better life.

They accepted injection therapy for its characteristics of low invasiveness, rapidity of execution, use of local anesthesia, lack of hospitalization and low complication rate. They would not have accepted more invasive surgical procedures preferring to remain incontinent.

In addition, the elderly woman is often affected by chronic diseases therefore more invasive surgical procedures are contraindicated.

Robinson et al. (17) suggested that women would choose to undergo less invasive procedures with a lower risk of complications, even though the chance of cure may be lower than with a major operation.

Although intervention for SUI is generally focused on minimizing urine leakage, the overall impact of treatment on patient QoL is arguably more important than the treatment outcome regarding leakage (2). This consideration is even truer for an aging patient. It is thus of great importance to inform elderly women of this treatment opportunity.

Another interesting question is if in elderly patients urodynamic assessment should be performed before injection therapy. Whilst there is no doubt regarding the need to perform urodynamic assessment before more invasive surgical procedures (colposuspension, suburethral slings), the evidence base to date supports the view that simple evaluation is adequate before injection therapy (14).

Studies published about injection therapy are numerous and often difficult to evaluate because of their heterogeneity: the various materials used, the technique (transurethral or periurethral), the injection site (bladder neck, mid-urethral), the heterogeneity of patients, the length of follow-up and the different urodynamic findings (presence of absence of DO). The results are also heterogeneous with percentages of improvement or cure between 40 and 100% (18), 15 and 94% (16). In literature, we found only one study on very old women (older than 75 years) with a percentage of 77% of patients cured or improved (19).

In a recent analysis of 500 cases, Mohr et al., in a review of literature, underlined in an elderly population a subjective and objective improvement in incontinence after bulking therapy (8).

In spite of the complexity of the theme and the lack of consistency in results, injection therapy should be seen as an important surgical therapy, in particular in patients who cannot or do not accept to undergo other forms of therapy.

The moderate reduction of the positive effects after 2 years also detected in other surgical procedures performed in patients with urinary incontinence and the occasional necessity to repeat the treatment do not reduce the validity of the therapy and allows a further period of good health. We must also emphasize that these patients have a low life expectancy and every year spent in a satisfactory manner is a good result.

CONCLUSIONS

The results of this study showed the effectiveness and low morbidity of Bulkamid® in women of 80 or more years with stress and mixed urinary incontinence. In these patients, often affected by chronic diseases and other health problems, this treatment should be considered as first line therapy.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Meyer L: To tilfælde af incontinentia urinæ helbredede ved parafininjektioner. Ugeskr Læger 1904;104:5.
2. Kotb AF, Campeau L, Corcos J: Urethral bulking agents: techniques and outcomes. Curr Urol Rep. 2009; 10: 396-400.
3. Lose G, Mouritsen L, Nielsen JB: A new bulking agent (polyacrylamide hydrogel) for treating stress urinary incontinence in women. BJU Int. 2006; 98: 100-4.

4. Bello G, Jackson IT, Keskin M, Kelly C, Dajani K, Studinger R, et al.: The use of polyacrylamide gel in soft-tissue augmentation: an experimental assessment. Plast Reconstr Surg. 2007; 119: 1326-36.
5. Lose G, Sørensen HC, Axelsen SM, Falconer C, Lobodasch K, Safwat T. An open multicenter study of polyacrylamide hydrogel (Bulkamid®) for female stress and mixed urinary incontinence. Int Urogynecol J. 2010; 21: 1471-7.
6. Kuhn A, Stadlmayr W, Lengsfeld D, Mueller MD: Where should bulking agents for female urodynamic stress incontinence be injected? Int Urogynecol J Pelvic Floor Dysfunct. 2008; 19: 817-21.
7. Faerber GJ: Endoscopic collagen injection therapy in elderly women with type I stress urinary incontinence.J Urol. 1996; 155: 512-4.
8. Mohr S, Siegenthaler M, Mueller MD, Kuhn A: Bulking agents: an analysis of 500 cases and review of the literature. Int Urogynecol J. 2013; 24: 241-7.
9. Chapple CR, Wein AJ, Brubaker L, Dmochowski R, Pons ME, Haab F, et al.: Stress incontinence injection therapy: what is best for our patients? Eur Urol. 2005; 48: 552-65.
10. Su TH, Hsu CY, Chen JC: Injection therapy for stress incontinence in women. Int Urogynecol J Pelvic Floor Dysfunct. 1999; 10: 200-6.
11. Bartholomew B, Grimaldi T: Collagen injection therapy for type III stress urinary incontinence. AORN J. 1996; 64: 75-83, 85-6.
12. Appell RA: Collagen injection therapy for urinary incontinence. Urol Clin North Am. 1994; 21: 177-8.
13. Monga AK, Robinson D, Stanton SL: Periurethral collagen injections for genuine stress incontinence: a 2-year follow-up. Br J Urol. 1995; 76: 156-60.
14. Peeker R, Edlund C, Wennberg AL, Fall M: The treatment of sphincter incontinence with periurethral silicone implants (macroplastique). Scand J Urol Nephrol. 2002; 36: 194-8.
15. Haab F, Zimmern PE, Leach GE: Urinary stress incontinence due to intrinsic sphincteric deficiency: experience with fat and collagen periurethral injections. J Urol. 1997; 157: 1283-6. Erratum in: J Urol 1997; 158: 188.
16. Lightner DJ, Itano NB, Sweat SD, Chrouser KL, Fick F: Injectable agents: present and future. Curr Urol Rep. 2002; 3: 408-13.
17. Robinson D, Anders K, Cardozo L, et al.: What do women want? Interpretation of the concept of cure. J Pelvic Med Surg. 2003;9:273-7.
18. Chapple CR, Wein AJ, Artibani W, Brubaker L, Haab F, Heesakkers JP, et al.: A critical review of diagnostic criteria for evaluating patients with symptomatic stress urinary incontinence. BJU Int. 2005; 95: 327-34.
19. Zullo MA, Ruggiero A, Montera R, Plotti F, Muzii L, Angioli R, et al.: An ultra-miniinvasive treatment for stress urinary incontinence in complicated older patients. Maturitas. 2010; 65: 292-5.

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ORIGINAL ARTICLE

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Associated factors and prevalence of erectile dysfunction in hemodialysis patients

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ABSTRACT

Purpose: The proposal of this study was to determine the prevalence and the associated factors of erectile dysfunction (ED) among hemodialysis (HD) patients.
Materials and Methods: This was a cross-sectional study based on data collected from HD male patients. Clinical, demographic and laboratory data of all patients were collected in three HD clinics from December 2010 to June 2011. Patients answered questions of erectile function domain from International Index of Erectile Function. Data were evaluated by descriptive analysis and by univariate (ULRA) and multivariate logistic regression analysis (MLRA).
Results: Three hundred and five patients participated of the study. The prevalence of ED was 68.19%. ED was associated with diabetes (DM), benign prostatic hyperplasia, glomerulonephritis as cause of chronic renal failure (CRF), smoking habits, lower creatinine levels (ULRA), use of calcium channel blocker (MLRA), aging, lower education level, alcohol consumption, DM (as cause of CRF) and coronary insufficiency (ULRA and MLRA).
Conclusions: ED was highly prevalent in the HD men. It was independently associated with aging, current use of alcohol, long alcohol use (even for those who do not drink more), lower education level, diabetes as cause of CRF, coronary insufficiency and use of channel blockers calcium.

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INTRODUCTION

Erectile dysfunction (ED) is a medical problem that alters patient's quality of life due to association with many problems as anxiety, loss of self-esteem, depression and marital misfit (1). The association of chronic renal failure (CRF) with ED is a well known fact (2). The population of CRF patients in hemodialysis (HD) is growing, in part, because of longer survival (3). This fact has highlighted the importance of quality of life of the HD patients (4), including the erectile function (EF).

The prevalence and determinants of ED are highly variable among HD patients. The determinants are not fully known and the prevalence ranges from 43 to 87% (5-13). These facts occur due to the lack of standardization of assessment of ED by previous researches (8-10).

In 1997, Rosen et al. reported the International Index of Erectile Function (IIEF) (14). This instrument has been shown to be a cross-culturally and psychometrically valid measurement of male ED. It is a brief, reliable, and valid self-administered questionnaire that standardizes

ED evaluation and allows the determination of the prevalence of this disorder.

In this study, we aimed to determine the prevalence and the associated factors of ED among HD patients.

MATERIALS AND METHODS

This was a cross-sectional study based on data collected in three HD centers, from December 2010 to June 2011. The HD centers are not linked or associated with each other or any University; they provide services for patients in the public health system and patients with and without health insurance and perform dialysis of approximately one third of the hemodialysis population of Goiânia, Brazil.

The present study was approved by the Ethics Committee of the Federal University of Goiás Clinical Hospital (under registration nº. 090/2011). Patients included in this work were male voluntary with 18 years of age or older undergoing HD therapy for at least three months. Patients were excluded if they did not complete the EF domain from IIEF, refused to participate in the study, did not sign the consent form or had cognitive or communication impairment.

The six questions of EF domain from IIEF (13) were self answered by each patient during HD sections. This study used the translated Portuguese version of the IIEF. The score for each item ranges from 0 to 5 for questions 1-5 and from 1 to 5 for the question 15. The severities of ED were defined as follows: normal (no ED): 25-30, mild: 19-24, mild to moderate: 13-18, moderate: 7-12, and severe: 1-6 (according to IIEF-1997).

It was considered the diagnosis of comorbidities. Clinical data described in this work were obtained from review of medical records. They included: body mass index (BMI), etiology of CRF, time on HD, presence of hypertension, Diabetes Mellitus (DM), heart or prostate diseases and current medications used.

The following socio demographic variables were collected from each patient in an interview during HD: age, civil status, education level, history of illegal drugs use, cigarettes or alcohol use.

Laboratory parameters were obtained by blood samples at midweek dialysis. These samples were collected up to 30 days before patient's interview. Laboratory data included: creatinine, hemoglobin, hematocrit, triglycerides, low density lipids (LDL)-cholesterol, high density lipids (HDL)-cholesterol, total cholesterol, albumin and parathormone (PTH) levels. To ascertain the adequacy of dialysis prescription for each patient, dialyzer clearance of urea X dialysis time/volume of distribution of urea (Kt/V) was estimated, using laboratory results gathered from chart review of the previous month to the study.

The data were tabulated on Microsoft® Excel 2007 and evaluated by Statistical Package for the Social Sciences (SPSS) 15 for Windows. A descriptive analysis of score of EF domain from IIEF and of the clinical, socio demographic and laboratory data from all patients was carried out. Continuous variables were divided into categories to facilitate analysis (categorical variable). All variables were evaluated by the proportion in each category. The univariate logistic regression analysis (ULRA) was performed on the EF domain from IIEF versus clinical, socio demographic and laboratory data. Those variables in which the ULRA had a P value less than 0.1 were considered for inclusion in multivariable logistic regression analysis (MLRA). Results were expressed as adjusted odds ratios and their 95% confidence interval. Statistical significance was set at $P < 0.05$ in all analysis.

RESULTS

Among 349 eligible patients, 305 (87.39%) participated of this study. Forty-four (12.60%) patients were excluded, 29 (8.30%) did not complete the EF domain from IIEF, 7 (2.00%) refused to take part of the study, 3 (0.85%) refused to sign a declaration of informed consent and 5 (1.43%) had cognitive or communication deficits. The mean ± SD age was 54.09 ± 13.17 years, the schooling time was 7.41 ± 4.60 years and the HD time was 50.65 ± 41.15 months. The underlying etiologies of renal failure of the patients who participated in the study included hypertension (34.42%), Diabetes Mellitus (18.36%),

glomerulonephritis (7.54%), polycystic kidney (5.24%), abuse of medications (3.93%), unknown (24.59%) and others (5.57%).

The prevalence of ED was 68.19%. The degree of ED among HD patients was mild in 9.83%, mild to moderate in 6.22%, moderate in 9.18%, and severe in 42.95% of patients. Among HD patients with ED, 11 (5.88%) received some type of treatment. Nine (4.82%) used oral drugs, 1 (0.53%) intracavernosal drugs and 1 (0.53%) patient received a penile prosthesis.

Most patients (81.31%) were using some antihypertensive. Despite this no antihypertensive drugs (diuretics, adrenergic inhibitors, direct vasodilators, calcium channel blockers, angiotensin converting enzyme inhibitors, angiotensin receptor blockers 1 and direct renin inhibitors) or other medications (erythropoietin, anxiolytic, anticonvulsant and antidepressant) were found to increase the probability of ED on ULRA.

ED was associated with, aging, lower education level (Table-1), history of cigarette use previous or current, duration of smoking (smoking or smoked), being an ex-smoker, higher pack-year index, pattern of high consumption of cigarettes (current or previous) (Table-2), history of alcohol consumption (current and past) current alcohol consumption, longer alcohol consumption (current or previous) (Table-3), the presence of diabetes (regardless of being or not the cause of the CRF), BPH or coronary insufficiency (Table-4), lower creatinine levels (Table-5), DM and glomerulonephritis (as underlying etiologies of renal failure) (Table-6) on ULRA.

Hypertension, history of drug use (previous or current), use of calcium channel blockers or adrenergic inhibitors had P between 0.05 and 0.10 on ULRA, therefore, these variable was assessed by MLRA.

ED was independently associated with aging, current use of alcohol, long alcohol use (even for those who do not drink anymore), lower education level, DM (as cause of CRF), coronary insufficiency and use of channel blockers calcium (Table-7). The aging, DM (as cause of CRF), and

Table 1 - Association of socio demographic data with Erectile Dysfunction in hemodialysis patients.

Socio demographic data	Without ED		With ED		P-values*	OR (95% CI)
	n	%	n	%		
Age (years)						
< 50	64	66.0	49	23.6		
≥ 50	33	34.0	159	76.4	< 0.001	6.293 (3.711-10.672)
Marital status						
Single	28	28.9	65	31.3		
Married	69	71.1	143	68.8	0.674	
Body mass index						
< 25	65	67.0	137	65.9		
≥ 25	32	33.0	71	34.1	0.844	
Educational level						
Incomplete elementary education	47	48.5	133	63.9		
Elementary school or up graduate	50	51.5	75	36.1	0.011	1.89 (1.16-3.07)

*Estimated by univariate logistic regression analysis; **ED =** Erectile Dysfunction; **OR =** odds ratio;
P = Statistical significance; **95% CI =** 95% confidence interval.

Table 2 - Association of smoking habits with Erectile Dysfunction in hemodialysis patients.

Smoking habits	Without ED		With ED		P-values*	OR (95% CI)
	n	%	n	%		
History of current or previous smoking						
No	54	55.7	85	40.9		
Yes	43	44.3	123	59.1	0.016	1.82 (1.12-2.96)
Active smoker[a]						
No	86	88.7	187	89.9		
Yes	11	11.3	21	10.1	0.741	0.88 (0.41-1.90)
Former smoker[b]						
No	65	67.0	106	51.0		
Yes	32	33.0	102	49.0	0.009	1.95 (1.18-3.23)
Nonsmokers[c]						
No	43	44.3	123	59.1		
Yes	54	55.7	85	40.9	0.016	1.82 (1.12-2.96)
Pattern of current cigarette smoking (smokers)						
Light[d]	7	63.6	14	70.0		
Moderate[e]	3	27.3	5	25.0		
Heavy[f]	1	9.1	1	5.0	0.647	0.76 (0.23-2.49)
Pattern of current cigarette smoking (smokers and former smokers)						
Light[d]	23	53.5	43	35.5		
Moderate[e]	12	27.9	42	34.7		
Heavy[f]	8	18.6	36	29.8	0.045	1.59 (1.01-2.51)
Years of smoking (smoking or smoked)						
< 20	22	51.2	32	26.2		
≥ 20	21	48.8	90	73.8	0.003	2.95 (1.43-6.06)
Pack-year index						
< 20	30	69.8	56	46.3		
≥ 20	13	30.2	65	53.7	0.009	2.68 (1.27-5.63)

*Estimated by univariate logistic regression analysis; [a]Smoked more than 100 cigarettes and currently smoke; [b]Smoked or had smoked up to 100 cigarettes and currently do not smoke; [c]Smoked or had smoked a maximum of 100 cigarettes and currently do not smoke; [d]Smoked more than 100 cigarettes and currently or previously smokes up to 10 cigarettes / day; [e]Smoked more than 100 cigarettes and currently or previously smokes 10 to 20 cigarettes / day; [f]Smoked more than 100 cigarettes and currently or previously smokes more than 20 cigarettes / day; **ED =** Erectile Dysfunction; **OR =** Odds ratio; **P =** Statistical significance; **95% CI =** 95% confidence interval.

Table 3 - Association of habit of use of alcohol and drugs with Erectile Dysfunction in hemodialysis patients.

Parameters	Without ED		With ED		P-values*	OR (95% CI)
	n	%	n	%		
History of alcohol use current or previous						
No	12	12.4	51	24.5		
Yes	85	87.6	157	75.5	0.017	2.30 (1.16- 4.55)
Current use of alcohol [a]						
No	70	72.2	182	87.5		
Yes	27	27.8	26	12.5	0.001	2.70 (1.47-4.94)
Former user of alcohol [b]						
No	39	40.2	77	37.0		
Yes	58	59.8	131	63.0	0.593	1.14 (0.70-1.87)
Never used alcohol [c]						
No	85	87.6	157	75.5		
Yes	12	12.4	51	24.5	0.017	2.30 (1.16-4.55)
Pattern of alcohol consumption (current alcohol user)						
Moderate [d]	22	81.5	23	88.5		
Excessive [e]	5	18.5	3	11.5	0.481	1.74 (0.37-8.18)
Pattern of alcohol consumption (former and current alcohol users)						
Moderate [d]	37	43.5	66	41.8		
Excessive [e]	48	56.5	92	58.2	0.792	1.07 (0.63-1.83)
Years use of alcohol (even if already not drink any more)						
< 20	42	49.4	45	28.7		
≥ 20	43	50.6	112	71.3	0.001	2.43 (1.41-4.21)
Current or previous history of illegal drug use						
No	85	87.6	196	94.2		
Yes	12	12.4	12	5.8	0.051	

*Estimated by univariate logistic regression analysis; [a]Currently use alcohol regularly; [b]Currently does not use alcohol but had already regularly used; [c]Never used alcohol. [d]Regular or previously use of alcohol up to 2 doses (350mL of beer or 150mL of wine or 50mL of distilled) / day; [e]Regular or previously use of alcohol, 5 doses / occasion or more / at least 1 time / week or three or more doses daily;
ED = Erectile Dysfunction; **OR =** Odds ratio; **P =** Statistical significance; **95% CI =** 95% confidence interval.

Table 4 - Association of clinical data with Erectile Dysfunction in hemodialysis patients.

Clinical data	Without ED		With ED		P-values*	OR (95% CI)
	n	%	n	%		
Diabetes						
No	88	90.7	126	60.6		
Yes	9	9.3	82	39.4	< 0.001	6.363 (3.036-13.339)
Hypertension						
No	21	21.6	51	24.5		
Yes	76	78.4	157	75.5	0.583	
Cardiac arrhythmia						
No	95	97.9	196.0	94.2		
Yes	2	2.1	12.0	5.8	0.168	
Coronary insufficiency						
No	96	99.0	180.0	86.5		
Yes	1	1.0	28.0	13.5	0.008	14.93 (2.00-111.45)
Congestive heart failure						
No	95	97.9	199	95.7		
Yes	2	2.1	9	4.3	0.334	
Cardiac valvulopathy						
No	97	100.0	204	98.1		
Yes	0	0.0	4	1.9		
Other heart diseases						
No	95	97.9	195	93.8		
Yes	2	2.1	13	6.3	0.134	
Benign prostatic hyperplasia						
No	95	97.9	179	86.1		
Yes	2	2.1	29	13.9	0.006	7.70 (1.80-32.95)
Prostate cancer						
No	97	100.0	206	99.0		
Yes	0	0.0	2	1.0		
Time on hemodialysis (months)						
< 48	50	52.1	122	59.2		
≥ 48	46	47.9	84	40.8	0.244	
Long of diagnosis of chronic renal failure(months)						
< 48	38	39.6	88	42.9		
≥ 48	58	60.4	117	57.1	0.584	

*Estimated by univariate logistic regression analysis; **ED =** Erectile Dysfunction; **HD =** hemodialysis; **OR =** Odds ratio; **P =** Statistical significance; **95% CI =** 95% confidence interval.

Table 5 - Association of laboratory values and Erectile Dysfunction in hemodialysis patients.

Laboratory parameters	Without ED		With ED		P-values*	OR (95% CI)
	n	%	n	%		
Hemoglobin (g/dL)						
< 10	24	24.7	51	24.5		
≥ 10	73	75.3	157	75.5	0.966	
Hematocrit (%)						
< 30	22	22.7	48	23.1		
≥ 30	75	77.3	160	76.9	0.939	
Albumin (g/100mL)						
< 3.5	3	3.1	9	4.4		
≥ 3.5	93	96.9	195	95.6	0.598	
Cholesterol (mg/dL)						
< 200	75	78.1	150	75.4		
≥ 200	21	21.9	49	24.6	0.603	
Cholesterol LDL (mg/dL)						
< 130	84	89.4	170	87.6		
≥ 130	10	10.6	24	12.4	0.669	
Cholesterol HDL (mg/dL)						
< 40	48	50.0	88	44.4		
≥ 40	48	50.0	110	55.6	0.371	
Triglycerides (mg/dL)						
< 150	47	49.0	85	42.7		
≥ 150	49	51.0	114	57.3	0.313	
Creatinine (mg/dL)						
< 8	15	15.5	66	31.7		
≥ 8	82	84.5	142	68.3	0.003	0.394 (0.211-0.734)
Parathormone (pg/mL)						
< 300	42	43.3	109	52.7		
≥ 300	55	56.7	98	47.3	0.129	
Kt/V						
< 1.2	21	21.6	34	16.7		
≥ 1.2	76	78.4	170	83.3	0.297	

*Estimated by univariate logistic regression analysis; **ED =** Erectile dysfunction; **HDL =** High density lipids; **Kt/V =** Dialyzer clearance of urea X dialysis time/volume of distribution of urea; **LDL =** Low density lipids; **OR =** Odds ratio; **P =** Statistical significance; **95% CI =** 95% confidence interval.

Table 6 - Association of cause of chronic renal failure with Erectile Dysfunction in hemodialysis patients.

Cause of chronic renal failure	Without ED		With ED		P-values*	OR (95% CI)
	n	%	n	%		
Medication abuse						
No	91	93.8	202	97.1		
Yes	6	6.2	6	2.9	0.177	0.45 (0.14-1.43)
Diabetes Mellitus						
No	92	94.8	157	75.5		
Yes	5	5.2	51	24.5	< 0.001	5.98 (2.30-15.51)
Glomerulonephritis						
No	84	86.6	198	95.2		
Yes	13	13.4	10	4.8	0.011	3.06 (1.29-7.26)
Hypertension						
No	57	58.8	143	68.8		
Yes	40	41.2	65	31.3	0.088	1.54 (0.94-2.54)
Polycystic disease						
No	91	93.8	197	94.7		
Yes	6	6.2	11	5.3	0.751	0.85 (0.30-2.36)
Unknown[a]						
No	75	77.3	155	74.5		
Yes	22	22.7	53	25.5	0.597	1.17 (0.66-2.06)
Others						
No	92	94.8	196	94.2		
Yes	5	5.2	12	5.8	0.828	1.13 (0.39-3.29)
Total	97	**100.0**	**208**	**100.0**		

*Estimated by univariate logistic regression analysis; [a]Patients with more than one possible cause of chronic renal failure; **ED =** Erectile Dysfunction; **OR =** Odds ratio; **P =** Statistical significance; **CI =** confidence interval.

coronary insufficiency increased the risk of ED in 5.24, 7.24 and 11.31 times respectively (Table-7). These three variables were those that had the greatest association with ED.

DISCUSSION

A high prevalence of 68.19% of ED in HD patients was found in our work. The prevalence of ED in HD patients has a wide variability among studies (43 to 87.7%) probably due to different methodologies and diagnostic criteria (5-13). The studies used different definitions and tools for ED assessment; some of them had low number of patients, were performed in a single-center evaluation and the populations evaluated differed from each other (6-9,12,13). Despite the great variability of ED in HD patients the most recent studies

Table 7 - Model of Multivariate Regression Logistic Analysis to identify factors independently associated with erectile dysfunction in hemodialysis patients.

Factor	P-values*	OR (95% CI)
Current use of alcohol	0.006	3.25 (1.41-7.51)
Educational level	0.006	2.66 (1.32- 5.37)
Diabetes	0.003	7.24 (1.99-26.36)
Age	< 0.001	5.24 (2.61-10.49)
Calcium channel blockers	0.037	2.26 (1.05-4.84)
Coronary insufficiency	0.025	11.31 (1.35-94.54)
Years of use of alcohol (even if do not drink any more)	0.036	2.28 (1.06-4.90)

*Estimated by multivariate logistic regression analysis; **OR =** Odds ratio; **P =** Level of statistical significance; **95% CI =** 95% confidence interval.

showed high prevalence. Severe ED rate of 42.95% observed in our study is similar to results of previous researches that observed proportions ranging from 25.4 to 45.8% (6,7,10-12).

Similar to our results, several researchers showed association of ED with increased age of HD patients (5-8,10-12,15,16). In the Massachusetts Male Aging Study (17) (2004), the prevalence of ED was strongly and independently associated with age. Despite this, the NIH Consensus Development Panel on Impotence (18) (1993) concluded that since other risk factors for ED appear with aging, ED could not be considered a direct result of aging. One of these age-related risk factors is the presence of atherosclerosis, which is also a known risk factor for ED (19). Atherosclerosis is responsible for the abnormal function and responsiveness of penile vasculature, and it can also contribute to the pathogenesis of ED (16).

There is no consensus about the impact of education status on ED. Although some studies showed no association between education level and ED (7,11), Moreira et al. (19) (2002) found that the education level is inversely correlated to ED and Johannes et al. (21) (2000) reported that the risk of developing age-related ED was higher in men with lower education degree. A possible reason is that poor education can be associated with low socioeconomic background. It is likely that people with lower education have more difficulty to health care access, so these people are subjected to higher rates of comorbidities associated with HD and/or less adequate treatment of uremia itself. Our data showed that independent of other factors, patients with lower education had more ED.

DM is the most common cause of CRF (15), and it is a risk factor for ED (17). As ED occurs in almost all diabetic patients with CRF (7,8) in our work, DM was also an independent risk factor of ED in male HD patients. Causes of ED in DM men are several: vascular disease (22), autonomic neuropathy, gonadal dysfunction, impaired neurogenic and endothelium mediated relaxation of penile smooth muscle (23,24) and additionally HD (6,7,10).

There is an association of ED and cardiovascular diseases (19). Endothelium dysfunction occurs in cardiovascular diseases and it may contribute to the pathogenesis of ED (25). The current study found an independent association between coronary insufficiency and ED.

In the present study, ED was positively and independently associated with current alcohol use and length of consumption of alcohol (past or current). Although individuals may be sexually disinhibited through the use of alcohol, clinical studies have shown that alcohol abuse causes irreversible damage to nerve endings in penis tissue, which is manifested as ED (26).

Rosas et al. (10) (2001) found among HD patients that history of prostatic diseases was a statistically significant predictor of ED in unadjusted analyses. Lue (26) (2000) regards possible association of ED and benign prostatic hyperplasia (BPH), however, Stolic and Bukumiric (19) (2010) indicated that BPH did not constitute significant parameters among ED patients. In the present research, the association between ED and BPH was observed, however, not independently of other factors.

Several researches showed association between ED and cigarette use (27-29). This occurred because smoking causes vasoconstriction in the penile venous plexus and thereby affects contraction of cavernous smooth muscle, which has a negative effect on EF (26). Despite this, other studies did not find this association (8,13,15,16,19). Association between ED and smoking habits (previous or current) not independent of other factors was found in this work.

Although high pre-dialysis creatinine levels significantly associated with sexual dysfunction are frequently reported (5,30), Messina et al. (7) (2007) found that pre-dialysis creatinine levels were significantly lower in patients with ED. Our study showed association between lower creatinine levels and ED only on unadjusted analysis for confounding factors, as in Rosas et al. (10) (2001) study. The decreased creatinine levels in the ED patients may be a reflection of reduced muscle mass in older patients that are most likely to present ED.

No association of ED and PTH in HD patients has been verified in the present work and others (9,11,19). However, Rosas et al. (10) (2001) found lower levels of PTH associated with ED only on unadjusted analysis for confounding factors. In small-scale, studies have been advocating that treatment of secondary hyperparathyroidism may result in significant improvement in sexual function in patients with CRF (31).

Patients with CRF usually do no present significant association between the use of antihypertensive drugs and ED (8,10). On the other hand, in a revision research that lists the principal drugs used by the general population that are associated with ED, antihypertensive drugs were frequently found (32). In the current study it was found an independent association between ED and the use of calcium channel blockers. It is difficult, however, to determine whether the erectile impairment in controlled hypertension is due to the influence of the disease, medications, or both (10).

There are many considerations in this study that limit our findings. First, our sample size was relatively small, so this limits the possibility of detecting some interactions such as ED and obesity or hypertension. Second, this is a cross-sectional study, so it is just possible to establish association links between ED and risk factors without certainty of a causal relation between them. Third, this study did not assess variables that have been linked to ED, such as autonomic neuropathy, peripheral vascular disease, residual renal function and levels of testosterone, prolactin, zinc and thyroid hormones.

CONCLUSIONS

In summary, in this research, the HD men had high prevalence of ED and the main variables associated were higher age, current use of alcohol, long alcohol use (even for those who do not drink more), lower education level, DM (as cause of CRF), coronary insufficiency and use of channel blockers calcium.

ABBREVIATIONS

BMI = body mass index
BPH = benign prostatic hyperplasia
CRF = chronic renal failure
DM = Diabetes Mellitus
ED = erectile dysfunction
EF = erectile function
HD = hemodialysis
HDL = high density lipids
IIEF = International Index of Erectile Function
Kt/V = dialyzer clearance of urea X dialysis time/ volume of distribution of urea
LDL = low density lipids
MLRA = multivariable logistic regression analysis
PTH = parathormone
SD = standard deviation
SPSS = Statistical Package for the Social Sciences
ULRA = univariate logistic regression analysis

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Makhlouf A, Kparker A, Niederberger CS:Depression and erectile dysfunction. Urol Clin North Am. 2007; 34: 565-74, vii.
2. Angulo J: Re: Erectile dysfunction in patients with chronic renal failure. Int Braz J Urol. 2007; 33: 845-6.
3. U S Renal Data System: USRDS 2012 Annual Data Report: Atlas of Chronic Kidney Disease and End-Stage Renal Disease in the United States, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases; Bethesda, MD, 2012; vol. 1, chapter 3.
4. Rosas SE, Joffe M, Franklin E, Strom BL, Kotzker W, Brensinger C, et al.: Association of decreased quality of life and erectile dysfunction in hemodialysis patients. Kidney Int. 2003; 64: 232-8.
5. Malekmakan L, Shakeri S, Haghpanah S, Pakfetrat M, Sarvestani AS, Malekmakan A. Epidemiology of erectile dysfunction in hemodialysis patients using IIEF questionnaire. Saudi J Kidney Dis Transpl. 2011; 22: 232-6.
6. Makarem AR, Karami MY, Zekavat OR: Erectile dysfunction among hemodialysis patients. Int Urol Nephrol. 2011; 43: 117-23.
7. Messina LE, Claro JA, Nardozza A, Andrade E, Ortiz V, Srougi M: Erectile dysfunction in patients with chronic renal failure. Int Braz J Urol. 2007 ; 33: 673-8.
8. Cerqueira J, Moraes M, Glina S: Erectile dysfunction: prevalence and associated variables in patients with chronic renal failure. Int J Impot Res. 2002; 14: 65-71.
9. Toorians AW, Janssen E, Laan E, Gooren LJ, Giltay EJ, Oe PL, Donker AJ, Everaerd W: Chronic renal failure and sexual functioning: clinical status versus objectively assessed sexual response. Nephrol Dial Transplant. 1997; 12: 2654-63.
10. Rosas SE, Joffe M, Franklin E, Strom BL, Kotzker W, Brensinger C, et al.: Prevalence and determinants of erectile dysfunction in hemodialysis patients. Kidney Int. 2001; 59: 2259-66.
11. Neto AF, de Freitas Rodrigues MA, Saraiva Fittipaldi JA, Moreira ED Jr.: The epidemiology of erectile dysfunction and its correlates in men with chronic renal failure on hemodialysis in Londrina,southern Brazil. Int J Impot Res. 2002; 14 Suppl 2:S19-26.
12. Collaborative Depression and Sexual Dysfunction in Hemodialysis Working Group, Vecchio M, Palmer S, De Berardis G, Craig J, Johnson D, et al.: Prevalence and correlates of erectile dysfunction in men on chronic haemodialysis: a multinational cross-sectional study. Nephrol Dial Transplant. 2012 ; 27: 2479-88.
13. Seck SM, Dahaba M, Diouf B, Cisse MM, Gueye S, Ka EF, et al.: The burden of erectile dysfunction in dialysis patients in Senegal. Hemodial Int. 2011; 15: 280-3.
14. Rosen RC, Riley A, Wagner G, Osterloh IH, Kirkpatrick J, Mishra A: The international index of erectile function (IIEF): a multidimensional scale for assessment of erectile dysfunction. Urology. 1997; 49: 822-30.
15. Naya Y, Soh J, Ochiai A, Mizutani Y, Ushijima S, Kamoi K, et al.: Significant decrease of the International Index of Erectile Function in male renal failure patients treated with hemodialysis. Int J Impot Res. 2002; 14: 172-7.
16. Ali ME, Abdel-Hafez HZ, Mahran AM, Mohamed HZ, Mohamed ER, El-Shazly AM, Gadallah AM, et al.: Erectile dysfunction in chronic renal failure patients undergoing hemodialysis in Egypt. Int J Impot Res. 2005; 17: 180-5. Erratum in: Int J Impot Res. 2005; 17: 390.
17. Feldman HA, Goldstein I, Hatzichristou DG, Krane RJ, McKinlay JB: Construction of a surrogate variable for impotence in the Massachusetts Male Aging Study. J Clin Epidemiol. 1994; 47: 457-67.
18. [No authors listed]: NIH Consensus Conference. Impotence. NIH Consensus Development Panel on Impotence. JAMA. 1993; 270: 83-90.
19. Stolic RV, Bukumiric ZM: Intima-media thickness of carotid arteries and erectile dysfunction in hemodialysis patients. Hemodial Int. 2010; 14: 510-4.
20. Moreira ED Jr, Lisboa Lôbo CF, Villa M, Nicolosi A, Glasser DB: Prevalence and correlates of erectile dysfunction in Salvador, northeastern Brazil: a population-based study. Int J Impot Res. 2002; 14 (Suppl 2): S3-9.
21. Johannes CB, Araujo AB, Feldman HA, Derby CA, Kleinman KP, McKinlay JB: Incidence of erectile dysfunction in men 40 to 69 years old: longitudinal results from the Massachusetts male aging study. J Urol. 2000; 163: 460-3.
22. Jevtich MJ, Edson M, Jarman WD, Herrera HH: Vascular factor in erectile failure among diabetics. Urology. 1982; 19: 163-8.
23. Whitehead ED, Klyde BJ: Diabetes-related impotence in the elderly. Clin Geriatr Med. 1990; 6: 771-95.
24. Saenz de Tejada I, Goldstein I, Azadzoi K, Krane RJ, Cohen RA: Impaired neurogenic and endothelium-mediated relaxation of penile smooth muscle from diabetic men with impotence. N Engl J Med. 1989; 320: 1025-30.
25. Crowe LC, George WH:Alcohol and human sexuality: review and integration. Psychol Bull. 1989; 105: 374-86.
26. Lue TF: Erectile dysfunction. N Engl J Med. 2000; 342: 1802-13.
27. Feldman HA, Johannes CB, Derby CA, Kleinman KP, Mohr BA, Araujo AB, et al: Erectile dysfunction and coronary risk factors: prospective results from the Massachusetts male aging study. Prev Med. 2000; 30: 328-38.

28. Derby CA, Mohr BA, Goldstein I, Feldman HA, Johannes CB, McKinlay JB: Modifiable risk factors and erectile dysfunction: can lifestyle changes modify risk? Urology. 2000; 56: 302-6.
29. Araujo AB, Johannes CB, Feldman HA, Derby CA, McKinlay JB: Relation between psychosocial risk factors and incident erectile dysfunction: prospective results from the MassachusettsMale Aging Study. Am J Epidemiol. 2000; 152: 533-41.
30. Soykan A, Boztas H, Kutlay S, Ince E, Nergizoglu G, Dileköz AY, et al.: Do sexual dysfunctions get better during dialysis? Results of a six-month prospective follow-up study from Turkey. Int J Impot Res. 2005; 17: 359-63.
31. Massry SG, Goldstein DA, Procci WR, Kletzky OA: Impotence in patients with uremia: a possible role for parathyroid hormone. Nephron. 1977; 19: 305-10.
32. Wein AJ, Van Arsdalen KN: Drug-induced male sexual dysfunction. Urol Clin North Am. 1988; 15: 23-31.

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Sexual Function in Male Patients with Metabolic Syndrome and Effective Parameters on Erectile Dysfunction

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ABSTRACT

Purpose: We aimed to investigate the relationship between metabolic syndrome and sexual function and effective parameters on erectile dysfunction (ED).
Materials and Methods: A total of 1300 individuals were included in this study between January 2009 and July 2012. All of individuals were asked to fill in an International Index for Erectile Function (IIEF) questionnaire. The presence of metabolic syndrome was determined when any three or more of the five risk factors were present according to the National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP)-III. Obese individuals were divided into six groups according to modified World Health Organization (WHO) definition. Effective parameters on erectile dysfunction were investigated in individuals with metabolic syndrome.
Results: Metabolic syndrome was detected in 455 individuals (35%). Mean domain scores of IIEF for all parameters were higher in individuals without metabolic syndrome than individuals with metabolic syndrome ($p < 0.05$). Mean domain scores of IIEF were lower in individuals with class 3 obesity than individuals with other obese groups ($p < 0.05$) for erectile dysfunction. There was statistical difference in terms of mean score of IIEF-Erectile function between smoking and nonsmoking groups ($p < 0.05$). Seventy percent of individuals with metabolic syndrome and 45% of individuals without metabolic syndrome had ED ($p < 0.001$). Logistic regression analysis revealed that waist circumference (WC) was the most important criteria for ED ($p < 0.05$).
Conclusions: Metabolic syndrome, smoking and obesity seem to be potential risk factors for ED. We recommend individuals with metabolic syndrome, smoking and obesity should be questioned about ED.

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Obesity; Erectile Dysfunction; Metabolic Syndrome X; Smoking

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INTRODUCTION

Metabolic syndrome is comprised of metabolic risk factors in one individual. This syndrome is also defined as a multidimensional risk factor for cardiovascular disease. It was initially described by a committee of experts from the World Health Organization (WHO) in 1998 (1).

The experts of the National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP)-III created an operational definition of metabolic syndrome in 2001. In this classification, abdominal obesity, high blood pressure (BP), high fasting blood glucose (FBG), high triglyceride (TG), and low high density lipoprotein (HDL) cholesterol are suggested as risk factors of metabolic syndrome. The existence of any three out of five factors is defined as metabolic syndrome (2).

In economically developed countries, the metabolic syndrome is very common and affects

up to 30% of the population, and its incidence continues to increase (3). Endothelial dysfunction occurs frequently in metabolic syndrome and is predictive of future cardiovascular events (4).

It affects the whole arterial system including those that supply blood to the penis (5). Montorsi et al. stated that erectile dysfunction (ED) existed approximately 39 months before cardiac events (6). Briefly, the relationship between metabolic syndrome and cardiovascular disease (CVD) has been established as well as the relationship between CVD and ED (7). However, association between ED and metabolic syndrome has been investigated in few studies (8).

In this study, we aimed to investigate the relationship between metabolic syndrome and sexual function and effective parameters on erectile dysfunction.

MATERIALS AND METHODS

A total of 1300 consecutive individuals who were admitted to urology and endocrinology clinics were included in the study. Individuals who had history of drug usage, history of surgeries and history of neurologic, psychogenic, cardiovascular diseases were excluded the study.

Individuals filled the IIEF questionnaire by themselves. Erectile Function, Orgasmic Function, Sexual Desire, Intercourse Satisfaction and Overall Satisfaction status were determined using IIEF (9).

All individuals underwent physical examination including measurement of height, weight, hip girth, and waist circumference (WC). Supine WC was measured at the level of umbilicus with the person breathing silently according to the WHO guidelines (10). Blood samples were obtained from all individuals in a fasting state for serum triglyceride (TG), high density lipoprotein (HDL) cholesterol, and fasting blood glucose (FBG) analysis.

The presence of metabolic risk factors were defined as: HDL-cholesterol < 40mg/dL, Blood Pressure ≥ 130/85mmHg; Fasting Glucose ≥ 110mg/dL; Triglycerides ≥ 150mg/dL; and Waist circumference > 102 cm2. The presence of metabolic syndrome was determined according to NCEP ATP-III guidelines (2). When a patient had three or more risk factors, he was assumed to have metabolic syndrome. Obese individuals were divided into six groups according to modified WHO definition (11).

Effective parameters on erectile dysfunction were investigated in individuals with metabolic syndrome.

Statistical analysis

Mean age and IIEF domain scores of individuals with and without metabolic syndrome were compared using an independent Student's t--test. χ^2-test was applied for association of each risk factor to ED. Statistical significance was defined as $p < 0.05$. The statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS.16.0) software program.

RESULTS

Metabolic syndrome was diagnosed in 455 (35%) of 1300 individuals and the rest (845 individuals) constituted the group of individuals without metabolic syndrome. The mean age of participating individuals in this study was 57.2 ± 9.2 years. Individuals with metabolic syndrome were significantly older than the individuals without metabolic syndrome ($p < 0.05$). Demographics and laboratory findings are shown in Table-1.

Mean domain scores of IIEF for all parameters were higher in individuals without metabolic syndrome than individuals with metabolic syndrome ($p < 0.05$). IIEF-Erectile function domain scores of individuals with and without metabolic syndrome were 15.3 ± 6.4 and 20.4 ± 4.8, respectively ($p < 0.001$). IIEF-Orgasmic function domain scores of individuals with and without metabolic syndrome were 4.3 ± 3.2 and 6.4 ± 4.4, respectively ($p < 0.05$). IIEF-Sexual desire domain scores of individuals with and without metabolic syndrome were 3.4 ± 2.1 and 5.4 ± 4.2, respectively ($p < 0.05$). IIEF-Intercourse satisfaction domain scores of individuals with and without metabolic syndrome were 8.4 ± 6.1 and 11.3 ± 5.3, respectively ($p < 0.001$). IIEF-Overall satisfaction domain scores of individuals with and without metabolic

Table 1 - Demographics and laboratory findings of the patients.

	With metabolic syndrome	Without metabolic syndrome
Patient number	455	845
Age (years)	61.3 ± 9.3	56.2 ± 8.2
WC (cm)	103.5 ± 6.3	94.3 ± 6.8
FBG	144.8 ± 35.3	109.2 ± 37.4
TG	236.3 ± 113.6	131.4 ± 56.4
HDL-cholesterol	34.7 ± 8.3	48.5 ± 10.4
Hypertension	232 (51%)	127 (15%)

FBG = Fasting blood glucose; **HDL =** High density lipoprotein; **IIEF-EF =** International Index for Erectile Function erectile function; **TG =** Triglyceride; **WC =** Waist circumference.

syndrome were 4.4 ± 2.2 and 6.5 ± 3.2, respectively (P < 0.05) (Table-2).

Sixty percent of individuals of the smoking group (202 individuals) and 45% of individuals in nonsmoking group (253 individuals) had ED among individuals with metabolic syndrome. Mean score of IIEF-Erectile function in smoking group was 13.4 ± 4.3 and in nonsmoking group was 16.7 ± 3.3. There was statistical difference in mean IIEF score between smoking and nonsmoking groups (p < 0.05) (Student's t test).

Obesity was detected in 380 of 455 individuals (83%) with metabolic syndrome. Mean score of IIEF-Erectile function was 18.4 ± 5.8 in class 1 obesity group, 17.6 ± 3.7 in class 2 obesity group and 13.4 ± 4.8 in class 3 obesity group. Mean score of IIEF-Erectile function was lower in individuals with class 3 obesity than individuals with other obese groups for erectile dysfunction (p < 0.05) (Table-3). Logistic regression analysis revealed that WC was the most important criteria for ED.

DISCUSSION

Obesity affects at least 400 million adults worldwide (12). In the USA, 40% of men are expected to be obese by 2020. ED affects physical and psychosocial health and has a significant impact on the quality of life of sufferers and the partners and families (13,14). ED is one of the most widespread chronic diseases in men (15). Currently, it is widely agreed that atherosclerosis vessels of the penis are the cause of organic ED in the majority

Table 2 - Relationship between the metabolic syndrome and parameters of IIEF questionnaire.

IIEF	With metabolic syndrome	Without metabolic syndrome	P
Erectile function	15.3 ± 6.4	20.4 ± 4.8	
Orgasmic function	4.3 ± 3.2	6.4 ± 4.4	
Sexual desire	3.4 ± 2.1	5.4 ± 4.2	< 0.05
Intercourse satisfaction	8.4 ± 6.1	11.3 ± 5.3	
Overall satisfaction	4.4 ± 2.2	6.5 ± 3.2	

IIEF-EF = International Index for Erectile Function

Table 3 - Relationship between the erectile function and obesity groups.

Obesity groups	Patients number	Mean score of IIEF-Erectile function	p
Class 1 obesity	164	18.4 ± 5.8	< 0.05
Class 2 obesity	128	17.6 ± 3.7	
Class 3 obesity	88	13.4 ± 4.8	
Total	380	17.2 ± 4.6	

of cases (16). Evidence of this can be seen from the fact that the risk factors for atherosclerosis, such as hypertension, diabetes mellitus (DM), dyslipidemia, sedentary lifestyle, obesity and smoking, are common in men with organic ED (15,17). Moreover, the severity of ED is known to correlate with the number and severity of the above listed disorders, while the combination of these factors raises the risk of developing ED (18). In our study we observed that the mean domain scores of IIEF for all parameters were higher in individuals without metabolic syndrome than individuals with metabolic syndrome.

Among the factors associated with ED are depression, hormonal changes, and vascular or neurologic damage after trauma or surgery. ED is also associated with different endocrine-metabolic disorders like type 2 diabetes mellitus (DM2), and components of metabolic syndrome (MS) such as hypertension, obesity, and dyslipidemia (19).

Metabolic syndrome is a group of risk factors that is responsible for endothelial dysfunction and atherosclerosis (20). Erectile dysfunction is a multifactorial disease. The most common cause of ED is penile vascular insufficiency (21). As shown in previous studies, endothelial dysfunction and ED share common pathophysiologic pathways (22). Cross-sectional studies have documented a concordance between the causes of ED and cardiovascular disease, that is, elements common to the metabolic syndrome (23,24). The relationship between metabolic syndrome and CVD has been established by Wilson et al. (25).

On the other hand, the metabolic syndrome is considered the most important public health threat of the twenty-first century (26). Men with cardiovascular diseases or metabolic syndrome often have erectile dysfunction, probably because of shared factors impairing hemodynamic mechanisms in both the penile and systemic vascular beds (27).

Obesity is a term applied to excess body weight with an abnormally high proportion of body fat. Several studies have indicated that obesity is an independent risk factor for CVD and ED (28,29). Abdominal obesity is associated with increased coagulability, endothelial dysfunction, and inflammation. Increased cytokine levels and other factors which cause insulin resistance and increased cardiovascular risk may be responsible for this condition (30,31).

In our study, the mean score of IIEF-Erectile function was significantly lower in individuals with class 3 obesity than individuals with other obese groups for erectile dysfunction. In addition, we detected that WC was the most important criteria for ED.

Some modifications to the WHO definitions have been made by particular bodies. The surgical literature breaks down “class III” obesity into further categories whose exact values are still disputed (11).

Numerous studies have demonstrated that endothelial dysfunction occurs early in the insulin resistant state and is predictive of future dysfunctional vascular diseases such as ED and CVD (20,32). There is accumulating evidence that waist hip ratio (WHR) and WC are better indicators of the metabolic syndrome and increased risk of CVD (33,34).

In a prospective study of risk factors for ED, the author found that obesity and smoking were positively associated, and physical activity was inversely associated with the risk of developing ED during the 14-year follow-up (35). In our

study, there was statistical difference in mean IIEF scores between smoking and nonsmoking groups.

In this manuscript the main weakness is that it was carried out on hospitalized individuals, who are not necessarily representative of the general population.

CONCLUSIONS

Metabolic syndrome is significantly associated with ED. Furthermore, there is a significant association between the increased severity of ED and metabolic syndrome. Abnormal WC is an independent and new metabolic risk factor for ED. Metabolic syndrome, smoking and obesity seem to be potential risk factors for ED. We recommend individuals with metabolic syndrome, smoking and obesity should be questioned about ED.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Alberti KG, Zimmet PZ: Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med. 1998; 15: 539-53.
2. [No authors listed]: Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults: Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection,Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). JAMA. 2001; 285: 2486-97.
3. Ford ES, Abbasi F, Reaven GM: Prevalence of insulin resistance and the metabolic syndrome with alternative definitions of impaired fasting glucose. Atherosclerosis. 2005; 181: 143-8.
4. Hsueh WA, Lyon CJ, Quiñones MJ: Insulin resistance and the endothelium. Am J Med. 2004; 117: 109-17.
5. Maas R, Schwedhelm E, Albsmeier J, Böger RH: The pathophysiology of erectile dysfunction related to endothelial dysfunction and mediators of vascular function. Vasc Med. 2002; 7: 213-25.
6. Montorsi F, Briganti A, Salonia A, Rigatti P, Margonato A, Macchi A, et al.: Erectile dysfunction prevalence, time of onset and association with risk factors in 300 consecutive patients with acute chestpain and angiographically documented coronary artery disease. Eur Urol. 2003 ; 44: 360-4; discussion 364-5.
7. Roumeguère T, Wespes E, Carpentier Y, Hoffmann P, Schulman CC: Erectile dysfunction is associated with a high prevalence of hyperlipidemia and coronary heart disease risk. Eur Urol. 2003; 44: 355-9.
8. Bansal TC, Guay AT, Jacobson J, Woods BO, Nesto RW: Incidence of metabolic syndrome and insulin resistance in a population with organic erectile dysfunction. J Sex Med. 2005; 2: 96-103.
9. Rosen RC, Riley A, Wagner G, Osterloh IH, Kirkpatrick J, Mishra A: The international index of erectile function (IIEF): a multidimensional scale for assessment of erectile dysfunction. Urology. 1997; 49: 822-30.
10. [No authors listed]: Physical status: the use and interpretation of anthropometry. Report of a WHO Expert Committee. World Health Organ Tech Rep Ser. 1995;854:1-452.
11. Sturm R: Increases in morbid obesity in the USA: 2000-2005. Public Health. 2007; 121: 492-6.
12. [No authors listed]: Obesity: preventing and managing the global epidemic. Report of a WHO consultation. World Health Organ Tech Rep Ser. 2000; 894: i-xii, 1-253.
13. Ruhm C: Current and future prevalence of obesity and severe obesity in the United States. In NBER Working Paper No 13181; 2007.
14. Hatzimouratidis K, Amar E, Eardley I, Giuliano F, Hatzichristou D, Montorsi F, et al.: Guidelines on male sexual dysfunction: erectile dysfunction and premature ejaculation. Eur Urol. 2010; 57: 804-14.
15. Nicolosi A, Moreira ED Jr, Shirai M, Bin Mohd Tambi MI, Glasser DB: Epidemiology of erectile dysfunction in four countries: cross-national study of the prevalence and correlates of erectiledysfunction. Urology. 2003; 61: 201-6.
16. Melman A, Gingell JC: The epidemiology and pathophysiology of erectile dysfunction. J Urol. 1999; 161: 5-11.
17. Feldman HA, Goldstein I, Hatzichristou DG, Krane RJ, McKinlay JB: Impotence and its medical and psychosocial correlates: results of the Massachusetts Male Aging Study. J Urol. 1994; 151: 54-61.
18. Parazzini F, Menchini Fabris F, Bortolotti A, Calabrò A, Chatenoud L, Colli E, et al.: Frequency and determinants of erectile dysfunction in Italy. Eur Urol. 2000; 37: 43-9.
19. Costanzo P, Knoblovits P, Rey Valzacchi G, Gueglio G, Layus O: Erectile dysfunction is associated with a high prevalence of obesity and metabolic syndrome. Rev Argent Endocrinol Metab 2008; 45: 142-8.
20. Lakka HM, Laaksonen DE, Lakka TA, Niskanen LK, Kumpusalo E, Tuomilehto J, et al.: The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. JAMA. 2002; 288: 2709-16.
21. Guay AT, Traish A: Testosterone deficiency and risk factors in the metabolic syndrome: implications for erectile dysfunction. Urol Clin North Am. 2011; 38: 175-83.

22. Sullivan ME, Thompson CS, Dashwood MR, Khan MA, Jeremy JY, Morgan RJ, et al.: Nitric oxide and penile erection: is erectile dysfunction another manifestation of vascular disease? Cardiovasc Res. 1999; 43: 658-65.
23. Corona G, Mannucci E, Schulman C, Petrone L, Mansani R, Cilotti A, et al.: Psychobiologic correlates of the metabolic syndrome and associated sexual dysfunction. Eur Urol. 2006; 50: 595-604; discussion 604.
24. Heidler S, Temml C, Broessner C, Mock K, Rauchenwald M, Madersbacher S, et al.: Is the metabolic syndrome an independent risk factor for erectile dysfunction? J Urol. 2007; 177: 651-4.
25. Wilson PW, Kannel WB, Silbershatz H, D'Agostino RB: Clustering of metabolic factors and coronary heart disease. Arch Intern Med. 1999; 159: 1104-9.
26. Traish AM, Guay A, Feeley R, Saad F: The dark side of testosterone deficiency: I. Metabolic syndrome and erectile dysfunction. J Androl. 2009; 30: 10-22.
27. Jackson G: The metabolic syndrome and erectile dysfunction: multiple vascular risk factors and hypogonadism. Eur Urol. 2006; 50: 426-7.
28. Rimm EB, Stampfer MJ, Giovannucci E, Ascherio A, Spiegelman D, Colditz GA, et al.: Body size and fat distribution as predictors of coronary heart disease among middle-aged and older US men. Am J Epidemiol. 1995; 141: 1117-27.
29. Esposito K, Giugliano F, Di Palo C, Giugliano G, Marfella R, D'Andrea F, et al.: Effect of lifestyle changes on erectile dysfunction in obese men: a randomized controlled trial. JAMA. 2004; 291: 2978-84.
30. McFarlane SI, Banerji M, Sowers JR: Insulin resistance and cardiovascular disease. J Clin Endocrinol Metab. 2001; 86: 713-8.
31. Guerre-Millo M: Adipose tissue hormones. J Endocrinol Invest. 2002; 25: 855-61.
32. Grundy SM: Obesity, metabolic syndrome, and coronary atherosclerosis. Circulation. 2002; 105: 2696-8.
33. Lundgren H, Bengtsson C, Blohme G, Lapidus L, Sjöström L: Adiposity and adipose tissue distribution in relation to incidence of diabetes in women: results from a prospectivepopulation study in Gothenburg, Sweden. Int J Obes. 1989; 13: 413-23.
34. Lean ME, Han TS, Morrison CE: Waist circumference as a measure for indicating need for weight management. BMJ. 1995; 311: 158-61.
35. Bacon CG, Mittleman MA, Kawachi I, Giovannucci E, Glasser DB, Rimm EB: A prospective study of risk factors for erectile dysfunction. J Urol. 2006; 176: 217-21

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ORIGINAL ARTICLE

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Efficacy of spermatic vein ligation in patients affected by high grade left varicocele

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ABSTRACT

Purpose: To study the effect of high grade varicocele treatment in infertile patients.
Materials and Methods: Seventy-five patients were selected by the following criteria: infertility persisting for more than 1 year; abnormal semen parameters; no other infertility-related disease; no obvious causes of infertility in the subject's partner; basal eco-color Doppler ultrasound demonstrating continuous reflux in the spermatic vein. All patients considered for the study had at least a six months period from the diagnosis to the surgery due to waiting list, choice of the patient or time needed to complete diagnostic evaluation of the couple. The surgical procedure was performed through an inguinal approach. All enrolled patients were counseled to have unprotected intercourse during the ovulation period in order to maximize the probability of pregnancy within the 6-month preoperative period. The achievement of pregnancy and semen parameters were recorded during the preoperative and postoperative period.
Results: Two of the seventy-five patients were excluded because of persistent varicocele after surgery. The preoperative pregnancy rate was 1.3% (1 couple). The postoperative pregnancy rate was 42.5%. The stratification of pregnancies by semester showed a significantly higher rate in the first postoperative period ($p = 0.0012$). Mean time to conception was 13.5 months. Mean preoperative sperm count was $17.6x10^6$/mL compared to $19.7x10^6$/mL in the postoperative period ($p < 0.0001$). Mean percentage of progressive sperm motility was 13.7%, compared to 17.6% in the postoperative period ($p < 0.0001$). Mean percentage of normal sperm morphology was 7.6%, compared to 15.2% postoperatively ($p < 0.0001$).
Conclusion: Surgical treatment of high grade varicocele proved to effectively treat associated infertility by improving seminal parameters and pregnancy rate in our patient cohort.

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INTRODUCTION

Varicocele is a common scrotal condition characterized by the elongation and enlargement of the network of veins leaving the testis that join to form the testicular vein.

The incidence is reported to be as high as 20-24% in the adult male population (1) with a higher prevalence in the left side. The condition is more common in men in infertile marriages, in which it affects 25-40% of men with abnormal semen analysis (2,3).

The exact association between reduced male fertility and varicocele is not known because prospective randomized studies on varicocele treatment in adults have given conflicting results (4-8). The largest study indicated a benefit (8,9), whereas meta-analysis of most of the prospective randomized trials did not (10). However, in previous studies, selection criteria based on the clinical and ultrasonographic grade of varicocele did not take into consideration a homogeneous population. It would be probable that a lower grade of varicocele does not affect fertility as well as the quality of seminal parameters, and a significant bias might exist.

For this reason, our intent was to study a series of infertile patients with high grade varicocele before and after surgical treatment. Our aim was to obtain reliable results from a homogeneous and selected patient population.

MATERIALS AND METHODS

From January 2006 to February 2011, we studied 75 patients with high grade left varicocele who presented with infertility. Patients were selected by the following criteria: (a) infertility persisting for more than 1 year despite regular, unprotected intercourse; (b) abnormal semen parameters as assessed by World Health Organization (WHO) guidelines 2010; (c) no other infertility-related disease; and (d) no obvious causes of infertility in the subject's partner.

All men underwent a standard diagnostic infertility evaluation (physical examination, blood tests, including hormonal dosages and testing for Y deletion and chromosomal defects when needed according to seminal and clinical features, eco-color Doppler ultrasound of the scrotum). The examination was performed after the patient stood for various minutes in a warm room; the scrotum was inspected and palpated in the upright position. In all cases, an ultrasound with color-Doppler study was performed using a linear 7.5 MHz probe both in supine and upright position. According to this method, varicocele was graduated as follows: 1st grade, reflux was visible only under Valsalva maneuver; 2nd grade, venous reflux was intermittent under basal conditions; 3rd grade, basal continuous reflux was demonstrated (11,12).

Infertility was defined, according to the WHO, as the inability of a sexually active, non-contracepting couple to achieve pregnancy in one year (13).

At least two preoperative semen analyses were obtained by masturbation after 3 days of abstinence from sexual activity, and the average value was considered.

All possible causes of male infertility were ruled out, including history of maldescended testis, infections, general diseases, or chronic medication. The partner was studied in all cases to rule out any cause of infertility (such as anovulation, endometriosis, tubal blockage, etc.)

All patients considered for the study had periods between 6 and 9 months (mean: 7.2 months, SD 0.8) from the diagnosis to the surgery due to waiting list, choice of the patient or time needed to complete diagnostic evaluation of the couple.

All the enrolled patients fulfilled the study inclusion criteria and were counseled to have unprotected intercourse during the ovulation period in order to maximize the probability of getting pregnant during the 6 months before intervention, when the patient was on the surgical waiting list.

The patients underwent spermatic vein ligature through an inguinal approach with optical magnification performed by a single-surgeon. Optical magnification using frontal loops (3x) was utilized to spare the spermatic artery and lymphatic ducts.

The achievement of pregnancy and semen parameters were recorded during the preoperative and postoperative period. Postoperative semen analyses were obtained 6 months after surgery.

Categorical data were examined by the chi-square test; continuous variables were tested by t-test assuming $p < 0.05$ as significant. The results were elaborated using the statistical program SigmaStat™ for Windows® V2.03.

RESULTS

All 75 patients had 3rd grade left varicocele. Two patients were excluded because of basal continuous reflux after surgery. The patients' mean age was 33.2 years (range 23-48

years). The mean study follow-up time was 32.4 months (range 24-47 months). The mean age of the partners was 28.5 years (range 23-39 years). No patient reported previous episodes of cryptorchidism, hydrocele, or testicular trauma, nor had they undergone surgery of the urogenital tract. No other causes of infertility were found.

The mean infertility period was 23 months (SD ± 8.4, range 12-39 months).

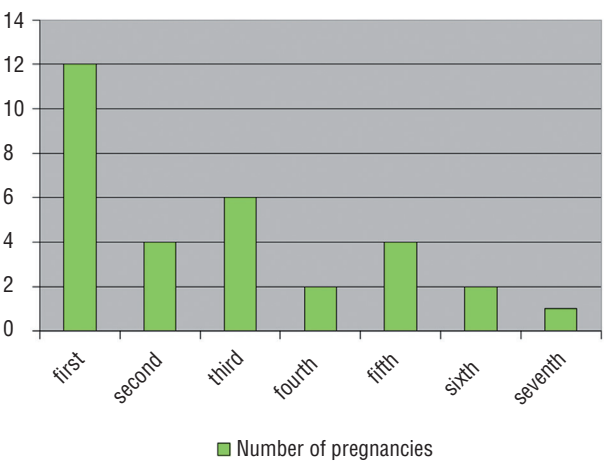
During the 6-9 months preoperative period while patients were on the surgery waiting list, pregnancy was achieved in 1 couple (1.3%).

The mean preoperative sperm count was $17.6x10^6$/mL compared to $19.7x10^6$/mL in the postoperative period ($p < 0.0001$). The mean preoperative percentage of progressive sperm motility was 13.7% compared to 17.6% in the postoperative period ($p < 0.0001$). The mean preoperative percentage of normal sperm morphology was 7.6% compared to 15.2% in the postoperative period ($p < 0.0001$) (Table-1).

During the first 6-month postoperative period, 12 couples got pregnant (16.5%). A comparison of the pregnancies occurring in the preoperative period to the occurrence during the first postoperative semester showed a significant difference ($p < 0.0001$). In the following months, 19 more pregnancies occurred. The stratification of pregnancies by semester showed a significantly higher rate during the first postoperative period ($p = 0.0012$) (Figure-1). The mean time to conception was 13.5 months.

The persistence rate of varicocele was 2.6%, and no minor or major postoperative complications were registered. All patients were discharged within 24 hours.

Figure 1 - Postoperative pregnancies stratified by semester.



DISCUSSION

The main point of discussion is the clear benefit for the rate of pregnancy when varicocele is treated in infertile patients if other causes of male infertility, as well as obvious causes of female infertility, are ruled out. However, this point is debated by the scientific literature because even prospective randomized controlled trials of varicocele treatment in adults have given conflicting results. Studies on this topic are divided into those that conclude there is no influence on infertility by treatment of varicocele (6,7,14,15) and those that find a real benefit on semen parameters and pregnancy rate (8,9).

However, the heterogeneous inclusion criteria, the small number of analyzed patients, and, in some cases, the high percentage of varicocele

Table 1 - Pregnancies and sperm characteristics before and after operation.

	Pre-op semester	1st post-op semester	p
Pregnancies	1 (1.3%)	12 (16.5%)	< 0.0001
Mean sperm count	17.6x106/mL	19.7x106/mL	< 0.0001
Mean percentage of progressive sperm motility	13.7%	17,6%	< 0.0001
Mean percentage of normal sperm morphology	7.6%	15.2%	< 0.0001

persistence after treatment do not allow to draw any final conclusion based on the evidence. We agree that the meta-analytic interpretation of these data does not provide information based on evidence that is useful in improving clinical practice (16). In particular, the high rate of patients who dropped out and/or were lost to follow-up calls into question the propriety of randomized studies comprised of an untreated arm that may appear unethical in such patients. In this regard, our method might be closer to real clinical practice than randomized clinical trials that have a higher percentage of randomization refusal. In our study, the same patients are their own controls because seminal parameters and pregnancy rate are measured before and after surgical treatment for varicocele in the same population.

Our positive results for pregnancy rate and improved seminal parameters may be explained by our selection criteria. Only 3rd grade varicocele according to color Doppler classification was taken into consideration differently from previous randomized studies that usually consider clinical classifications, which are known to have low sensitivity (3).

Another bias present in previous studies is the different treatment used to correct the venous reflux and, in some cases, the high persistence rate of varicocele. In our series, treatment was the same in all patients and consisted in the microsurgical inguinal ligation that, in our hands, had a lower persistence rate (2.6%).

Our study was prospective but not randomized, and this is the main limitation. We do not know if the simple counseling done during the waiting list months could be a treatment comparable to surgical treatment of varicocele. To answer this question, we should have considered a randomized non-operated group of patients treated by counseling alone. However, data on efficacy of counseling alone with no surgical procedures in infertile couples with associated varicocele are lacking therefore we are not able to assess if counseling alone might increase pregnancy rate in this selected group of patients.

In the absence of any significant data on counseling efficacy we think it is unethical not to treat patients strongly motivated to have children.

CONCLUSIONS

The surgical treatment of high grade varicocele effectively treats infertility, improving seminal parameters and pregnancy rate. Increased improvement occurs early after treatment. A microsurgical inguinal approach seems to be a good treatment because the incidence of persistence is acceptable.

Study limitations: We did not have a control group for comparison with our treatment data. We agree that a control would be ideal to provide high level evidence of treatment benefits, but is ethically questionable to exclude a well-defined patient group from a recommended therapy.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Pfeiffer D, Berger J, Schoop C, Tauber R: A Doppler-based study on the prevalence of varicocele in German children and adolescents. Andrologia. 2006; 38: 13-9.
2. Levinger U, Gornish M, Gat Y, Bachar GN: Is varicocele prevalence increasing with age? Andrologia. 2007; 39: 77-80.
3. Liguori G, Trombetta C, Garaffa G, Bucci S, Gattuccio I, Salamè L, et al.: Color Doppler ultrasound investigation of varicocele. World J Urol. 2004; 22: 378-81.
4. Nieschlag E, Hertle L, Fischedick A, Behre HM: Treatment of varicocele: counselling as effective as occlusion of the vena spermatica. Hum Reprod. 1995; 10: 347-53.
5. Zini A, Azhar R, Baazeem A, Gabriel MS: Effect of microsurgical varicocelectomy on human sperm chromatin and DNA integrity: a prospective trial. Int J Androl. 2011; 34: 14-9.
6. Baazeem A, Belzile E, Ciampi A, Dohle G, Jarvi K, Salonia A, et al.: Varicocele and male factor infertility treatment: a new meta-analysis and review of the role of varicocele repair. Eur Urol. 2011; 60: 796-808.
7. Nieschlag E, Hertle L, Fischedick A, Abshagen K, Behre HM: Update on treatment of varicocele: counselling as effective as occlusion of the vena spermatica. Hum Reprod. 1998; 13: 2147-50.
8. Hargreave TB. Varicocele: overview and commentary on the results of the WHO varicocele trial. Proceedings of the VIth International Congress of Andrology, Salzburg, Austria. Current Advances in Andrology. Waites GM, Frick J, Baker GW (eds). Bologna. Monduzzi Editore. 1997; pp. 31-44.

9. Madgar I, Weissenberg R, Lunenfeld B, Karasik A, Goldwasser B: Controlled trial of high spermatic vein ligation for varicocele in infertile men. Fertil Steril. 1995; 63: 120-4.
10. Evers JL, Collins JA . Assessment of efficacy of varicocele repair for male subfertility: a systematic review. Lancet. 2003; 361: 1849-52.
11. Grasso M, Lania C, Castelli M, Galli L, Franzoso F, Rigatti P: Low-grade left varicocele in patients over 30 years old:the effect of spermatic vein ligation on fertility. BJU Int. 2000; 85: 305-7.
12. Grasso M, Lania C, Castelli M, Galli L, Rigatti P: Bilateral varicocele: impact of right spermatic vein ligation on fertility. J Urol. 1995; 153: 1847-8.
13. World Health Organization. WHO Manual for the Standardised Investigation and Diagnosis of the Infertile Couple. Cambridge: Cambridge University Press; 2000.
14. Nilsson S, Edvinsson A, Nilsson B: Improvement of semen and pregnancy rate after ligation and division of the internal spermatic vein: fact or fiction? Br J Urol. 1979; 51: 591-6.
15. Yamamoto M, Hibi H, Hirata Y, Miyake K, Ishigaki T: Effect of varicocelectomy on sperm parameters and pregnancy rate in patients with subclinical varicocele: a randomized prospective controlled study. J Urol. 1996; 155: 1636-8.
16. Ficarra V, Cerruto MA, Liguori G, Mazzoni G, Minucci S, Tracia A, et al.: Treatment of varicocele in subfertile men: The Cochrane Review--a contrary opinion. Eur Urol. 2006; 49: 258-63.

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Genital prevalence of HPV types and co-infection in men

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ABSTRACT

Introduction: HPV infection is a highly prevalent sexually transmitted disease and there is evidence of the relationship of HPV infection and the development of genital warts, penile intraepitelial neoplasia, invasive penile carcinoma and cervical cancer. However, there is sparse data regarding the prevalence of HPV types and co-infection of different HPV types among men.

Objectives: To assess the prevalence of HPV subtypes infections and rates of co-infection among men.

Materials and Methods: 366 men were evaluated from March to October 2010. Men were referred to our institution for HPV diagnostic evaluation based on the following criteria: 1. presence of a genital wart; 2. presence of an atypical genital lesion; 3. absence of symptoms and a partner with a HPV diagnosis; 4. absence of symptoms and a desire to undergo a full STD diagnostic evaluation. Genital samples were collected from the urethra, penile shaft, scrotum and anus with Digene® collection and preservation kit and submitted to HPV genotype microarray detection (Papillocheck®). All men were tested for the low-risk HPV types 6-11-40-42-43-44 and for the high-risk HPV types 16-18-31-33-35-39-45-51-52-53-56-58-59-66-68-70-73-82.

Results: Of the 366 men, 11 were tested inconclusive and were excluded from the analysis. 256 men (72.1% of the men from the cohort referred to our institution) tested positive with genotype micro-array detection and 99 tested negative. The most prevalent HPV-subtypes in the studied population were 6, 42, 51 and 16. Co-infection was found in 153 men. Of those, 70 (19.7%) had a co-infection by 2 types, 37 (10.4%) by 3 types; 33 men (9.2%) by 4 types; 8 men (2.2%) by 5 types; 1 man (0.3%) by 6 types; 1 man (0.3%) by 7 types; 2 men (0.6%) by 8 types and 1 man (0.3%) by 9 types.

Conclusion: The most frequent HPV types were 6, 16, 42 and 51. Co-infection was found in 59% of our patients. This information is vital to drive future public health policies including massive public vaccination campaign.

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INTRODUCTION

Infection by human papillomavirus (HPV) is the cause of several different diseases in men and women (1,2). Anogenital HPV related infection is the most prevalent common viral sexually transmitted infection worldwide (3). The World Health Organization (WHO) estimates that the prevalence of HPV infection is between nine and thirteen percent or about 630 million people (4). In the United States, CDC (Centers for Disease Control) estimates that approximately 26,000 new cancers attributable

to HPV occurs each year, including 18,000 among females and 8,000 among males (5). Genital warts are more frequently associated to HPV types 6 and 11 whereas HPV 16 and 18 are more commonly associated to cervical cancers.

HPV infection also prevails as the most common viral infection among the Brazilian population. Female HPV genotyping data shows prevalence rates of 57%, 23%, 5%, 4% and 3% for HPV genotypes 16, 18, 31, 33 and 56, respectively (6). A recent large epidemiological study demonstrated that among Brazilian men the prevalence of at least one type of HPV was 86.0%, while more than one HPV type, including high and low risk HPV, was identified in 39.5% of the samples (7). Nevertheless, data on Brazilian HPV infection and disease among males is scarce.

The aim of our study was to describe HPV genotypes prevalence data and to evaluate rates of co-infection with more than one HPV subtype in our male population.

MATERIAL AND METHODS

Enrollment

From March to October 2010, 366 men were referred to the Urological Division of Fleury Medicine and Health from private medical offices for evaluation of penile HPV related disease. Mean age was 35.6 ± 2.6 years (range 18-81 years). Patients were enrolled in a prospective manner and underwent genotyping, cytology and peniscopy. The study was approved by the Ethics Committee of Fleury Medicine and Health and informed consent was obtained from each patient.

Inclusion criteria

Males with age > 18 years old with active genital warts lesions; males with atypical penile lesions; males with a partner with a genital diagnosis of HPV and asymptomatic individuals willing to have a complete sexual transmitted diseases (STD) evaluation.

Diagnostic Techniques

After receiving proper information on the diagnostic methods, men were positioned in supine position. First, samples from the penile shaft, glans, balanopreputial sulcus and urethra were collected for cytology.

HPV genotyping materials were collected with a Digene® kit (Digene, USA). Samples were collected from the same sites as cytology examination. For cytology, specimens were preserved using the ThinPrep® (Hologic, USA) collection and preservation solution. HPV genotype micro-array detection was performed with Papillocheck® (Greiner Bio One, Germany). All men were tested for the following low-risk (LR) HPV types 6-11-40-42-43-44/55 as well as for the following high-risk (HR) HPV types 16-18-31-33-35-39-45-51-52-53-56-58-59-66-68-70-73-82.

Co-infection was defined as an infection with two or more HPV types.

All patients underwent peniscopy by a single physician. Acetic acid (5%) was spread in the genitalia to show small flat lesions. When indicated, biopsies were obtained using local anesthetics (infiltration of lidocaine 2%). Samples were examined by the Pathology Department.

Statistical analysis

All clinical data were collected and prospectively input in an Access® database (Microsoft, Redmond, Washington, USA). SPSS® (SPSS Inc., Chicago, Illinois, USA) were used for the statistical analysis.

RESULTS

From the cohort of men referred to our institution for HPV diagnostic evaluation, 256 men (72.1%) tested positive with genotype micro-array detection and 99 tested negative. Eleven inconclusive cases were excluded from analysis. So, our final sample for analysis was composed of 355 males at risk for HPV infection.

Overall HPV prevalence was 72.1%. The HPV prevalence distribution is shown on Table-1. Low risk HPV types represented 52.6% of our positive tests. Co-infection was found in 153 men, representing 59.7% of men who tested positive with an overall HPV co-infection prevalence of 43.1%. Of those, 70(19.7%) had co-infection by 2 types; 37 (10.4%) by 3 types; 33 men (9.2%) by 4

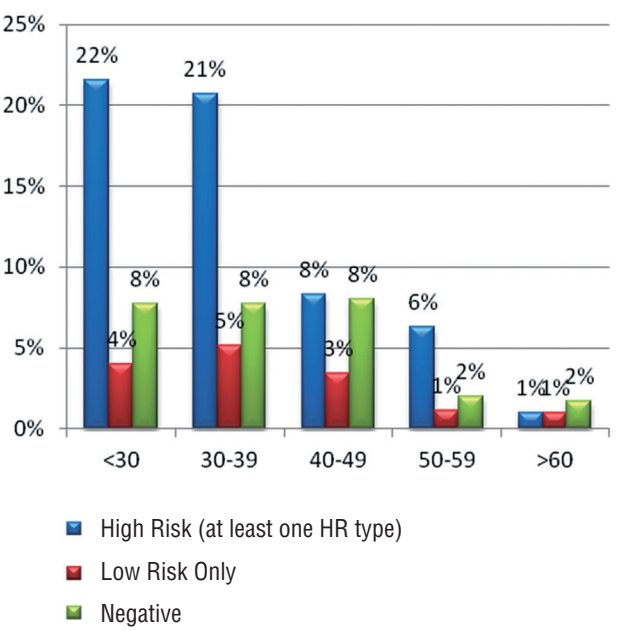
Table 1 - HPV types prevalence.

HPV type	Prevalence (%)
6	17.7
42	13.8
51	11.0
16	11.8
44-55	9.0
39	8.7
53	8.7
56	7.6
31	5.6
59	5.9
68	6.8
52	5.9
66	6.5
11	5.9
40	5.6
43	5.4
33	45
58	3.9
73	3.4
18	2.8
70	3.7
82	2.0
35	1.1
45	1.4

types; 8 men (2.2%) by 5 types; 1 man (0.3%) by 6 types; 1 man (0.3%) by 7 types; 2 men (0.6%) by 8 types and 1 man (0.3%) by 9 types.

Overall, at least one high-risk-HPV (HR-HPV) type was found in 58% of the men and in 81.1% of the men tested positive. HPV infection was highly prevalent among middle-aged groups (50-59 years old), young adults (30-39 years) and among youth (18-29 years old), respectively 78.8%, 76.9% and 76.7%. HPV infection distribution shows that at least one HR-HPV type infection prevailed in most of men when divided by age (Figure-1). There was no correlation between high or low risk HPV infections and age. Also, we could not identify any preferential distribution of any HPV type and co-infection.

Figure 1 - HPV infection distribution according to age groups.



DISCUSSION

Our study demonstrated some notable findings. At first, this is one of the largest studies on HPV's infection prevalence and its genotype distribution among males.

In this study, HPV was highly prevalent, with 72.1% of men testing positive for the virus. These results are consonant with previous published data showing prevalence ranging from 1.3 to 72.9% (3,8-13). Our high number of infected men is probably due to selection, as our sample is

composed by men already referred for HPV testing with some degree of suspicious. This fact might also be the cause of our high number of at least one high-risk HPV type infection (58%) among our population. Regarding the observed high prevalence of high-risk HPV types among our studied population, it was also observed by others and may be consequence of the local restrict HPV vaccination policies (14).

In our study, the four more frequent observed HPV types were HPV 6, 42, 51 and 16, representing 54% of the total amount. This information is consistent with current published data where HPV types 6 and 16 are the most common types observed among men and women (6,7,15,16).

The genotyping description of the HPV infection distribution is an important diagnostic tool once men act as a reservoir for women's infection. Therefore, HPV infection in men greatly affects disease risk in women and must be considered that, in fact, men is less frequently submitted to medical, in special, genital evaluation compared to women (17,18).

Furthermore, the knowledge of HPV prevalence, its subtype and age distribution might guide public health policies and prevention measures, including vaccination. In this regard, a quadrivalent HPV vaccine (HPV4) covering HPV types 6/11/16/18 is currently licensed for vaccination of 9-26 years old men.

Our findings should be interpreted in the context of the study design. Although our research scope was HPV prevalence, there are several limitations in this study. First, our cohort may not represent the entire population, as the studied patients already had a specific demand on HPV diagnosis prior to be referred to our laboratory. Therefore, a selection bias might be inferred since patients may represent a high risk population (e.g: previously treated for HPV, visible genital lesion or had a partner with diagnosed HPV). Moreover, our population was composed of a specific subset of the Brazilian population with full access to medical care. All patients were referred from private offices and had private medical insurance. Finally, in this study we could not study the epidemiology of the disease, including its transmission and potential influence of social and sexual habits on infection as well lack of follow-up data on each patient.

Future research should correlate these findings with women HPV prevalence. This correlation is of great importance considering the impact of the vaccination campaign as well as the transmission rate of the most prevalence HPV types found in our study.

CONCLUSIONS

This study demonstrates critical data on HPV prevalence and its subtype distribution among males with different age distribution. In our population, the most frequent HPV types were 6, 16, 42 and 51. Also, co-infection was found in almost 60% of our patients. In the future, this information should be useful to monitor the prevalence of vaccine-targeted HPV types after the introduction of vaccines.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Anic GM, Lee JH, Stockwell H, Rollison DE, Wu Y, Papenfuss MR, et al.: Incidence and human papillomavirus (HPV) type distribution of genital warts in a multinational cohort of men: the HPV in men study. J Infect Dis. 2011; 204: 1886-92.
2. Giuliano AR, Lee JH, Fulp W, Villa LL, Lazcano E, Papenfuss MR, et al.: Incidence and clearance of genital human papillomavirus infection in men (HIM): a cohort study. Lancet. 2011; 377: 932-40. Erratum in: Lancet. 2011; 377: 2006.
3. Giuliano AR, Anic G, Nyitray AG: Epidemiology and pathology of HPV disease in males. Gynecol Oncol. 2010; 117: S15-9.
4. Cranston RD: Anal cancer prevention: how we are failing men who have sex with men. Sex Transm Infect. 2008; 84: 417-9.
5. [No Authors]: Centers for Disease Control and Prevention (CDC): Human papillomavirus-associated cancers - United States, 2004-2008. MMWR Morb Mortal Wkly Rep. 2012; 61: 258-61.
6. Anic GM, Giuliano AR: Genital HPV infection and related lesions in men. Prev Med. 2011; 53(Suppl 1): S36-41.

7. de Lima Rocha MG, Faria FL, Gonçalves L, Souza Mdo C, Fernandes PÁ, Fernandes AP: Prevalence of DNA-HPV in male sexual partners of HPV-infected women and concordance of viral types in infected couples. PLoS One. 2012; 7: e40988.
8. Dunne EF, Markowitz LE: Genital human papillomavirus infection. Clin Infect Dis. 2006; 43: 624-9.
9. Wikström A, Hedblad MA, Syrjänen S: Penile intraepithelial neoplasia: histopathological evaluation, HPV typing, clinical presentation and treatment. J Eur Acad Dermatol Venereol. 2012; 26: 325-30.
10. Vaccarella S, Plummer M, Franceschi S, Gravitt P, Papenfuss M, Smith D, et al.: Clustering of human papillomavirus (HPV) types in the male genital tract: the HPV in men (HIM) study. J Infect Dis. 2011; 204: 1500-4.
11. Nyitray AG, da Silva RJ, Baggio ML, Lu B, Smith D, Abrahamsen M, et al.: The prevalence of genital HPV and factors associated with oncogenic HPV among men having sex with men and men having sexwith women and men: the HIM study. Sex Transm Dis. 2011; 38: 932-40.
12. Barzon L, Militello V, Pagni S, Franchin E, Dal Bello F, Mengoli C, et al.: Distribution of human papillomavirus types in the anogenital tract of females and males. J Med Virol. 2010; 82: 1424-30.
13. Shigehara K, Sasagawa T, Kawaguchi S, Kobori Y, Nakashima T, Shimamura M, et al.: Prevalence of human papillomavirus infection in the urinary tract of men with urethritis. Int J Urol. 2010; 17: 563-8.
14. Afonso LA, Moyses N, Alves G, Ornellas AA, Passos MR, Oliveira Ldo H, et al.: Prevalence of human papillomavirus and Epstein-Barr virus DNA in penile cancer cases from Brazil. Mem Inst Oswaldo Cruz. 2012; 107: 18-23.
15. Dunne EF, Nielson CM, Stone KM, Markowitz LE, Giuliano AR: Prevalence of HPV infection among men: A systematic review of the literature. J Infect Dis. 2006; 194: 1044-57.
16. de Lima SF Jr, Fernandes MC, Heráclio Sde A, de Souza PR, Maia Mde M: Prevalence of human papillomavirus genotypes: comparison between three detection methods in patients of Pernambuco, Brazil. Rev Bras Ginecol Obstet. 2011; 33: 315-20.
17. Castellsagué X, Iftner T, Roura E, Vidart JA, Kjaer SK, Bosch FX, Muñoz N, et al.: Prevalence and genotype distribution of human papillomavirus infection of the cervix in Spain: the CLEOPATRE study. J Med Virol. 2012; 84: 947-56.
18. Monsonego J: Genital infection with HPV in men: research into practice. Lancet. 2011; 377: 881-3.

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ORIGINAL ARTICLE

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Evaluation of the metabolism of glycosaminoglycans in patients with interstitial cystis

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ABSTRACT

Introduction: Painful bladder syndrome/interstitial cystitis (PBS/IC) pathogenesis is not fully known, but evidence shows that glycosaminoglycans (GAG) of bladder urothelium can participate in its genesis. The loss of these compounds facilitates the contact of urine compounds with deeper portions of bladder wall triggering an inflammatory process. We investigated GAG in urine and tissue of PBS/IC and pure stress urinary incontinence (SUI) patients to better understand its metabolism.

Materials and Methods: Tissue and urine of 11 patients with PBS/IC according to NIDDK criteria were compared to 11 SUI patients. Tissue samples were analyzed by histological, immunohistochemistry and immunofluorescence methods. Statistical analysis were performed using t Student test and Anova, considering significant when $p < 0.05$.

Results: PBS/IC patients had lower concentration of GAG in urine when compared to SUI (respectively 0.45 ± 0.11 x 0.62 ± 0.13 mg/mg creatinine, $p < 0.05$). However, there was no reduction of the content of GAG in the urothelium of both groups. Immunofluorescence showed that PBS/IC patients had a stronger staining of TGF-beta, decorin (a proteoglycan of chondroitin/dermatan sulfate), fibronectin and hyaluronic acid.

Conclusion: the results suggest that GAG may be related to the ongoing process of inflammation and remodeling of the dysfunctional urothelium that is present in the PBS/IC.

ARTICLE INFO

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Cystitis, Interstitial; Glycosaminoglycans; Hyaluronic Acid

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INTRODUCTION

Painful bladder syndrome/interstitial cystitis (PBS/IC) is a chronic syndrome that affects the lower urinary tract and which diagnosis is based on urinary symptoms and chronic pelvic pain or discomfort related to the bladder (1). Its aetiopathology is not fully known, but it is believed that the glycosaminoglycans (GAGs) and proteoglycans (PGs) that line the bladder urothelium can actively participate in its genesis. They potentially help to prevent infections, stone formation, and tissue damage related to radiation carcinogenesis and interstitial cystitis. The biological activity of this layer is generated by the anionic charge of the polysaccharides forming the GAG. They are highly hydrophilic and are adhered to the surface of transitional cell bladder. That avidity for water molecules creates a barrier that prevents migration of solutes from urine through epithelium (2,3). Their capacity to prevent part of the urinary solutes to reach the interstitium has been demonstrated in studies that found increased urea and potassium absorption by bladder wall when the GAG layer is damaged.

Urine and tissue levels of GAG have been studied in PBS/IC and in animal models but their results are not uniform (2,4-18). Such variation suggests that there is no complete understanding of how and why GAG is produced and degraded in PBS/IC, if GAG expression is the cause or the reaction for PBS/IC. Or both cause and reaction. The mechanisms of changes in GAG levels that can occur during the course of the syndrome are not precisely described although some have correlated disease severity to symptoms (4). We investigated GAG behavior in an attempt to understand its metabolism and the chemical reactions related to the expression of those polisaccharides in tissue and urine.

MATERIAL AND METHODS

Female patients with confirmed diagnosis of PBS/IC (n = 11) according to the NIDDK criteria except for glomerulations were subjected to clinical questionnaire assessing age, date of onset of symptoms, coexisting diseases, severity of symptoms, type of previous treatment and quality of life index. They underwent cystoscopy for urine collection followed by bladder biopsy. In the same procedure bladder hydrodistention was done for 3 minutes and 80cmH_20 pressure (19). Mean age was 48 (26-73) years.

The control group (n = 11) was composed of female patients with pure stress urinary incontinence (SUI) that also underwent bladder biopsies at the time of cystoscopy as a part of surgical correction of the underlying disease. Again a new sample of urine was collected at the time of the surgery and bladder biopsy was taken. These patients also had previous negative urinary culture and an urodynamic study realized 3 months earlier in order to exclude eventual non-inhibited detrusor contraction. This group was chosen as the control group because a bladder cold biopsy would add little risk to them, as cystoscopy was mandatory in their surgical treatment. Mean age was 64 (50-79) years.

The study was approved by the ethics committees and a consent form was signed by participants.

The tissue samples were referred to the following analysis: histological, immunohistochemistry and immunofluorescence, and RNA expression by real time PCR.

Extraction of Tissue Glycosaminoglycans

Tissue samples from six patients with interstitial cystitis and 7 controls (SUI) were processed and analyzed biochemically for the content of sulfated GAG, as previously described (20-22). Briefly, the bladder tissue samples were shredded into 10 volumes of acetone to remove water and lipids. The ketonic powder obtained was then subjected to proteolysis with the enzyme maxatase 4mg/ml (Biocon Industrial of Brazil, Rio de Janeiro, Brazil) in Tris-HCL50mM, NaCl1M, pH 8.0, "overnight". Peptides and nucleic acids were precipitated with trichloroacetic acid 90%, and the GAG precipitated from the supernatant by addition of methanol. After incubation at -20°C, 18 hours, the precipitate (containing GAGs) was dried and resuspended in distilled water (1mL for each mg of dry tissue). The identification of the different GAG obtained by the procedure above was done by agarose gel electrophoresis in 1.3 diaminopropane acetate buffer 0.05 M, pH 9.0 (PDA). Five mL of each sample as well as 5µL of a mixture of standard GAG (containing 1mg/mL of chondroitin sulfate, dermatan sulfate and heparan sulfate, all of Seikagaku Kogyo Co., Tokyo, Japan) were applied to the same gel and subjected to electrophoresis, 100V, one hour. The starting point of electrophoresis corresponds to the negative pole as a function of the negative charge of GAG they migrate toward the positive pole. Thus, this system can discriminate, in order of decreasing electrophoretic mobility, three major GAG: chondroitin sulfate (CS), the dermatan sulfate (DS) and heparan sulfate (HS), respectively. Finally, the GAG were precipitated in the gel by the addition of cetyltrimethyl ammonium bromide (CETAVLON, Sigma-Aldrich, St. Louis, MO) 0.1% for a minimum of two hours at room temperature. After drying under heat and ventilation, the gel was stained with toluidine blue solution 0.1% acetic acid 1% and 50% ethanol for about 15 minutes. The excess dye was removed with a solution of 1% acetic acid in 50% ethanol. Quantification of GAG present in different samples was performed by optical densitometry equipment in A595nm QuickScan (Helena Laboratories, Seiko,

Japan) and compared to the existing content of the standard mix. The values were expressed as mg/mg of dry powder.

Extraction and quantification of sulfated GAGs and hyaluronic acid in the urine

For analysis of sulfated GAG, 8mL of each urine were concentrated and washed twice with distilled water (8mL) in tubes of Amicon Ultra concentration 3kDa (Millipore Corporation, Carrigtwohill, Ireland) to a final volume of 20mL. Five mL of the solution were subjected to electrophoresis as described above, and the GAG present in the sample quantified by comparison with a standard mix of GAG (containing 1mg/mL of chondroitin sulfate, dermatan sulfate and heparan sulfate, all of Seikagaku Kogyo Co, Tokyo, Japan). The content of GAG present in different samples was expressed as mg/mg creatinine. The analysis of the HA method was performed as previously described (23,24). Briefly, 100mL/well of each urine (diluted 1:2 in assay buffer Tris-HCl0, 05M, pH 7.75 + 1% BSA) and the same amount of a standard curve (0-1000ng/mL) from human umbilical cord HA (Sigma-Aldrich, St. Louis, MO) were applied in triplicate in ELISA plates (Perkin--Elmer Life Sciences-WallacOy, Turku, Finland) pre coated with an HA-binding protein extracted from bovine nasal cartilage (21). After incubation at 4ºC, 12 hours, the plates were washed six times with wash buffer (Tris-HCl0, 05M, pH 7.75) followed by the addition of HA binding protein labeled with biotin (1mg/mL) diluted in assay buffer. The plates were then shaken at room temperature for 2 hours and washed 12 times with the wash buffer to remove protein not complexed to the biotinylated HA retained on board. Finally, the presence of biotin on the plate was determined by adding 100mL/well of streptavidin labeled with europium (Perkin-Elmer Life Sciences-WallacOy, Turku, Finland) diluted 1:10,000 in assay buffer, 30 minutes. To measure the fluorescence emitted by europium, 200mL of a solution Enhancement (Perkin-Elmer Life Sciences-WallacOy, Turku, Finland) were added to each well and read on equipment Victor 2 (Perkin-Elmer Life Sciences--WallacOy, Turku, Finland). HA values were expressed in ng/mg of creatinine.

Immunofluorescence of tissue

The tissue samples of patients with PBS/IC (n = 6) and control patients with SUI (n = 4) were washed in PBS and immediately fixed in 4% paraformaldehyde solution for 2 hours at room temperature. Then the fragments were embedded in freezing medium for fluorescence Tissue freezing medium - Tissue Tek (SakuraFinetek USA, Inc., Torrance, CA, USA) and frozen on dry ice and isopentane, and kept in a freezer at -70ºC, which were placed on silanized slides. The following cuts were made in a cryostat (Leica, Wetzlar, Germany) with a thickness of about 5mm. For immunofluorescence reactions the sections were washed five times with PBS and one time with PBS containing 0.1M glycine At this stage three antibody combinations were made in separate slides for analysis: 1) anti-CD44 (Santa Cruz Bioechnology Inc., CA, USA) vs. probe of biotinylated HA binding protein (prepared in our laboratory according to Martins et al., 2003); 2) anti-decorim (Seikagaku, Japan) vs. anti-TGF-β (Santa Cruz Bioechnology Inc., CA, USA); 3) anti-SINDEC (Bioechnology Inc. Santa Cruz, CA, USA) vs. anti--fibronectin (Bioechnology Inc. Santa Cruz, CA, USA). The slides were incubated with the antibodies mentioned above and also with the acid-binding protein, for 2 hours at room temperature. At the end of incubation, the slides were washed 3 times in PBS, and now incubated with secondary antibody or streptavidin conjugated with fluorescent marker Alexa FluorTM 488 or 594 or 546 or 630 or 430 (the number corresponds to the wavelength of excitation of the fluorophore conjugated) diluted 1:300 in PBS. After 30 min. of incubation, the slides were washed five times in PBS and nuclei were marked with DAPI (4'6-diamidino-2-phenylindole, dihidrocloride - marking in blue) 1:10,000 in PBS containing 0.01% saponin (detergent that permeates the cell membrane) for 30 min. Next, the slides were washed five times in PBS, washed again in ultrapure water and finally mounted with coverslips in histological Fluormont G (Electron Microscopy Sciences, Hatfield, PA, USA). The slides were observed and analyzed with a fluorescence microscope (Nikon Eclipse, TE300 and TE800, Tokyo, Japan) and confocal microscope (LSM500 meta - Carl Zeiss, Germany). The controls for the immunostaining experiments were performed omitting the primary antibody.

RESULTS

Table-1 summarizes the demographics of the patients.

Although the average concentration of GAG in tissue was higher in patients with PBS/IC (3.3mg/mg dry tissue, range: 0.58 to 7.08) than in the control group (2.7mg/mg dry tissue, range: 0.15 to 5.3), this difference was not statistically significant (p = 0.62) (Figure-1). Chondroitin sulfate (CS) and dermatan sulfate (DS) were compared to heparin sulfate (HS): CS + DS were the main component in the urothelium (mean CS + DS: PBS/IC 74.3%, control 78%; mean HS: PBS/IC 25.7%; control 22%).

In urine we found a statistically significant decrease ($p < 0.05$) of GAG in the PBS/IC group (0.45 ± 0.11mg/mg creatinine) compared to

Table 1 - Demographics of IC/PBS and SUI patients.

Pacient	Age (Year)	Symptoms	Urinary culture at surgery
IC/PBS 1	27	Vesical pain, nicturia	Negative
IC/PBS 2	40	Vesical pain, nicturia	Negative
IC/PBS 3	53	Supra pubic pain, nicturia	Negative
IC/PBS 4*	64	Nicturia	E.coli
IC/PBS 5	65	Vesical pain, nicturia, anal pain, dispareunia	Negative
IC/PBS 6	73	Vesical pain, nicturia, frequency	Negative
IC/PBS 7	43	Vesical pain, nicturia, dispareunia	Negative
IC/PBS 8	27	Vesical pain, nicturia	Negative
IC/PBS 9	57	Supra pubic pain, nicturia, dispareunia, frequency	Negative
IC/PBS 10	26	Supra pubic pain, nicturia, dispareunia, frequency	Negative
IC/PBS 11	48	Supra pubic pain, nicturia, dispareunia, frequency	Negative
SUI 1	68	Stress urinary incontinence	Negative
SUI 2	53	Stress urinary incontinence	Negative
SUI 3	71	Stress urinary incontinence	Negative
SUI 4	64	Stress urinary incontinence	Negative
SUI 5	61	Stress urinary incontinence	Negative
SUI 6	59	Stress urinary incontinence	Negative
SUI 7	64	Stress urinary incontinence	Negative
SUI 8	50	Stress urinary incontinence	Negative
SUI 9	79	Stress urinary incontinence	Negative
SUI 10	66	Stress urinary incontinence	Negative
SUI 11	54	Stress urinary incontinence	Negative

* This patient was excluded because of UTI.

the control group (0.62 ± 0.13mg/mg creatinine) (Figure-2). The percentage of GAG found in the urine of PBS/IC and controls was similar.

Although the average concentration of HA was lower in the PBS/IC group (1.71 ± 2.06ng/mg creatinine) compared to the control group (2.46 ± 2.07ng/mg creatinine), this difference was not significant (p = 0.48).

The immunofluorescence study revealed an intense labeling for HA without a corresponding increase in the expression of its receptor, CD44 (Figure-3). The expression of TGF-β and decorim was marked with greater intensity in two

Figure 1 - Urothelium concentration of sulfated GAG of IC/BS and control (SUI) patients. Vertical bars represent the standard deviations. Statistically significant deviation was not found between groups (p = 0.62).

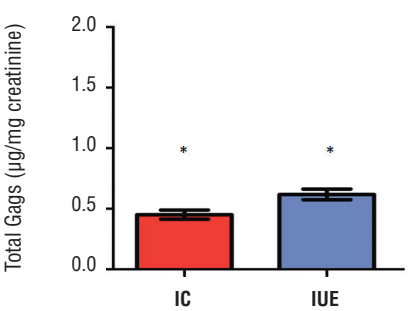


Figure 2 - Urinary concentration of sulfated GAG of IC/PBS and control (SUI) patients. Vertical bars represent the standard deviations. Statistically significant difference was found between groups (p < 0.05).

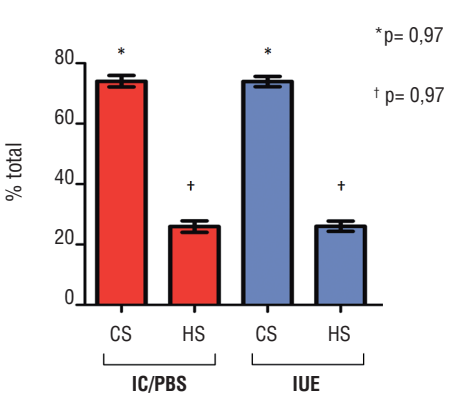
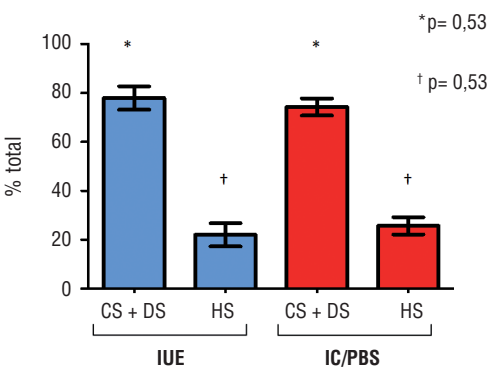


Figure 3 - Immunofluorescence of hyaluronic acid (HA) and its receptor (CD44) in the bladder urothelium. The tissue of SIU patients (CTR) and IC/PBS patients (PAT) were labeled with anti-HA receptor (green) and a protein biotinylated HA binding (red). The nuclei were stained with DAPI (blue). The overlap of the markings appears in the right pane (merged). Scale bar: 50µm.



patients compared to controls. There was also an intense labeling for fibronectin in patients compared to controls. Syndecan-4 had a significant decrease in expression.

DISCUSSION

In the present study we were able to show that urinary levels of GAG are lower in PBS/IC patients when compared to controls but the relative proportion of each GAG is the same in both groups. On the other hand, tissue concentration of GAG was similar. HA in urine was similar in both groups but in immunofluorescence HA labeling was more intense in PBS/IC patients. The same happened for fibronectin, decorin and TGF-β.

Urinary GAG content in PBS/IC patients has been shown to be decreased (25,26), similar (27,28) and increased (4,13,18). Our results showed a significant decrease in urinary excretion of sulfated GAG of patients with PBS/IC compared to the controls. Moreover, unlike previous studies, the technique employed in this study showed that the reduction in urinary GAG excretion in patients with PBS/IC occurred uniformly, with no predilection for any urinary GAG. Our study was not managed to analyze urinary GAG and the activity of the disease, such as degree of symptoms, glomerulation and histologic findings since they are not

reliable isolated markers today (29). As for the HA, although the average urinary concentration was lower in the PBS/IC group but there was no significant difference when compared to control. Distinct methods of extraction could explain such variation. But it is possible that PBS/IC level of activity may influence urinary GAG level. As previously stated, correlation between urinary GAG level and symptoms has already been described (4,13,18). We were not able to make such correlation.

However, urinary GAG level does not correlate to tissue GAG level. GAG expression in tissue has already been studied and no change in the content of GAG in the urothelium of patients with PBS/IC was described (8). The present study did not found statistically significant difference in the concentration of sulfated GAG in the urothelium of PBS/IC patients compared to controls. Hurst and Zebrowski (14) found the HS as the predominant GAG in the urothelium of patients with PBS/IC (55% of total GAG) but in our study the major GAG present in those urothelium was a combination of CS + DS which accounted for about 80% of the total. That proportion was not different from that of the controls meaning they had a decrease of total GAG in tissue but not a predilection for any GAG. Different methods of extraction and analysis of the different GAG could justify such differences. Moreover, the small size of the fragments availabe for the study may have hindered the analysis and the interpretation of these data.

Growth factors may be also increased in patients with PBS/IC (30). We have demonstrated by immunofluorescence increased expression of TGF-β. Simultaneously the expression of decorin was increased, a proteoglycan composed of CS and DS chains involved in the regulation of various cellular functions such as proliferation, adhesion and migration, which is able to mediate the binding of TGF-β with its membrane receptor (31). As the action of TGF-β depends on decorin, it makes sense that decorin is increased in a scenario of increased TGF-β. We also noticed an intense labeling of fibronectin in tissues of PBS/IC, especially in the deeper layers of tissue. Fibronectin is a glycoprotein with important role of scaffold in the extracellular matrix, regulating the cellular architecture and matrix components. Those higher expressions may represent modifications in the extracellular in an attempt to recovery urothelial damage. But one can not assure they are specific to PBS/IC. Variations are described in literature and this topic also remains controversial (9,15,16).

Syndecan is a transmembrane receptor that mediates cell adhesion to fibronectin, playing a key role in healing. Its lower expression was consistent with a previous study from our group that observed in animal models loss of expression of HS and syndecan on the surface of the urothelium of rats after induction of inflammation with DMSO (9). As shown in the study, it may be results from desquamation of the dysfunctional urothelium.

Another important step in this study was the evaluation of the expression of HA and its receptor CD44 in the urothelium by immunostaining. Hyaluronic acid is a key component of the extracellular matrix due to its viscoelastic properties and its hygroscopic capacity, promoting hydration and turgidity. The HA forms a base to which other proteoglycans can bind (32). It was remarkable that AH showed intense labeling in the urothelium of PBS/IC compared to controls, while CD44 did not labeled significantly different. That could represent a paradox at a glance. The CD44 is the major membrane receptor for HA and is responsible, among other things, by its binding to and internalization into cells where this element is digested by lysosomal hyaluronidase and the digestion products are reused for new synthesis (33). The combination of intense marking HA without a corresponding increase in the marking of its receptor may indicate a change of its turnover in the urothelium of PBS/IC ending up this compound to be "stored" in the interstitial. That pattern may represent a response to inflammation. In studies of mice lacking the CD44 receptor (knock-out) accumulation of hyaluronic acid is seen during bleomycin-induced lung inflammatory process. The result is excessive and uncontrolled severity of the inflammatory process leading to death of the animal (34).

Although we found a heterogeneous pattern of immunofluorescence for all patients with

PBS/IC, our findings are in agreement with other researchers, that analyzed biomarkers by immunohistochemistry in the urothelium of patients with cystitis and found distinct patterns of marking, sometimes with predominance of either one of a set of matrix components, but all the profiles different from those found in normal urothelium (15,16). Whereas the inflammatory involvement of the urothelium does not occur uniformly, our current results and also those of Hauser et al. (16) suggest different profiles of involvement and expression of various inflammatory components of the extracellular matrix and depending on the different stages of inflammation and/or remodeling that each segment of the bladder is subject. That is why a pattern of GAG expression has not been achieved. Because those differences, one may speculate that they are not PBS/IC specific inflammatory process but rather a non-specific reaction to any aggression. In attempts to recovery, modeling of the extracellular matrix takes place in a dysfunctional urothelium. As the process is going on, distinct expressions may be identified and help to understand the phase of the disease.

The main limitation of this study is the small number of patients. Control patients were those with urinary incontinence subjected to surgical correction because bladder biopsy would add little risk to them. Even in those patients finding a volunteer was a challenge that would be more difficult when looking for healthy controls. The same limitation in the number of PBS/IC patients was found because the necessity of normalizing their selection for research restricts in up to 60% of those with clinically PBS/IC (35). There may also have had some bias in patient selection because our hospital is a tertiary referral for IC/PBS. Another important issue was the small size of bladder biopsies. They were used for all the analyses and could not be taken in big bites because PBS/IC bladders have typically a high degree of inflammation and were done in the same procedure of hydrodistention.

CONCLUSIONS

The alterations in GAG may be related to an ongoing process of inflammation and tissue remodeling in a dysfunctional urothelum, reflecting the pathophysiology of the disease.

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CONFLICT OF INTEREST

None declared.

REFERENCES

1. Hanno PM, Burks DA, Clemens JQ, Dmochowski RR, Erickson D, Fitzgerald MP, et al.: AUA guideline for the diagnosis and treatment of interstitial cystitis/bladder pain syndrome. J Urol. 2011; 185: 2162-70.
2. Lilly JD, Parsons CL: Bladder surface glycosaminoglycans is a human epithelial permeability barrier. Surg Gynecol Obstet. 1990; 171: 493-6.
3. Parsons CL: The role of the urinary epithelium in the pathogenesis of interstitial cystitis/prostatitis/urethritis. Urology. 2007; 69(4 Suppl): 9-16.
4. Lokeshwar VB, Selzer MG, Unwala DJ, Estrella V, Gomez MF, Golshani R, Kester RR, Klumpp DJ, Gousse AE: Uronate peaks and urinary hyaluronic acid levels correlate with interstitial cystitis severity. J Urol. 2006; 176: 1001-7.
5. Parsons CL, Greenspan C, Mulholland SG: The primary antibacterial defense mechanism of the bladder. Invest Urol. 1975; 13: 72-8.
6. Parsons CL, Greenspan C, Moore SW, Mulholland SG: Role of surface mucin in primary antibacterial defense of bladder. Urology. 1977; 9: 48-52.
7. Parsons CL, Danielson B, Feldstrom B: Inhibition of sodium urate crystal adherence to bladder surface by polysaccharide. J Urol. 1985; 134: 614-6.
8. Nickel JC, Emerson L, Cornish J: The bladder mucus (glycosaminoglycan) layer in interstitial cystitis. J Urol. 1993; 149: 716-8.

9. Soler R, Bruschini H, Truzzi JC, Martins JR, Camara NO, Alves MT, et al.: Urinary glycosaminoglycans excretion and the effect of dimethyl sulfoxide in an experimental model of non-bacterial cystitis. Int Braz J Urol. 2008; 34: 503-11; discussion 511.
10. Soler R, Bruschini H, Truzzi J.C, Alves M.T, Martins J.R, Dreyfuss J, et al.: Role of urinary and urothelial glycosaminoglycans concentrations and DMSO local interaction in an experimental model of non-bacterial cystitis. Eur Urol. 2007; 2 (Suppl 6): 102.
11. Nickel JC, Downey J, Morales A, Emerson L, Clark J: Relative efficacy of various exogenous glycosaminoglycans in providing a bladder surface permeability barrier. J Urol. 1998; 160: 612-4.
12. Moldwin RM, Sant GR: Interstitial cystitis: a pathophysiology and treatment update. Clin Obstet Gynecol. 2002; 45: 259-72.
13. Lokeshwar VB, Selzer MG, Cerwinka WH, Gomez MF, Kester RR, Bejany DE, Gousse AE: Urinary uronate and sulfated glycosaminoglycan levels: markers for interstitial cystitis severity. J Urol. 2005; 174: 344-9.
14. Hurst RE: Structure, function, and pathology of proteoglycans and glycosaminoglycans in the urinary tract. World J Urol. 1994 12: 3-10.
15. Hurst RE, Roy JB, Min KW, Veltri RW, Marley G, Patton K, et al.: A deficit of chondroitin sulfate proteoglycans on the bladder uroepithelium in interstitial cystitis. Urology. 1996; 48: 817-21.
16. Hauser PJ, Dozmorov MG, Bane BL, Slobodov G, Culkin DJ, Hurst RE: Abnormal expression of differentiation related proteins and proteoglycan core proteins in the urothelium of patients with interstitial cystitis. J Urol. 2008; 179: 764-9.
17. Hanno P: Potassium sensitivity test for painful bladder syndrome/interstitial cystitis: con. J Urol. 2009; 182: 431-2, 434.
18. Akçay T, Konukoğlu D: Glycosaminoglycans excretion in interstitial cystitis. Int Urol Nephrol. 1999; 31: 431-5.
19. Forrest JB, Moldwin R: Diagnostic options for early identification and management of interstitial cystitis/painful bladder syndrome. Int J Clin Pract. 2008; 62: 1926-34.
20. Dietrich CP, Dietrich SM: Electrophoretic behaviour of acidic mucopolysaccharides in diamine buffers. Anal Biochem. 1976; 70: 645-7.
21. Martins JR, Gadelha ME, Fonseca SM, Sampaio LO, De L Pontes PA, Dietrich CP, et al.: Patients with head and neck tumors excrete a chondroitin sulfate with a low degree of sulfation: a new tool for diagnosis and follow-up of cancer therapy. Otolaryngol Head Neck Surg. 2000; 122: 15-8.
22. Perosa SR, Porcionatto MA, Cukiert A, Martins JR, Passeroti CC, Amado D, et al.: Glycosaminoglycan levels and proteoglycan expression are altered in the hippocampus of patients with mesial temporal lobe epilepsy. Brain Res Bull. 2002; 58: 509-16.
23. Martins JR, Passerotti CC, Maciel RM, Sampaio LO, Dietrich CP, Nader HB: Practical determination of hyaluronan by a new noncompetitive fluorescence-based assay on serum of normal and cirrhotic patients. Anal Biochem. 2003; 319: 65-72.
24. Martins JR, Furlanetto RP, Oliveira LM, Mendes A, Passerotti CC, Chiamolera MI, et al.: Comparison of practical methods for urinary glycosaminoglycans and serum hyaluronan with clinical activity scores inpatients with Graves' ophthalmopathy. Clin Endocrinol (Oxf). 2004; 60: 726-33.
25. Parsons CL, Hurst RE: Decreased urinary uronic acid levels in individuals with interstitial cystitis. J Urol. 1990; 143: 690-3.
26. Hurst RE, Parsons CL, Roy JB, Young JL: Urinary glycosaminoglycan excretion as a laboratory marker in the diagnosis of interstitial cystitis. J Urol. 1993; 149: 31-5.
27. Erickson DR, Ordille S, Martin A, Bhavanandan VP: Urinary chondroitin sulfates, heparan sulfate and total sulfated glycosaminoglycans in interstitial cystitis. J Urol. 1997; 157: 61-4.
28. Erickson DR, Xie SX, Bhavanandan VP, Wheeler MA, Hurst RE, Demers LM, et al.: A comparison of multiple urine markers for interstitial cystitis. J Urol. 2002; 167: 2461-9.
29. Hanno P, Lin A, Nordling J, Nyberg L, van Ophoven A, Ueda T, et al.: Bladder Pain Syndrome Committee of the International Consultation on Incontinence. Neurourol Urodyn. 2010; 29: 191-8.
30. Schwalenberg T, Stolzenburg JU, Ho TP, Mallock T, Hartenstein S, Alexander H, et al.: Enhanced urothelial expression of human chorionic gonadotropin beta (hCGβ) in bladder pain syndrome/interstitial cystitis (BPS/IC). World J Urol. 2012; 30: 411-7.
31. Shi Y, Massagué J: Mechanisms of TGF-beta signaling from cell membrane to the nucleus. Cell. 2003; 113: 685-700.
32. Nusgens BV: [Hyaluronic acid and extracellular matrix: a primitive molecule?]. Ann Dermatol Venereol. 2010; 137: S3-8.
33. Knudson W, Chow G, Knudson CB: CD44-mediated uptake and degradation of hyaluronan. Matrix Biol. 2002; 21: 15-23.
34. Krasiński R, Tchórzewski H: Hyaluronan-mediated regulation of inflammation. Postepy Hig Med Dosw (Online). 2007; 61: 683-9.
35. Hanno PM, Landis JR, Matthews-Cook Y, Kusek J, Nyberg L Jr.: The diagnosis of interstitial cystitis revisited: lessons learned from the National Institutes of Health Interstitial CystitisDatabase study. J Urol. 1999; 161: 553-7.

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ORIGINAL ARTICLE

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Long-term results of permanent memotherm urethral stent in the treatment of recurrent bulbar urethral strictures

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ABSTRACT

Purpose: To evaluate the long term outcomes of permanent Memotherm urethral stent in the treatment of recurrent bulbar urethral stricture.
Materials and Methods: Twenty patients who underwent permanent Memotherm urethral stent implantation due to recurrent bulbar urethral stricture following previous unsuccessful surgical procedure from 1996 to 2002 were included in the study. Long-term outcomes of the patients were evaluated.
Results: The overall success rate was 87.5% at the end of the tenth year. There was discomfort in implantation area in eight patients about 1 month following the procedure. These patients were treated with alpha-blocker and anti-inflammatory drugs. Stone formation was observed at the urethral stent implantation area in two patients. Post-void dripping has been observed in 15 patients up to the postoperative 3rd month. Stress urinary incontinence was observed in a patient with a 1-year follow-up. Partial stent migration was observed in two patients. None of the patients experienced pain during erection.
Conclusion: Memotherm urethral stent is a minimal invasive surgical procedure which can be safely and effectively used in patients with recurrent urethral stricture.

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Key words:
Urethral Stricture; Stents; Urinary Sphincter, Artificial

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INTRODUCTION

Recurrent urethral stricture is one of the biggest problems in Urology. Internal urethrotomy, intermittant urethral dilatation and open urethroplasty in the treatment of urethral strictures may not give the desired result every time. This situation negatively affects the quality of life in patients with recurrent urethral stricture. As an alternative treatment to traditional methods urethral stents have been used since 1985 in the treatment of urethral strictures and successful results have been reported by many centers (1,2).

We retrospectively evaluated the long term (10 years) outcomes of Memotherm permanent urethral stent.

MATERIALS AND METHODS

Twenty patients who were treated with Memotherm permanent urethral stent due to recurrent urethral stricture from 1996 to 2002 were included in the study. Memotherm is a thermoactive stent constructed of nitinol. Memotherm stent expands at body temperature and contracts at colder temperatures, so it is easy to remove. It is a wall rather than a mesh stent like the Urolume which might limit the hyperplastic reaction.

The mean age was 48 years (23-76) and the average length of the stricture was 2.5 cm (0.5-5.5) respectively. Mean duration of urethral stenosis was 3.7 years (1.3-6.2) and all patients experienced internal urethrotomy at least 3 times

previous to urethral stent implantation. The mean follow-up was 12 (10-16) years. Demographic data of the patients are provided in Table-1.

Prior to surgery physical examination, urinalysis, routine serum tests, uroflowmetry, postvoiding residual urine volume measurement (PVR), retrograde urethrography, urethroscopy and ultrasound scan (US) of the urinary tract were performed. All patients used 2nd generation cephalosporins for prophylaxis.

In the first two patients the stent was placed gently in the urethra beginning 0.5 cm proximal to the stricture just after internal urethrotomy procedure under spinal anesthesia with 0 degree optical image. Urethrotomy was performed at 12 o'clock direction and we observed urethral stent migration through the internal urethrotomy incision in both patients. In our opinion the urethral tissue where the incision was performed was weak and epithelization had possibly occurred into the urethral stent causing a partial obstruction in the stent lumen and thus the urethral stent migrated through the weak tissue at 12 o'clock position. We thought that the migration rate could probably be decreased by urethral catheterization for a period of time allowing the re-epithelization of urethral tissue around the urethral catheter. Therefore we decided to change the subsequent surgical procedures. The latter 18 patients primarily underwent internal urethrotomy under spinal anesthesia and the patients maintained a urethral Foley catheter for two weeks following the procedure. At the postoperative 2nd week urethral catheter was removed and the stent was gently placed in the urethra beginning 0.5 cm proximal to the stricture with 0 degree optical image under local anesthesia in an outpatient basis. We performed the procedure under local anesthesia to see the voluntary contractions of external urethral sphincter and thus inserting urethral stent to the optimal position in the urethra. For the urethral stenosis close to the external urethral sphincter, care was taken during the stent implantation not to include the sphincter. No Foley catheter was inserted after the urethral stent placement. All patients were evaluated with uroflowmetry, PVR measurement, and urethroscopy (to evaluate stent epithelization) in the postoperative first month. All patients were followed at 3 months intervals for the first year than yearly. PVR, average (Q_{ave}) and maximum urinary flow rate (Q_{max}) values of the patients measured prior to the surgery, at the 1st and 10th year postoperatively are shown in Table-2.

Wilcoxon binary sample test was used for statistical analysis and $p < 0.05$ was considered to indicate statistical significance.

Table 1 - Patients' characteristics and causes of urethral stenosis.

Mean age (year)	48 (23-76)
mean stenosis duration (year)	3.7 (1.3-6.2)
The average length of stricture (cm)	2.5 (0.5-5.5)
The mean follow-up (year)	12 (10-16)
Previous failed surgeries	**Number of patients (%)**
Internal urethrotomies	16 (80)
Open urethroplasty	3 (15)
Railroad catheterization	1 (5)
Etiology	**Number of patients (%)**
Trauma	9 (45)
After endoscopic urethral venture	7 (25)
Post-infectious	1 (5)
Attempt wrong urethral catheter	2 (10)
Idiopathic	1 (5)

RESULTS

The mean age of the patients was 48 (23-76) years. Patients were followed for an average 12 (10-16) years. The stent was placed under spinal anesthesia in the first two patients. But the stents migrated. These stents were positioned using repositioning forceps. The stent was placed under local anesthesia in the latter 18 patients. None of these patients had stent migration after the surgical procedure was modified as explained previously.

Table 2 - Uroflowmetry and PVR measurements preoperatively, at the postoperative 1st and 10th year.

	Preoperative	1-year follow-up	10-year follow-up	p value
Mean Q_{max} (mL/s)	6.9 (4-11)	23.7 (14-32)	22.4 (13-33)	< 0.05
Mean Qaverage (mL/s)	3.7 (2-5)	11.2 (8-14)	10.5(7-13)	< 0.05
PVR (mL)	190 (100-330)	37(0-50)	22 (0-80)	< 0.05

The mean preoperative Q_{max} was 6.9(4-11) mL/s, Q_{ave} 3.7(2-5) mL/s and PVR was 190(100-330) mL. The mean Q_{max} was 23.7(14-32) mL/s and 22.4(13-33) mL/s, the mean Q_{ave} was 11.2(8-14) mL/s and 10.5(7-13) mL/s , the mean PVR was 37(0-50) mL and 22(0-80) mL at the 1st and 10th year follow-up, respectively. The data are summarized in Table-2.

The urethral opening was obtained in 18 of 20 patients (90%) at the end of the first year following single procedure (Figure-1). Secondary stent implantation was performed in two patients. None of our patients underwent neither internal urethrotomy or transurethral resection for hyperplastic reaction. The stone formation occured at urethral stent implantation area in two patients (Figure-2). These stones were fragmented using endoscopic lithotripsy. The stents were observed as re-epithelialized in the 2nd month after the stones have been fragmented and no patients experienced extraction of the stent due to stone formation. Partial stent migration was observed in two patients. Firstly the urethra was irrigated with cold saline and then these stents were repositioned using re-positioning forceps. Eight patients experienced discomfort in the implantation area at the first month following the procedure. These patients were treated with alpha-blockers and anti-inflammatory drugs. No stent extraction was performed due to discomfort in the implantation area. Fifteen patients have experienced post-voiding dripping for 3 months following the stent placement procedure. But post-voiding dripping decreased in 10 patients by the 10-year follow-up. Stress urinary incontinence was observed in one patient with a 1-year follow-up. None of the patients presented with pain during erection. Complications are listed in Table-3. The overall success rate was 87.5% at the postoperative 10th year.

DISCUSSION

Despite the advances in technology endoscopic treatment of urethral stenosis still does not have the desired outcomes. Frequent recurrence of stenosis and lack of curative treatment

Figure 1 - Appearance of the urethra at the postoperative 1st and 10th year respectively.

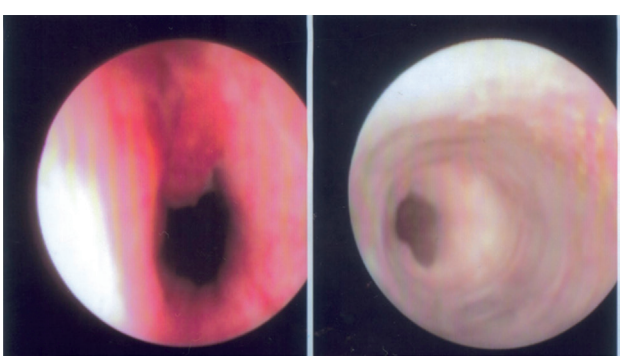

Figure 2 - Stone formation in implantation area.

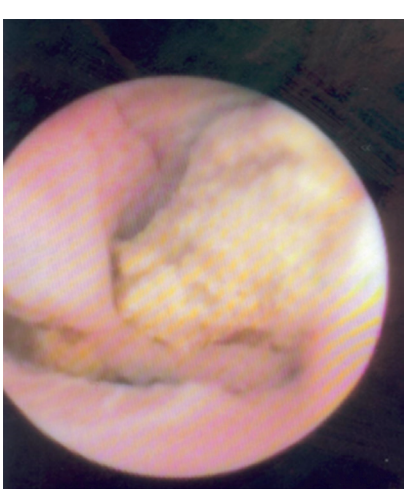

Table 3 - Complication rates.

	Number of patients (%)
Discomfort in implantation area	8 (40)
Partial stent migration	2 (10)
Stone formation in implantation area	2 (10)
Dripping after micturition	15 (75)

have a negative impact on patients' psychology. The long-term treatment success rate is 20-45% after the first internal urethrotomy. Success rate after urethroplasty using a variety of techniques is up to 90-95%. However, urethroplasty success rate decreases to 40% in complex urethral strictures (3). Due to low success rates of internal urethrotomy and difficulty in the urethroplasty technique, clinicians searched for alternative methods which can be used in the treatment of urethral strictures including metallic urethral stents (Urolume, Memotherm) (4).

Milroy et al. reported a 63% success rate at long term follow-up of the permanently implantable 'Urolume' stent in 1993 (5). Also Sertcelik et al. reported their clinical experience with Urolume in 2000. In that study they reported an 87% success rate at a mean of 3.8-year follow-up in 60 patients who had recurrent bulbar urethral stenosis (6).

Memotherm is a thermoactive stent constructed of nitinol. Memotherm can be extracted easily and repositioned since it does not stick to the tissue. Ponce et al. have achieved complete success in four patients with complicated urethral stenosis using Memotherm metalic stent with long-term follow-up (7).

In the present study Memotherm stent has been inserted immediately after internal urethrotomy operation in the first two patients and stent migration has been observed following the procedure. Urethrotomy was performed at 12 o'clock direction and we observed urethral stent migration through the internal urethrotomy incision in both patients. In our opinion the urethral tissue where the incision was performed was weak and epithelization possibly occurred into the urethral stent causing a partial obstruction in the stent lumen and thus the urethral stent migrated through the weak tissue at 12 o'clock position. We thought that the migration rate could probably be decreased by urethral catheterization for a period of time allowing the re-epithelization of urethral tissue around the urethral catheter. Therefore we decided to change the subsequent surgical procedures. The surgical method has been modified and eighteen patients underwent Memotherm stent insertion observing the voluntary contractions of external urethral sphincter. At the postoperative 2nd week urethral catheter was removed and the stent was gently placed in the urethra beginning 0.5 cm proximal to the stricture with 0 degree optical image under local anesthesia in an outpatient basis. We performed the procedure under local anesthesia to see the voluntary contractions of external urethral sphincter and thus inserting urethral stent to the optimal position in the urethra. No stent migration has been observed in these 18 patients. In contrast with the previous studies, none of our patients presented with partial migration after the surgical procedure has been changed (3,7,8). We think that stent implantation should be performed at least 2 weeks following the internal urethrotomy procedure to achieve optimal epithelization. The high success rate (87.5%) presented here was probably due to the modified surgical procedure which was explained above. In the present study eight patients reported discomfort in the implantation area in the first postoperative month and the patients were treated with alpha-blocker and anti-inflammatory drugs. In contrast with the previous studies, none of our patients experienced the extraction of the stent due to discomfort in the implantation area (3).

Two patients presented with stone formation in the implantation area during the follow-up and the patients were treated with endoscopic stone fragmentation. None of the patients underwent stent extraction following the endoscopic fragmentation of the urethral stones. At urethroscopy performed in the first month following the endoscopic stone fragmentation, sufficient urethral opening and epithelization over the stent was observed. In our experience we observed that

stent extraction is not necessary for the endoscopic treatment of urethral stones. To the best of our knowledge this is the first study reporting stone formation in the implantation area.

None of our patients underwent neither internal urethrotomy nor transurethral resection for hyperplastic reaction. Post-voiding dripping which has been observed in 15 patients, continued up to 3 months after the stent placement. But this decreased in 10 patients by the 10-year follow-up. In reviewing the literature, this may be due to post-voiding of urine accumulated in the stent (8). The rate of dripping after micturition decreased after stent epithelization in the long-term follow-up (3).

In the present study the mean preoperative Q_{max} was 6.9(4-11) mL/s, Q_{ave} 3.7(2-5) mL/s and PVR was 190(100-330) mL. The mean Q_{max} was 23.7(14-32) mL/s and 22.4(13-33) mL/s, the mean Q_{ave} was 11.2(8-14) mL/s and 10.5(7-13) mL/s, the mean PVR was 37(0-50) mL and 22(0-80) mL at the 1- and 10-year follow-up, respectively. Sertcelik et al. reported the mean PVR in patients with urethral stenosis as 210 (100-350) mL, and at 1 and 7 years after stent placement it was 36 (0-700) mL and 27.5 (0-100) mL, respectively. They also reported the Q_{max} before stent placement as 6.8 (4-10) mL/s and at 1 and 7 years after stent placement it was 22.4 (16-33) mL/s and 22.2 (12-35) mL/s, respectively (3). Ricciotti et al. reported the mean Q_{max} as 24.4 (18-33) mL/s and the PVR was 36 (0-80) mL at 6 months after the stent placement. They also reported complete epithelization in 20 of 21 patients with recurrent urethral stenosis and 18 F urethral calibration at the 6-month follow-up (9).

Since the study presented here is retrospective and non-randomized, it can potentially have selection bias. In addition, the number of patients included in the study is relatively low to support the usefulness and efficacy of a modified surgical technique. All patients presented here had bulbar urethral stricture and we couldn't make a comparison between patients who had urethral strictures in different localizations including penile, bladder neck etc. Further high numbered, prospective and randomized studies are necessary to support our findings.

In conclusion we assessed the Memotherm urethral stent implantation in the treatment of recurrent bulbar urethral stricture. Memotherm stent expands at body temperature and contracts at colder temperatures, so it is easy to remove. It is a wall rather than a mesh stent like the Urolume which might limit the hyperplastic reaction. In spite of various opinions about the treatment of patients with recurrent urethral stricture, in our opinion urethral stent implantation is a minimal invasive technique that can be safely and effectively used as a primary surgical procedure in the treatment of recurrent urethral stricture. Urethral stent implantation should be the preferred method after two consecutive unsuccessful internal urethrotomy procedures. In our experience implantation of the stent using the surgical technique explained at least 2 weeks following the internal urethrotomy procedure would potentially increase the success rates. This finding should be supported with further prospective and randomized studies.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Braf Z, Chen J, Sofer M, Matzkin H: Intraprostatic metal stents (Prostakath and Urospiral): more than 6 years' clinical experience with 110 patients. J Endourol. 1996; 10: 555-8.
2. Parikh AM, Milroy EJ: Precautions and complications in the use of the urolume wallstent. Eur Urol. 1995; 27: 1-7.
3. Sertcelik MN, Bozkurt IH, Yalcinkaya F, Zengin K: Long-term results of permanent urethral stent Memotherm implantation in the management of recurrent bulbar urethral stenosis. BJU Int. 2011; 108: 1839-42.
4. Milroy EJ, Chapple C, Eldin A, Wallsten H: A new treatment for urethral strictures: a permanently implanted urethral stent. J Urol. 1989; 141: 1120-2.
5. Milroy E, Allen A: Long-term results of urolume urethral stent for recurrent urethral strictures. J Urol. 1996; 155: 904-8.
6. Sertcelik N, Sagnak L, Imamoglu A, Temel M, Tuygun C: The use of self-expanding metallic urethral stents in the treatment of recurrent bulbar urethral strictures: long-term results. BJU Int. 2000; 86: 686-9.

7. Ponce Campuzano A, González Satué C, Rodríguez Tolrà J, Trilla Herrera E, Serralach i Milà N: Treatment of urethral stenosis with thermo-expandable prosthesis "Memotherm". Our experience. Arch Esp Urol. 2000; 53: 253-8.
8. Gottfried HW, Schlmers HP, Gschwend J, Brändle E, Hautmann RE: Thermosensitive stent (Memotherm) for the treatment of benign prostatic hyperplasia. Arch Esp Urol. 1994; 47: 933-43; discussion 943-6.
9. Ricciotti G, Bozzo W, Perachino M, Pezzica C, Puppo P: Heat-expansible permanent intraurethral stents for benign prostatic hyperplasia and urethral strictures. J Endourol. 1995; 9: 417-22.

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EDITORIAL COMMENT

This is an important article that certainly adds to existing knowledge about the long term results of permanent memotherm urethral stent for urethral strictures. Urologists have to deal with scarce specific data to make projections about clinical evolution of these complex situations. The current paper assesses the feasibility of the procedure and the (satisfying) outcome for most of the patients. Interestingly, stone formation in the stent area was first described for nitinol urethral devices. Unfortunately, the long term evolution after stone removal was not described, since follow-up was restricted to a month period.

Currently there is no consensus among researchers on which intervention is best for urethral stricture disease in terms of results, and also costs and adverse effects (1). Undoubtely, memotherm stent is a valid alternative in cases that have had previous failed surgical procedures.

REFERENCES

1. Barbagli G, Sansalone S, Djinovic R, Romano G, Lazzeri M: Current controversies in reconstructive surgery of the anterior urethra: a clinical overview. Int Braz J Urol. 2012; 38: 307-16; discussion 316.

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ORIGINAL ARTICLE

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Validation of a novel non-biological bench model for the training of percutaneous renal access

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ABSTRACT

Purpose: The percutaneous renal access (PRA) is the most critical step of percutaneous renal surgery (PRS). For the training of PRA in the lab, a novel non-biological bench model was developed and set for validation test.

Materials and Methods: Experts in PRS (> 60 cases) and novices were included to perform fluoroscopy guided PRA on the model. Overall time, X-ray exposure time and puncture attempts were recorded to establish construct validity. After accomplishment, the experts rated the model using a standardized questionnaire for face and content validity based on a 5-point Likert scale, with 1 denoting very bad and 5 as excellent. Baseline and post-training data of novices were analyzed for skill acquisition.

Results: 9 experts and 30 novices were finally included. The overall appraisal was 4 by the experts, and consensus of all experts was reached for the model as an excellent training tool. Significant difference between experts and novices was detected with the experts using less total time 183.11 ± 29.40 vs. 278.00 ± 50.30 seconds ($P < 0.001$), shorter X-ray exposure time 109.22 ± 19.93 vs. 183.13 ± 38.83 seconds ($P < 0.001$), and fewer attempts 1.28 ± 0.44 vs. 2.35 ± 0.65 ($P < 0.001$). After training, the novices demonstrated significant skill improvement in total and fluoroscopy time, and number of attempts ($P < 0.001$).

Conclusions: Our non-biological model provides a new method for PRA training. The face, content and construct validity were demonstrated. This model allows contact with PRA skills and could be applied to the first step in the learning curve.

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Key words:
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INTRODUCTION

Percutaneous renal surgery (PRS) is regarded as one of the advanced techniques in endourology. Successful percutaneous renal access (PRA) is the most important integral step of the overall procedure. Most endourologists acquire the necessary skills and experience in the operating theater. Learning curve analysis of percutaneous nephrolithotomy has suggested that surgical competence is achieved after 60 cases and surgical excellence after 115 cases (1,2). However, with the advancement of simulation in medical education, surgical skills can be practiced and acquired by the training in the laboratory before entering the operating theatre (3,4). For the purposes of learning and training for PRS in the laboratory, we developed a novel non-biological bench model which allows for percutaneous renal puncture, aspiration, tract dilation, sheath introduction, endoscopic pelvicaliceal system inspection, and intrarenal lithotripsy. The present study was designed to evaluate the appropriateness and effectiveness of the model as a training modality for PRA.

MATERIALS AND METHODS

In 2009, a novel non-biological bench model was designed in Wu Jie Ping Urology center of Peking University Shougang Hospital and manufactured with mixed silicon materials by Yingkou Guidong Medical Apparatus Co. Ltd (Yingkou, Liaoning, China). It is 36cm X 32cm X 12cm in dimension and has three parts - a kidney with dilated pelvicaliceal system, a ureteral stamp, and non-transparent perirenal tissue about 4cm in thickness (Figure-1). Each model has 13 calyces at different directions for PRA training (Figure-2). The texture is made to simulate that of human body. Artificial stones can be pre-placed in the kidney for relevant manipulations. Both fluoroscopy and ultrasound guided PRA practice were feasible, and repetitive puncture and usage by multiple trainees were proved during experimentation. Genuine surgical equipment and tools were applied at the user's preference, including C-arm (SIREMOBIL Compact L, Siemens, Muenchen, Germany), ultrasound set (Flexfocus 400, BK Medical, Herlev, Denmark),

Figure 1 - a) non-biological model; b) cross section of the model.

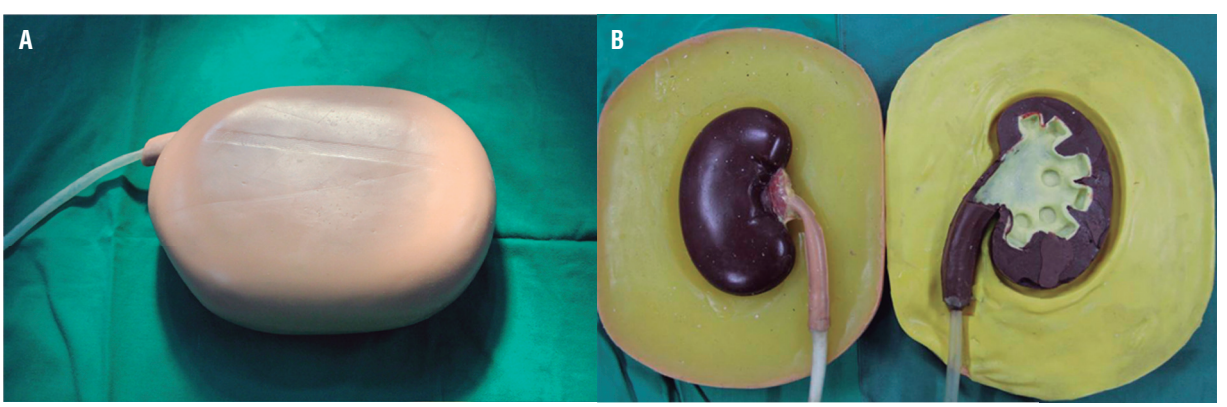
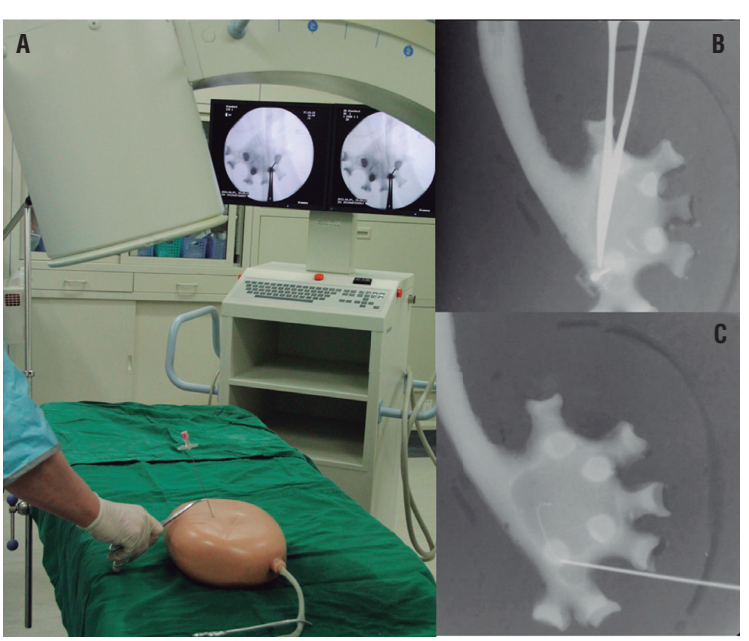


Figure 2 - a) Practice of fluoroscopy guided PRA; b) Puncture, C-arm at 20 degrees; c) Guidewire placement, C-arm upright



lithotripter (Lithoclast Master, EMS, Nyon, Switzerland), 8.6-9.8F semirigid ureteroscope (Olympus, Hamburg, Germany), 26F rigid nephroscope (Olympus, Hamburg, Germany), 16F peel-away sheath (Urovision, Bad Aibling, Germany), Amplatz serial dilators and sheaths (Cook Medical, Spencer, USA), and Alken coaxial telescopic metal dilators (Olympus, Hamburg, Germany).

With the success from experiments, we started a study for validation of the model as a training tool of fluoroscopy guided PRA. Experts (> 60 cases of PCNL) were invited to perform PRA in the same fashion as in real operating theatre. An independent observer judged the successful PRA. Only experts with successful PRA were considered for the questionnaire-based assessment of face and content validity. The experts rated the models using the questionnaire, which was based on a 5-point Likert scale, with 1 denoting very bad and 5 as excellent. Novices (urologists without prior PRA experience) received an orientation course in terms of standard fluoroscopy-guided PRA, the model anatomy of the pelvicaliceal system after injecting contrast medium and their intended direction of the access tract, and observed a live procedure demonstration. Baseline assessment of puncture and wire placement skills (twice) was done by a single, independent expert observer noting the novice perform PRA in an appropriate way. Objective parameters such as overall procedural time, x-ray exposure time, and access attempts were noted (pretest) for evaluation of construct validity. Then the novices received two 1-hour sessions of supervised training to facilitate PRA skill learning. The novices further attempted to perform PRA twice using the same scenario (posttest) after 24 hours. Differences in objective parameters between the posttest and pretest demonstrated the skill acquisition of novices. SPSS 13.0 software (IBM Software, USA) was used for data comparative analysis. $P \leq 0.05$ was regarded as of statistical significance.

RESULTS

From October 2010 to February 2012, 11 experts and 37 novices in PRS participated in the present study. Although all 11 experts successfully accomplished the fluoroscopy guided PRA, only 9 fulfilled the questionnaire on face and content validity. A total of 37 novices performed PRA on the model, and complete evaluation of 30 was available. Those excluded were either of unavoidable circumstances or novices with incomplete baseline or posttest data.

At the experts' view, the overall appraisal and graphics of the model were 4 (1 as very bad and 5 as excellent). Simulation complexity was given 3 because of the dilated collecting system by majority of experts. All experts had consensus of the model as an excellent training tool for PRA. Most experts claimed practice on this model covered most key steps of PRS and agreed on the possibility of using the model as an assessment tool. However, further validation studies are considered necessary. Overall face and content validity are summarized in Table-1. In the analysis for construct validity, experts significantly outperformed the novices with shorter time on task, reduced time of X-ray exposure, and fewer attempts

Table 1 - Results of face and content validity (experts).

Questionnaire	Subjective field	Median score (range, 1-5)
1	Overall appraisal	4 (3-5)
2	Simulation of ease/complexity	3 (3-4)
3	Graphics	4 (3-5)
4	Training tool	5 (5)
5	Assessment tool	4 (3-5)

to puncture (Table-2). In pretest and posttest comparison, novices demonstrated significant acquisition of skills in reducing the total procedural time and fluoroscopy time, and decreasing the number of attempts to puncture (Table-3).

Table 2 - Construct validity (experts and novices).

Data parameters studied	Expert (n = 9; mean ± SD)	Novices (n = 30; mean ± SD)	F	p-value
Total time (S)	183.11 ± 29.40	278.00 ± 50.30	28.73	< 0.001[a]
X-ray exposure time (S)	109.22 ± 19.93	183.13 ± 38.83	29.84	< 0.001[a]
Number of attempts	1.28 ± 0.44	2.35 ± 0.65	21.61	< 0.001[a]

[a] Statistically significant($p \leq 0.05$).

Table 3 - Acquisition of skills (novices).

Data parameters studied	Novice pretest (mean ± SD)	Novice posttest (mean ± SD)	F	p-value
Total time (S)	278.00 ± 50.30	189.93 ± 52.18	44.30	< 0.001[a]
X-ray exposure time (S)	183.13 ± 38.83	121.97 ± 32.81	43.43	< 0.001[a]
Number of attempts	2.35 ± 0.65	1.43 ± 0.50	37.60	< 0.001[a]

[a] Statistically significant($p \leq 0.05$).

DISCUSSION

Simulation based surgical education has been addressed for years (5). It is shown surgical skills can be acquired through deliberate practice in the laboratory outside the operating theatre (3,6). Within this purpose, however, few models have been established in the literature for PRA training and they are of 3 categories: ex-vivo models, virtual reality simulator, and non-biological trainer or models (7).

In ex-vivo or biological models, porcine kidneys are used for its similarity in anatomy and size to that of human (8) and are either hidden in foam layer, embedded in silicone gel, enclosed in chicken carcass, or wrapped in full thickness skin flap (9-13). Fluoroscopy or ultrasound guidance, or both, could be used for PRA practice. These models were claimed to be low cost and simple to set up. The equipment used in clinical practice could be employed. Teaching and skill acquisition were practicable. However, no vigorous validation has been performed. When a specific wet lab, the wet-lab-use-only equipment and tools, organ harvesting, storage and preparation were all taken into account, the cost practicing on such models would not be low.

In recent years, virtual reality simulators (VRS) have been given more attention. A great advantage of VRS is that, apart from various scenarios of different levels for repetitive practice, it can provide objective data of certain critical parameters for trainees and evaluators to analyze (14). The PERC

Mentor™ (Simbionix, Lod, Israel) is such a VRS designed to train PRA skills under fluoroscopy, including X-ray orientation, puncture, aspiration, and guidewire manipulations. In 2005, Knudson et al. (15) and colleagues demonstrated the face, content and construct validity of this particular VRS through a prospective randomized study. And then a few reports affirmed its usefulness as a training adjunct with discounted overall realism and tactile feedback (16,17). Tract dilation and intrarenal manipulations are not provided.

In the aspect of non-biological bench models, they are scarce in the literature. In 2011 EAU annual meeting, Schöppler et al. (18) in their poster announced to have developed such a model with a plastic cup cut in half on which some plastic tubes were fixed. The apparatus was then positioned in a box filled with agar-agar for drying before PRA training. No detailed description was provided. The Limbs & Things Ltd. (Bristol, UK) has another commercial silicon PRA trainer. It has two separate parts each for ultrasound and fluoroscopy guided practice, £500 and 680 respectively. The models are semitransparent with molded, non-dilatable collecting system inside. In our experimentations, the trainer became leaking after 1-2 punctures and was easy to see through from outside, hindering realistic simulation. The coarse tactile feeling of the silicon material was unpleasant for tract dilation.

Our non-biological bench model was the first in the literature of its kind designed by experienced endourologists together with a dedicated manufacturer willing to modify at the feedback of experimentations. It was to let trainees understand the concept of using fluoroscopy in 3D manner. Complexity of procedure was not the training objective. Some may argue it is too easy, but this model was meant for the first step in the learning curve. The texture was adjusted close to that of human with a good sealing effect because of the consistent nature of mixed silicon material. Almost unlimitedly repetitive puncturing was allowed without significant leakage. Tract dilation, sheath insertion, pelvicaliceal inspection and intrarenal lithotripsy with preplaced stones could also be performed. Each model cost about $550 and was ready for use. For the consideration of cost-effectiveness, we recommend tract establishment be performed after multiple trainees have successfully placed their guidewires in the collecting system. In this way, each setup could allow up to 6 trainees to practice complete PRS procedure at a time. Since no ethics and animal disease were involved, little extra investment on the lab equipment and environment was required. Genuine clinical equipment and tools could be employed and any space safe for fluoroscopy would be sufficient. Exposure to the equipment, tools, actual X-ray, and proper lead wear was also helpful for simulating real operating theatre environment.

In our study, all experts successfully accomplished the PRA procedure. According to the experts' comment, practice on this model covered most key steps of PRS. The tactile feedback was close-to-reality in puncturing and tract dilatation. With the summary of questionnaire, face and content validity were demonstrated. For a training model to be effective, construct validity, which distinguishes the level of training (19,20), has to be proved. In the present study, this was clearly demonstrated by comparing the results of experts with those of novices. With the supervised training of 2 hours, the improvement of trainees in terms of time and number of attempts reached close to expert level. Experts did not have pre-test hands--on practice and simplicity of the model and "hot hands" effect even with a 24h wash-out may play a role. However, a learning process was detected by the pretest and posttest analysis, meaning the improvement of skills had taken place.

Like any other non-biological model, our training model has certain limits. It has no overlapping ribs and does not simulate the movement of kidney or complications of bleeding and periorgan injuries. Though the face, content and construct validity were demonstrated in our study, an appropriate curriculum was to be set up. Parallel studies may be required to compare the effectiveness among different modalities before a structured curriculum for PRA training is built up. Transferability means that the skills acquired in the lab can be translated into the performance in the true clinical settings (21). However, this is extremely difficult to fulfill. Live animal model with an anesthesiated pig had been created by us in 2008 (unpublished data) similar to that reported by

Mishra et al. (14) who used such one to replicate a live surrogate for assessment of predictive validity or transferability of the PERC Mentor. Similar treatment may be applied to test the transferability of other training tools, including our model.

CONCLUSIONS

Our non-biological bench model provides a new method for PRA training. It is feasible for both fluoroscopy and ultrasound guided PRA practice and is ready for repetitive and multiple use. The face, content and construct validity as a training tool were demonstrated in our study. This model allows contact with PRA skills and could be applied to the first step in the learning curve.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Tanriverdi O, Boylu U, Kendirci M, Kadihasanoglu M, Horasanli K, Miroglu C: The learning curve in the training of percutaneous nephrolithotomy. Eur Urol. 2007; 52: 206-11.
2. Acosta E, Temkin B: Dynamic generation of surgery specific simulators -- a feasibility study. Stud Health Technol Inform. 2005; 111: 1-7.
3. Scott DJ, Cendan JC, Pugh CM, Minter RM, Dunnington GL, Kozar RA: The changing face of surgical education: simulation as the new paradigm. J Surg Res. 2008; 147: 189-93.
4. Wignall GR, Denstedt JD, Preminger GM, Cadeddu JA, Pearle MS, Sweet RM, et al.: Surgical simulation: a urological perspective. J Urol. 2008; 179: 1690-9.
5. Scott DJ, Cendan JC, Pugh CM, Minter RM, Dunnington GL, Kozar RA: The changing face of surgical education: simulation as the new paradigm. J Surg Res. 2008; 147: 189-93.
6. Rosen KR: The history of medical simulation. J Crit Care. 2008; 23: 157-66.
7. Mishra S, Jagtap J, Sabnis RB, Desai MR: Training in percutaneous nephrolithotomy. Curr Opin Urol. 2013; 23: 147-51.
8. Sampaio FJ, Pereira-Sampaio MA, Favorito LA: The pig kidney as an endourologic model: anatomic contribution. J Endourol. 1998; 12: 45-50.
9. Earp PP: Percutaneous renal surgery--new model for learning and training. Int Braz J Urol. 2003;9: 151-4.
10. Strohmaier WL, Giese A: Ex vivo training model for percutaneous renal surgery. Urol Res. 2005; 33: 191-3.
11. Hammond L, Ketchum J, Schwartz BF: A new approach to urology training: a laboratory model for percutaneous nephrolithotomy. J Urol. 2004; 172: 1950-2.
12. Häcker A, Wendt-Nordahl G, Honeck P, Michel MS, Alken P, Knoll T: A biological model to teach percutaneous nephrolithotomy technique with ultrasound- and fluoroscopy-guided access. J Endourol. 2007; 21: 545-50.
13. Zhang Y, Ou TW, Jia JG, Gao W, Cui X, Wu JT, et al.: Novel biologic model for percutaneous renal surgery learning and training in the laboratory. Urology. 2008; 72: 513-6.
14. Mishra S, Kurien A, Patel R, Patil P, Ganpule A, Muthu V, et al.: Validation of virtual reality simulation for percutaneous renal access training. J Endourol. 2010; 24: 635-40.
15. Knudsen BE, Matsumoto ED, Chew BH, Johnson B, Margulis V, Cadeddu JA, et al.: A randomized, controlled, prospective study validating the acquisition of percutaneous renal collecting system accessskills using a computer based hybrid virtual reality surgical simulator: phase I. J Urol. 2006; 176: 2173-8.
16. de la Rosette JJ, Laguna MP, Rassweiler JJ, Conort P: Training in percutaneous nephrolithotomy--a critical review. Eur Urol. 2008; 54: 994-1001.
17. Mishra S, Kurien A, Ganpule A, Muthu V, Sabnis R, Desai M: Percutaneous renal access training: content validation comparison between a live porcine and a virtual reality (VR) simulation model. BJU Int. 2010; 106: 1753-6.
18. Schoppler G.M., Heinzelbecker J, Krombach P, Alken, P: A training model for the puncture of ultrasound- and fluoroscopy- guilded percutaneous nephrolithotomy (abstract). Eur Urol Suppl. 2011; 10: 134.
19. Aucar JA, Groch NR, Troxel SA, Eubanks SW: A review of surgical simulation with attention to validation methodology. Surg Laparosc Endosc Percutan Tech. 2005; 15: 82-9.
20. Gallagher AG, Ritter EM, Satava RM: Fundamental principles of validation, and reliability: rigorous science for the assessment of surgical education and training. Surg Endosc. 2003; 17: 1525-9.
21. Seymour NE, Gallagher AG, Roman SA, O'Brien MK, Bansal VK, Andersen DK, et al.: Virtual reality training improves operating room performance: results of a randomized, double-blinded study. Ann Surg. 2002; 236: 458-63; discussion 463-4.

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ORIGINAL ARTICLE

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Analysis of the effect of renal excretory system cooling during thermal radiofrequency ablation in an animal model

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ABSTRACT

Objective: Analysis of renal excretory system integrity and efficacy of radiofrequency ablation with and without irrigation with saline at 2ºC (SF2).
Materials and Methods: The median third of sixteen kidneys were submitted to radiofrequency (exposition of 1 cm) controlled by intra-surgical ultrasound, with eight minutes cycles and median temperature of 90ºC in eight female pigs. One excretory renal system was cooled with SF2, at a 30ml/min rate, and the other kidney was not. After 14 days of post-operatory, the biggest diameters of the lesions and the radiological aspects of the excretory system were compared by bilateral ascending pyelogram and the animals were sacrificed in order to perform histological analysis.
Results: There were no significant differences between the diameters of the kidney lesions whether or not exposed to cooling of the excretory system. Median diameter of the cooled kidneys and not cooled kidneys were respectively (in mm): anteroposterior: 11.46 vs. 12.5 (p = 0.23); longitudinal: 17.94 vs. 18.84 (p = 0.62); depth: 11.38 vs. 12.25 (p = 0.47). There was no lesion of the excretory system or signs of leakage of contrast media or hydronephrosis at ascending pyelogram.
Conclusion: Cooling of excretory system during radiofrequency ablation does not significantly alter generated coagulation necrosis or affect the integrity of the excretory system in the studied model.

ARTICLE INFO

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INTRODUCTION

The rationale of the use of radiofrequency (RF) in medicine is based on the generation of heat in order to destroy cells.

At temperatures 60-100ºC range, there is instant protein coagulation that frequently evolves to coagulation necrosis (1). Consequently, the ideal working temperature varies from 50 to 100ºC throughout the target tissue (2).

However, heat is heterogeneously distributed. Generally, there is a major heating close to the needle and lower temperature in the outer tissues, since there is no uniform heat conductivity to the surroundings (2). A simple needle is capable to produce a maximum lesion of 1.6 cm of diameter (3).

Although not extensively studied, the use of saline in the excretory system before or during radiofrequency ablation may interfere in the heat

distribution and consequently in the efficacy and complications of the method.

We evaluated the integrity of the excretory system and efficacy of renal radiofrequency in an animal model with and without irrigation of the excretory system, using saline at 2ºC.

MATERIALS AND METHODS

After approval of the local Ethical Committee in Animal Research (protocol 041/03) and technique standardization during a pilot study using 12 kidneys of 6 animals, 16 kidneys of 8 female pigs free of specific pathogens (SPF - Specific Pathogen Free) of Large White breed, with ages from 54 to 62 days of life, weighting 15.5 to 26 kg, were studied.

The animals were adequately anesthetized (sodic thiopental, 2mg/kg; midazolam 0.1 mg/kg; alfentanil 0.1 mg/kg and pancuronium bromete 0.1mg/kg), submitted to oro-tracheal intubation and mechanic ventilation (Takaoka®) and placed on a horizontal dorsal decubitus. Then, it was performed a median longitudinal laparotomy, the kidneys were identified and the bladder was incised.

One of the ureteral meatus was catheterized with a double-lumen 7 Fr catheter in order to perform cooling using saline at 2ºC (using a digital thermometer Testo 106-T1® for control) at a medium flow of 30ml/min. Eight kidneys were randomly distributed, 4 animals at right and 4 at left, and the perfusion was initiated two minutes before the ablation, as proposed by Margulis et al (4). One of the ports of the catheter was used to infuse and the other to collect the cooled saline.

A generator model 1500 (RITA Medical Systems, Mountain View, California, maximum potency of 150W) was used to apply radiofrequency under direct vision in the medium third of both kidneys of each animal (n = 16), using a 1cm exposition needle and ultrasound control, keeping a constant distance between the center of the ablation area and the excretory system in all cases. It was used a cycle of 8 minutes and a medium temperature of 90ºC. The catheter was removed and the bladder was sutured.

After ablation was completed, the medium temperature of the liquid that was returning from the double-lumen catheter was 13.5ºC. Animal temperature was controlled intra-rectally and varied from 37 to 37.5ºC.

After 14 days, the integrity of the collecting system was evaluated using bilateral ascending pyelogram in vivo, injecting 5ml of iodine contrast after incision of the bladder and catheterization of both ureters.

The kidneys were removed and opened (convex part) and the biggest dimensions of the lesion were recorded: longitudinal, anteroposterior and depth, using a digital capiler rule as previously proposed (5,6).

Cellular viability was confirmed by histological aspect and presence of coagulation necrosis after hematoxilin-eosin stain. Next, the animals were sacrificed and the histopathologic exam was performed by a blind examiner, who was not aware of which side was cooled.

The size of the studied population (8 animals, 16 kidneys) was based on a pilot study using 14 animals (28 kidneys) and similar protocols in the literature. The “SAS System for Windows” (Statistical Analysis System), version 6.12. “SAS Institute Inc.”, 1989-1996, Cary, NC, USA and the t-Student tests were used to compare the measures. Significance level was 5% ($p < 0.05$).

RESULTS

The sample was quite homogeneous, given the animals weight and the kidney dimensions, with elevated p values (Table-1). There was no lesion of the excretory systems, signs of contrast leakage or hydronephrosis during ascending pyelogram.

All kidneys showed a lesion with a central area of coagulation necrosis, and the tubules and glomerules lost their cellular limits, with intense eosinophilia in the cytoplasm and areas with total destruction of cellular architecture and no nucleus. In the middle of the area of necrosis, some vessels with thrombosis and recanalization were observed, showing that heat ablation also damaged vessels, adding ischemic lesion to the area previously irrigated by those vessels. In the transition area with viable locals, it was possible to identify a well defined circle around the central

Table 1 - Sample data of the animals submitted to thermoablation using radiofrequency.

	MEDIAN	SD	MIN.	MAX.	P-VALUE
Animal (kg)	21.44	3.58	15.1	26	
High (C) (mm)	111.38	5.7	94.5	111.7	p = 0.995
High (mm)	101.39	7.24	90.3	111	
Width (C) (mm)	50.18	4.38	44.2	56.7	p = 0.232
Width (mm)	51.69	3.78	44.9	56.7	
Thickness (C) (mm)	23.86	1.56	22.3	26.9	p = 0.329
Thickness (mm)	24.63	1.86	22.6	28.1	

SD = Standard deviation; **MIN =** Minimum; **MAX =** Maximum; **(C) =** Cooled kidney

necrosis. It is a transition area with acute tubular necrosis (ATN), with hypereosinophilia of the tubular cytoplasm, nuclear basophilia and alteration of the glomerular architecture with inflammatory infiltrate. Cell viability in the area is uncertain, and it may or may not show tissue recovery.

There was no significant difference between the groups in terms of area of lesion, whatever considered diameter (Tables 2 and 3).

Media of diameters of lesion of the cooled and not cooled kidneys were, respectively (mm):

Anteroposterior: 11.46 vs. 12.5 (p = 0.23)
Longitudinal: 17.94 vs. 18.84 (p = 0.62)
Depth: 11.38 vs. 12.25 (p = 0.47)

There were no differences between the closest and the more distant areas in the irrigated kidneys. The microscopic aspects of lesions were very similar, independently of cooling of excretory system. There was no difference at histopathology and coagulation necrosis area between the groups (p > 0.05) (Figure-1).

In the urothelium areas close to the tissue submitted to RF it was observed a light distortion of the urothelial architecture. The umbrella cells, that form the most superficial layer of the urothelium, were bigger in size, vacuolated, suggesting entrance of liquid in the intracellular space due to alteration of permeability. There was also inflammatory process and some areas of subepithelium fibrosis. In some regions there was a massive lost of

Table 2 - Median size of the lesions of cooled and not cooled kidneys (mm).

	MEDIAN	SD	MIN.	MAX.	P - VALUE
AP - C	11.46	1.3	10	13.2	p = 0.232
AP	12.5	2.21	9.3	16.9	
Longitudinal - C	17.94	2.81	13.1	22.2	p = 0.625
Longitudinal	18.84	3.71	13.3	25.4	
Depth - C	11.38	1.77	7.6	13.3	p = 0.467
Depth	12.25	2.41	7.8	15.8	

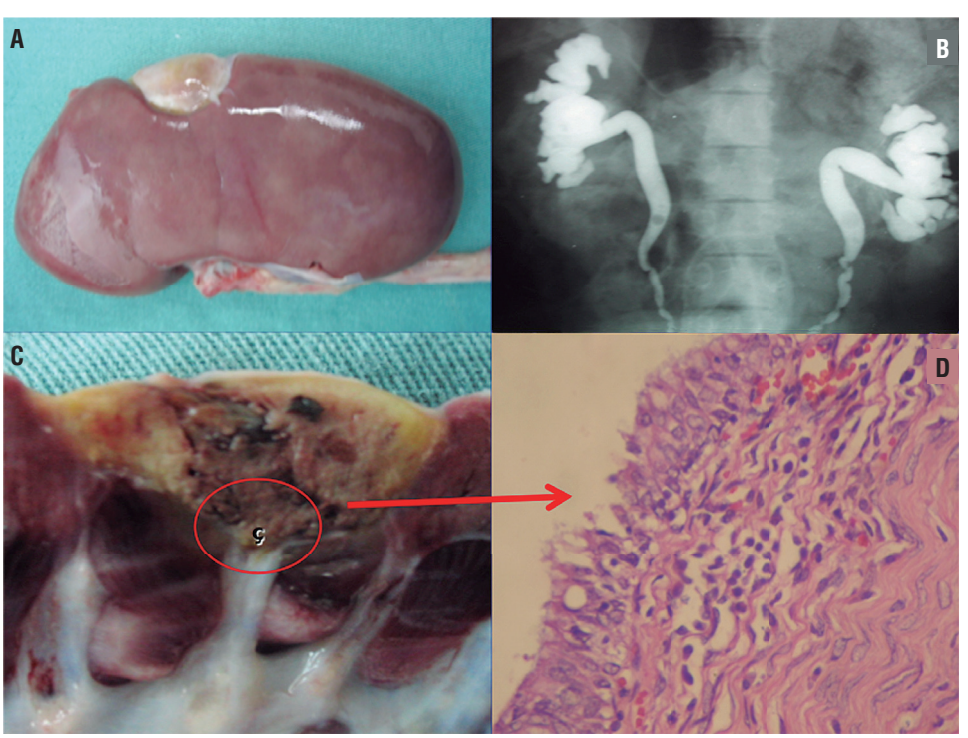
SD = Standard deviation; **MIN =** Minimum; **MAX =** Maximum; **AP =** Anteroposterior; **(C) =** Cooled kidney

Table 3 - Size of the lesions in each animal (mm).

	Cooled kidney			Kidney not cooled		
	AP	Long.	Depth	AP	Long.	Depth
Pig 1	11.3	17.8	13.5	10	13.1	7.6
Pig 2	13.2	18	14.2	10	17.4	11.3
Pig 3	12	22.5	15.8	13.2	16.6	12.7
Pig 4	16.9	25.4	11	11.7	18.9	13.3
Pig 5	13.1	17.2	12.5	12.2	17.4	12
Pig 6	13	19.7	12.1	10.2	21	11.6
Pig 7	9.3	13.3	7.8	13	22.2	12.2
Pig 8	11.2	16.8	11.1	11.4	16.9	10.3

AP = Anteroposterior; **Long =** Longitudinal

Figure 1: A) external aspect of the lesion; B) Ascending pyelogram without alterations; C) macroscopic aspect of the excretory system; D) Microscopy, 40X, urothelium.



cellular layers, with a single layer of paving stone cells. However, the alterations were discrete without cellular necrosis. Actually the urothelium showed signs of regeneration, with recovery of layers. Microscopic aspects of urothelium were very similar in the kidneys submitted to cooling or not.

DISCUSSION

Heat lesion of urothelium during thermoablation of renal tumor with radiofrequency is one of the complications of this form of treatment (7).

In the studied model, cooling of excretory system did not alter coagulation necrosis or interfere with the integrity of urothelium after 14 days of surgery, adding data to previous studies that limited evaluation to 74.8 to 107 days of protocol.

In order to define the sample size, we considered the study by Chang et al (8) that used six animals. Also, the present study was based on a pilot protocol that allowed the necessary standardizations with 12 kidneys of 6 animals, finishing with 28 kidneys and 14 animals. There was also intra-individual pairing: the same animal was submitted to two different forms of RF ablation allowing the use of a small population in order to obtain relevant statistical data (4), sparing experimental animals according to the best practices of animal experimentation.

We used ablation under direct vision, since porcine perirenal fat is scarce (9), favoring lesion of neighbor structures. In humans, this layer is less than 1 cm of thickness, with better protection and thermal isolation during RF ablation.

We defined a median temperature of 90ºC, capable to destroy tissues (1) during eight minutes, in one cycle, since this pattern proved to be effective during the pilot study.

The way to evaluate the renal lesion created by RF is also not standardized in literature, since it is quite irregular. Some use a formula to calculate the volume of an ellipse (10), others use only the greatest diameter (11) or both diameters multiplied. We believe that the analysis of the three biggest diameters (longitudinal, anteroposterior and depth), as described by Gettman and Rehman (5,6) is more accurate since allows tridimensional analysis of the lesion.

Lesions have a wedge form, probably due to the segmental pattern of renal circulation, with the flow running from the medulla to the cortex. When central flow is interrupted, the area downstream becomes ischemic and suffers infarct. So the tissue necrosis after RF ablation is obtained not only by heat but also by occlusion of vessels, followed by ischemia. The present study shows that the longitudinal diameter is around 6mm greater than the anteroposterior and depth diameters. These data were obtained in normal renal tissue. Maybe in tumor tissue the interaction with RF may be different.

The organ to be submitted to RF may be accessed by laparotomy, laparoscopy or percutaneously, the latter cheaper and less aggressive, that can be performed under local anesthesia and sedation (12). The advantage of ablation by laparotomy or laparoscopy, using an intra-surgical ultrasound is the capacity of direct visualization of the tumor and possible concurrent tumor lesions.

Image control during the procedure is fundamental to avoid lesions of neighborhood organs or even incomplete ablation. Unfortunately, there is still no image method able to fulfill this task and identifies the needle and its eventual repositioning (13).

In the present study, confirmation of cellular viability by histopathology is justified since there is no adequate correlation of radiologic image and histopathology after renal radiofrequency (14). The median temperature of the returning liquid by the double-lumen catheter of 13.5ºC is in accordance to other experiments in literature (4,15).

Anidjar et al (16), in 1999, studied the experimental model of heat ureteral lesion in pigs, coagulating all median upper ureter circumference using an electrical scalpel. After a median interval of 9 days, there was a marked hydronephrosis in all animals. Besides, current literature about lesions of excretory systems during tumor ablation by radiofrequency shows that in the majority of cases the lesion occurs in the first 15 days after the procedure (7,17). Also, maximal extension of necrosis is detected after 7 days of RF (7,18), which corroborates our analysis after 14 days of protocol.

In relation to the absence of difference of the size of lesion whether the kidney was cooled or not, Landman et al experiment (15) showed that during cooling performed exclusively by ureteral catheter, intraparenchymal temperature was not altered, since renal temperature depends mainly of arterial flow, that keeps the organ stable, as long as the flow is not obstructed. In that manner, it is possible to understand the results, independently of the cooling effect of the excretory system, proved but not completely explained.

On the other hand, although not statistically significant, it is important to observe a constant difference of 1mm less in the media of the lesion diameters of the cooled kidneys (Tables 2 and 3). It is important to confirm the clinical importance of this aspect since it can alter the efficacy of RF during cooling of excretory system of human beings. Long term studies with oncological data are necessary (19).

Hwang et al (20) also showed evidences, although fragile, that cooling of excretory system may alter the clinical results of RF. They observed a variation of the median diameter of the ablated area of cooled kidneys in relation to those not cooled after 7 days of post-operatory: 7.5% ($P = 0.002$) and 9.6% ($P = 0.008$), respectively.

The size of the lesion is not constant, and each situation is different in terms of interaction with the energy source and tissue response. The variation of the lesion size probably is related to the high blood flow of kidneys (15).

The comparison of the results of the present study with those related to biliary duct cooling during thermoablation with RF (21) shows that cooling is capable to reduce significantly the possibility of fistula or stenosis of biliary ducts. Some possible explanations can be discussed why the same was not observed in the urothelium. First, the epithelium linings are different. Second, urine and bile components are different and may have different electrical proprieties. And finally, space disposition of the biliar tree and of the urinary tract are different.

In the kidney, major complications occur after lesion of ureter or pelvic-ureteral junction, away from the renal cortex. In the liver, intra-hepatic lesion of the biliar ducts may present significant damages.

Also, the exposition of the needle was smaller in the present study (1cm) in relation to previous ones (3cm) (20) and intentionally was not place upon the excretory tract (7). So our results are related to lesions close to the urothelium and further studies are needed for more profound lesions with bigger damage to the urothelium and to test the consequences of cooling.

Some aspects differentiate our study: the use of in vivo retrograde pyelogram (most studies used ex vivo (20)), lower morbidity of retrograde cooling in relation to antegrade (that requires nephrostomy), avoiding dissemination, bleeding and additional trauma (20), use of hystopathology to measure the lesion, ablation under direct vision and use of intrasurgical ultrasound in order to maximize accuracy of ablation, data collection and hystological interpretation without knowledge of cooling, avoiding bias and the use of saline in order to avoid complications in the event of leakage and absorption.

Main limitations of our study are the absence of median and long terms data and not use of an oncological model, as occurred with other studies (4,7,20) as well as the low exposition of the needle (1cm) avoiding lesion of the excretory tract, although the clinical significance of the lesions is questionable (4,7,20).

CONCLUSIONS

Cooling of the excretory tract during ablation of renal parenchyma did not alter significantly the integrity of the urothelium and did not interfere with the area of coagulation necrosis generated by RF in relation to conventional ablation, without cooling, microscopically and radiologically.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Goldberg SN, Gazelle GS, Solbiati L, Livraghi T, Tanabe KK, Hahn PF, et al.: Ablation of liver tumors using percutaneous RF therapy. AJR Am J Roentgenol. 1998; 170: 1023-8.

2. Goldberg SN, Gazelle GS, Halpern EF, Rittman WJ, Mueller PR, Rosenthal DI: Radiofrequency tissue ablation: importance of local temperature along the electrode tip exposure in determining lesionshape and size. Acad Radiol. 1996; 3: 212-8.
3. Goldberg SN, Gazelle GS, Dawson SL, Rittman WJ, Mueller PR, Rosenthal DI Tissue ablation with radiofrequency: effect of probe size, gauge, duration, and temperature on lesion volume. Acad Radiol. 1995; 2: 399-404.
4. Margulis V, Matsumoto ED, Taylor G, Shaffer S, Kabbani W, Cadeddu JA: Retrograde renal cooling during radio frequency ablation to protect from renal collecting system injury. J Urol. 2005; 174: 350-2.
5. Rehman J, Landman J, Lee D, Venkatesh R, Bostwick DG, Sundaram C.: Needle-based ablation of renal parenchyma using microwave, cryoablation, impedance- and temperature-based monopolarand bipolar radiofrequency, and liquid and gel chemoablation: laboratory studies and review of the literature. J Endourol. 2004; 18: 83-104.
6. Gettman MT, Lotan Y, Corwin TS, Smith TG, Napper CA, Lindberg G, et al.: Radiofrequency coagulation of renal parenchyma: comparison of effects of energy generators on treatment efficacy. J Endourol. 2002; 16: 83-8.
7. Brashears JH 3rd, Raj GV, Crisci A, Young MD, Dylewski D, Nelson R, et al.: Renal cryoablation and radio frequency ablation: an evaluation of worst case scenarios in a porcine model. J Urol. 2005; 173: 2160-5.
8. Chang I, Mikityansky I, Wray-Cahen D, Pritchard WF, Karanian JW, Wood BJ: Effects of perfusion on radiofrequency ablation in swine kidneys. Radiology. 2004; 231: 500-5.
9. Hwang JJ, Walther MM, Pautler SE, Coleman JA, Hvizda J, Peterson J, et al.: Radio frequency ablation of small renal tumors: intermediate results. J Urol. 2004; 171: 1814-8.
10. Burdío F, Güemes A, Burdío JM, Navarro A, Sousa R, Castiella T, et al.: Bipolar saline-enhanced electrode for radiofrequency ablation: results of experimental study of in vivo porcine liver. Radiology. 2003; 229: 447-56. Erratum in: Radiology. 2004; 230: 303.
11. Rendon RA, Gertner MR, Sherar MD, Asch MR, Kachura JR, Sweet J, et al.:Development of a radiofrequency based thermal therapy technique in an in vivo porcine model for the treatment of smallrenal masses. J Urol. 2001; 166: 292-8.
12. Gillams AR: Thermal ablation of liver metastases. Abdom Imaging. 2001; 26: 361-8.
13. Rhim H, Goldberg SN, Dodd GD 3rd, Solbiati L, Lim HK, Tonolini M, Cho OK: Essential techniques for successful radio-frequency thermal ablation of malignant hepatic tumors. Radiographics. 2001; 21: S17-35; discussion S36-9.
14. Weight CJ, Kaouk JH, Hegarty NJ, Remer EM, O'Malley CM, Lane BR, et al.: Correlation of radiographic imaging and histopathology following cryoablation and radio frequency ablation for renal tumors. J Urol. 2008 ; 179: 1277-81; discussion 1281-3.
15. Landman J, Rehman J, Sundaram CP, Bhayani S, Monga M, Pattaras JG, et al.: Renal hypothermia achieved by retrograde intracavitary saline perfusion. J Endourol. 2002; 16: 445-9.
16. Anidjar M, Mongiat-Artus P, Brouland JP, Meria P, Teillac P, Le Duc A, et al.: Thermal radiofrequency induced porcine ureteral stricture: a convenient endourologic model. J Urol. 1999; 161: 298-303.
17. Johnson DB, Saboorian MH, Duchene DA, Ogan K, Cadeddu JA: Nephrectomy after radiofrequency ablation-induced ureteropelvic junction obstruction: potential complication and long-term assessment of ablation adequacy. Urology. 2003; 62: 351-2.
18. Schulman CC, Zlotta AR: Transurethral needle ablation of the prostate (TUNA). A new treatment of benign prostatic hyperplasia using interstitialradiofrequency energy. J Urol. 1995; 101: 33-6.
19. Cantwell CP, Wah TM, Gervais DA, Eisner BH, Arellano R, Uppot RN, et al.: Protecting the ureter during radiofrequency ablation of renal cell cancer: a pilot study of retrograde pyeloperfusion withcooled dextrose 5% in water. J Vasc Interv Radiol. 2008; 19: 1034-40.
20. Hwang SI, Cho JY, Kim SH, Jun SR, Lee HJ, Moon KC, et al.: Protection of the renal collecting system during radiofrequency ablation with antegrade cold dextrose infusion. Radiology. 2010; 256: 759-66.
21. Dominique E, El Otmany A, Goharin A, Attalah D, de Baere T: Intraductal cooling of the main bile ducts during intraoperative radiofrequency ablation. J Surg Oncol. 2001; 76: 297-300.

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ORIGINAL ARTICLE

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The effect of Sertraline, Paroxetine, Fluoxetine and Escitalopram on testicular tissue and oxidative stress parameters in rats

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ABSTRACT

Introduction: The aim of this study was to evaluate the effect of selective serotonin reuptake inhibitors (SSRIs) on testicular tissue and serum malondialdehyde (MDA) levels in rats.
Materials and methods: A total of 40 male Wistar albino rats, 5.5-6 months old, were equally divided at random into five groups: group 1 was the control group, group 2 received sertraline 10mg/kg (p.o), group 3 was administered fluoxetine 10mg/kg (p.o), group 4 received escitalopram 10mg/kg (p.o), and group 5 (n = 8) was administered paroxetine 20mg/kg. Each dose was administered orally for two months. Johnsen's criteria were used to categorize spermatogenesis. Johnsen's method assigns a score of 1 to 10 to each tubule cross-section examined. In this system, a Johnsen score of 9 and 10 indicates normal histology. Serum luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone levels were evaluated. Serum MDA levels were also measured.
Results: The mean Johnsen scores were 9.36 ± 0.33, 9.29 ± 0.32, 8.86 ± 0.48, 9.10 ± 0.56, and 8.33 ± 0.90 in control group, sertraline group, fluoxetine group, escitalopram group, and paroxetine group, respectively. The Johnsen score was significantly lower for paroxetine group compared with the control group ($p < 0.05$). The mean FSH level increased only in the sertraline group. With the exception of the fluoxetine group, the testosterone levels were lower in all groups compared with the control group. The total testosterone level was significantly lower in the sertraline group compared with the control group [40.87 (22.37-46.8) vs. 15.87 (13.53-19.88), $p < 0.01$]. There were no significant differences between the groups with respect to the MDA and LH levels ($p = 0.090$ and $p = 0.092$).
Conclusion: These data suggest that SSRIs have a negative effect on testicular tissues. This negative impact is markedly greater in the paroxetine group. To determine the exact mechanism of action of these drugs on testicular tissue, well-designed randomized controlled clinical studies are needed on a larger population.

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INTRODUCTION

The incidence of infertility ranges from 10% to 15% among couples (1,2). Examining the etiology of infertility among couples reveals isolated female factors to be the cause in 40% to 50% of cases, isolated male factors to be the cause in 30% of the occurrences, and both male and female factors to be the cause in the remaining 20% of cases (1). Therefore, male factors are either directly or indirectly involved in approximately 50% of infertility cases. Spermatogenesis is performed

by germ cells in the seminiferous tubules. The basic process includes stimulation of Sertoli cells by follicle-stimulating hormone (FSH), which is secreted from the pituitary gland, and the stimulation of testosterone synthesis following Leydig cell stimulation by luteinizing hormone (LH), which is also secreted from the pituitary. For this reason, FSH, LH, and testosterone are used as markers of spermatogenesis and testicular activity in males. Abnormalities or disruptions in sperm production are indicated by deterioration of sperm number and movement and may result in infertility (3). Many factors affect the male reproductive system and lead to infertility. Among the etiological factors of male infertility, varicocele, sexual factors, congenital anomalies, urogenital infections, endocrine disorders, and immunological factors are all important, and idiopathic causes of semen alteration account for up to 75% of cases (4). Additionally, obesity, radiation, climate, environment, occupation, and drug usage may also affect male fertility (5-8). Antidepressants and antipsychotic agents are extensively used, both in the short and long term, depending on various indications. Among the agents used in psychiatric practice are monoamine oxidase inhibitors, tricyclic antidepressants, selective serotonin reuptake inhibitors (SSRIs), serotonin and noradrenaline reuptake inhibitors, selective noradrenaline reuptake inhibitors, noradrenaline and dopamine reuptake inhibitors, noradrenergic and specific serotonergic antidepressants, and benzodiazepines, with SSRI medications accounting for the majority of drugs prescribed (9). SSRI drugs include fluoxetine, fluvoxamine, sertraline, paroxetine, citalopram, and escitalopram. SSRIs are frequently prescribed, and their use is directly proportional to the frequency of mental disorders, observed in 6-18% of the population (10,11). Approximately 80% of psychiatric disorders diagnosed are depression and anxiety, for which SSRIs are the first-line drug in treating these conditions (10,11). Both disorders are observed more frequently at reproductive ages, i.e., between the ages of 15 and 50 years. Indeed, SSRIs are frequently used, and they constituted 65% of all new drugs prescribed for 20.5 million psychiatric patients in the year 2000. Approximately 6 million males have been reported to use SSRIs (11). Among these, sertraline, paroxetine, and fluoxetine are very well known drugs used to treat premature ejaculation, particularly in urological practice (12).

Oxidative stress is a disturbance in the balance between the production of reactive oxygen species (ROS) and antioxidant defenses, which can damage DNA, proteins, and lipids, ultimately leading to apoptosis or necrosis in living cells. Several factors cause oxidative stress, including drugs. In the last decade, the SSRI class of drugs has been reported to be associated with sexual dysfunction, gastrointestinal disorders, insomnia, headaches, anorexia, weight loss, nausea, diarrhea, and palpitations as well as both male and female infertility (13). The relationship between the serotonergic system and male reproductive system has been evaluated only in a limited number of studies. In this study, the effects of SSRIs on testicular tissue and serum malondialdehyde (MDA) levels were investigated.

MATERIALS AND METHODS

This study was approved by the local ethics committee (ethical approval number 2011-HADYEK-047). A total of 40 male Wistar albino rats, 5.5-6 months old, were used in the study. The experimental animals were housed at 18-22°C throughout the study period of 8 weeks and had free access to rat food and tap water ad libitum. All surgical procedures were performed under xylazine/ketamine anesthesia in sterile conditions. The rats were randomly divided into five groups of eight as follows: group 1 was the accepted control group, group 2 received 10mg/kg sertraline, group 3 was administered 10mg/kg fluoxetine, group 4 received 10mg/kg escitalopram, and group 5 was administered 20mg/kg paroxetine. Each dose was administered orally for two months, as previously reported in the literature (14-16). All groups were compared to the control group. In addition, the sertraline group, fluoxetine group, escitalopram group, and paroxetine group were compared to identify their differences in Johnsen scores.

Both testes of all rats were harvested for pathologic examination. Each testis was cut into two halves, placed in a 10% formalin solution, processed by routine histological methods, and embe-

dded in paraffin blocks. The sections were cut by a rotary microtome and stained with hematoxylin and eosin. The stained sections were studied under a light microscope to evaluate spermatogenesis. Johnsen's criteria were used to categorize spermatogenesis. This system describes the preservation of spermatogenesis, on a scale from 1 to 10, according to the absence or presence of the main cell types arranged in order of maturity. A Johnsen score of 9 or 10 indicates normal histology, a score of 8 signifies hypospermatogenesis, a score of 3-7 implies maturation arrest, a score of 2 indicates germinal cell aplasia (Sertoli cells only), and a score of 1 represents tubular fibrosis (Table-1). The germinal epithelium of at least 50 tubules was assessed for each testis, and the mean Johnsen' score was calculated for each rat. Blood samples from the inferior vena cava were stored in heparin-free tubes for biochemical analyses. After centrifugation (2000 x g for 15 min at +4°C), the serum samples were stored and frozen at -70°C.

Biochemical Analysis

Blood samples were drawn into Vacutainer serum separator tubes and allowed to clot for 20 minutes at room temperature before the serum was separated by centrifugation (1500 x g for 10 min at 4°C). The serum samples were then separated from the clot within one hour of blood collection, transferred to a clean test tube, and stored at -70°C until examination. Rat LH, FSH (Cusabio Biotech, China), and testosterone (Uscn Life Science Inc., China) were measured using ELISA kits according to the manufacturers' instructions. Serum MDA levels were also measured using a method based on reaction with thiobarbituric acid (TBA) at 90-100°C (17). Serum MDA levels were considered to indicate lipid peroxidation and oxidative stress.

Statistical analysis

The Kruskal-Wallis test was used to compare continuous data between groups. For multiple comparisons, the Bonferroni-adjusted Mann-Whitney U test was employed. Continuous data are given as the median and interquartile range (quarter 1 to quarter 3). A p-value of < 0.05 was considered significant. Analyses were performed using SPSS 19 (IBM SPSS Statistics 19, SPSS Inc., IBM Co., Somers, NY).

RESULTS

The mean Johnsen scores were 9.36 ± 0.33, 9.29 ± 0.32, 8.86 ± 0.48, 9.10 ± 0.56, and

Table 1 - Modified Johnsen score system.

Johnsen score	Description
10	Full spermatogenesis
9	Slightly impaired spermatogenesis, many late spermatids, disorganized epithelium
8	Less than five spermatozoa per tubule, few late spermatids
7	No spermatozoa, no late spermatids, many early spermatids
6	No spermatozoa, no late spermatids, few early spermatids
5	No spermatozoa or spermatids, many spermatocytes
4	No spermatozoa or spermatids, few spermatocytes
3	Spermatogonia only
2	No germinal cells, Sertoli cells only
1	No seminiferous epithelium

8.33 ± 0.90 for the control group, sertraline group, fluoxetine group, escitalopram group, and paroxetine group, respectively (Table-2). The Johnsen score was significantly lower for paroxetine group when compared with control group (Figures 1 and 2 and Table-2). There were no statistically significant differences in Johnsen score between the other groups ($p > 0.05$). FSH levels were lower in the fluoxetine group, escitalopram group, and paroxetine group compared with the control group ($p < 0.001$). The mean FSH level increased only in the sertraline group. In contrast, the FSH levels were significantly decreased in groups 3 and 5 compared with group 2 ($p < 0.001$) (Table-3). With the exception of the fluoxetine group, the testosterone levels were lower in all groups compared with the control group. The total testosterone level was significantly lower in the sertraline group compared with the control group [40.87 (22.37-46.8) vs. 15.87 (13.53-19.88), $p < 0.01$] (Table-3). The serum LH and serum MDA levels did not significantly differ between the groups ($p = 0.090$ and $p > 0.092$, respectively).

Table 2 - The spermatogenesis results according to Johnsen Score System in the testicular tissues of rats.

Groups	n	Mean ± Std. Deviation	p
Control	8	9.36 ± 0.33	
Sertraline	8	9.29 ± 0.32	
Fluoxetine	8	8.86 ± 0.48	0.021
Escitalopram	8	9.10 ± 0.56	
Paroxetine	8	8.33 ± 0.90[a,b,d]	
Total	**40**	**8.99 ± 0.65**	

[a:] Different from Group 1, [b:] Different from Group 2, [d:] Different from Group 4.

Figure 1a - Spermatogenesis with a Johnsen score of 9.8 in the control group. A seminiferous tubule with normal spermatogenesis is seen.

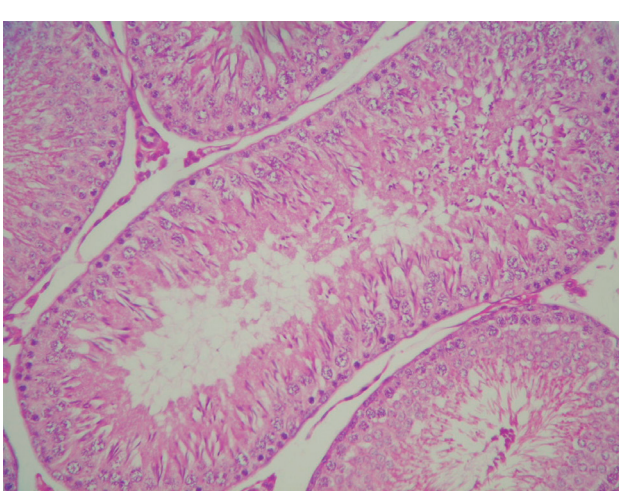


Figure1b - Complete spermatogenesis. Spermatogonia, spermatocytes, spermatids and many spermatozoa are seen from the basement membrane toward the lumen of the seminiferous tubule (H-E, x400).

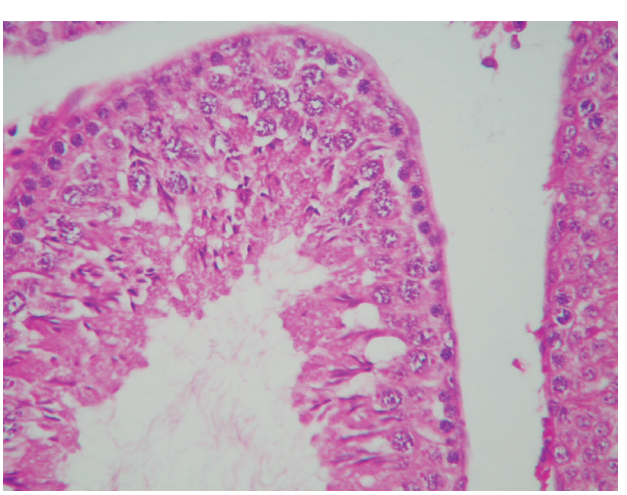


Figure 2a - Spermatogenesis with a Johnsen score of 7.8 in paroxetine group (H-E, x100).

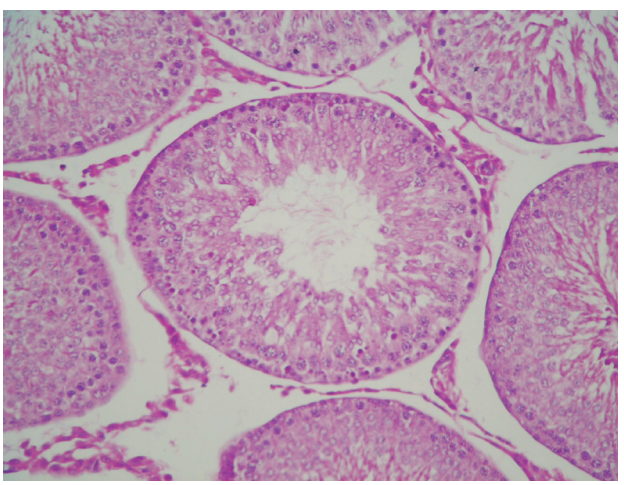
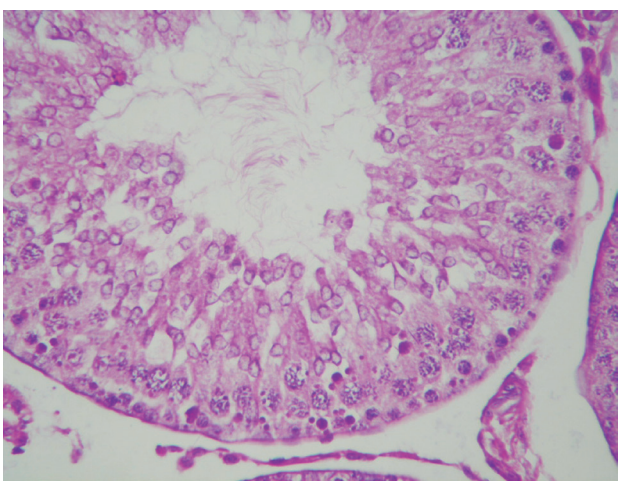


Figure 2b - Spermatogonia, spermatocytes and many early spermatids are seen. No late spermatids and spermatozoa are present (H-E, x400).



DISCUSSION

Infertility is defined as the inability of couples to achieve pregnancy within 12 months despite regular and unprotected intercourse (1). Drugs have been reported to play a possible role in the etiology of male infertility. Recently, it has been reported that SSRIs may affect semen parameters (13). SSRIs increase the amount of serotonin in the synaptic clefts by inhibiting serotonin reuptake pumps. To elucidate the relationship between infertility and the use of SSRIs, we need to investigate the relationship between serotonin and the urinary system. In the urinary system, serotonin receptors have been reported to be located in the vas deferens and are responsible for contraction (18). Serotonin receptors have also been identified in the testes, where they have been shown to play a role in the regulation of testicular blood flow (19). Moreover, serotonin receptors are also present in the epididymis on epithelial, neuroendocrine, and mast cells (20). In the epididymis, the serotonin receptors 5-HT2A and 5-HT3 play a role in sperm maturation. Tryptophan hydroxylase, which converts tryptophan to serotonin, has been detected in epithelial and neuroendocrine cells in the epididymis and is also likely to support the local synthesis of serotonin in this region. Studies suggest that serotonin receptors are located in Sertoli cells and are likely to play a role in spermatogenesis (21). Testosterone is known to be synthesized by LH released from Leydig cells in the interstitial area and to play a role in spermatogenesis. Serotonin receptors, particularly 5-HT2, have been co-localized with LH, and studies have shown that serotonin receptors can bind LH to Leydig cells or play a role in the synthesis of testosterone in Leydig cells. Therefore, serotonin may affect sperm function. The relationship between serotonin receptors and spermatozoa has also been investigated. It has been speculated that 5-HT2A and 5-HT3, detected in the tail of the sperm, play a role in the activity of spermatozoa (22). According to several studies, a lack or excess of serotonin may also lead to a deterioration of sperm parameters (23,24).

The first study on the relationship between male infertility and the use of antidepressants was published more than four decades ago. In 1966, Simpson incidentally identified a spermatogenesis disorder in a patient using trimipramin, a tricyclic antidepressant, with the diagnosis of schizophrenia (25). Several years later, subsequent studies confirmed this observation (26). Most of the ensuing studies were conducted in the mid-1980s using tricyclic antidepressants, as it was before the introduction of SSRIs into clinical practice.

Serotonin plays a role in spermatogenesis, and excess serotonin can lead to sperm dys-

Table 3 - The Serum hormones and MDA levels of all groups and statistical comparisons.

	Groups	Median (IQR)	p
LH (mIU/mL)	Control	6.22 (3.16-11.03)	0.090
	Sertraline	3.55 (1.64-6.33)	
	Fluoxetine	2.57 (1.92-5.17)	
	Escitalopram	2.45 (1.04-3.89)	
	Paroxetine	2.52 (1.59-5.72)	
FSH (mIU/mL)	Control	2.7 (1.71-3.6)	<0.001
	Sertraline	4.77 (3.42-7.48)	
	Fluoxetine	1.4 (1.11-1.95) [a]	
	Escitalopram	1.72 (0.82-2.29) [a]	
	Paroxetine	1.87 (1.35-2.16) [a]	
Testosterone (ng/mL)	Control	40.87 (22.37-46.8) [a]	0.001
	Sertraline	15.87 (13.53-19.88)	
	Fluoxetine	42.05 (34.31-54.75) [a]	
	Escitalopram	39.72 (33.07-47.27) [a]	
	Paroxetine	38.4 (34.28-44.37) [a]	
MDA (µmol/L)	Control	2.15 (1.35-2.88)	0.092
	Sertraline	3.2 (2.48-3.95)	
	Fluoxetine	2.8 (2.03-3)	
	Escitalopram	2.65 (2.23-3.2)	
	Paroxetine	2.25 (1.83-2.73)	

IQR = Interquartile range (quarter 1 to quarter 3). [a]: There was statistical significant differences from group 2.

LH = Luteinizing hormone; **FSH** = Follicle-stimulating hormone; **MDA =** Malondialdehyde

function. The negative effects of increased serum and urinary serotonin levels on semen parameters have been reported in many studies (23,27). In this context, in their study investigating 70 infertile patients aged 20 to 40 years old, Gonzales et al. reported that increased serum serotonin levels are associated with deterioration in sperm number and function (23). In a clinical study, Tanrikut and Schlegel reported the detailed examination of two patients with primary infertility and a history of SSRI use. Semen analysis conducted after the first and second months following the discontinuation of SSRI use showed the normalization of semen parameters in both patients (28). The fact that the semen parameters returned to normal in the time period specified suggests that SSRI usage may have decreased these sperm parameters due to an emission or ejaculation disorder, rather than a sperm production disorder (28). Indeed, it has been reported that SSRIs may cause emission and ejaculation disorders (29). In the study of Tanrikut and Schlegel, it was reported that 78% of cases

showed DNA fragmentation, which has been shown to be significantly associated with the use of SSRIs (28). In another study conducted by Tanrikut et al., 35 healthy male subjects with a mean age of 33.9 ± 11.1 years were administered paroxetine for 5 weeks (30). This study showed no significant change in semen parameters following drug intake, whereas the DNA fragmentation rate increased from 13.8% to 30.3% after drug intake. Consequently, the researchers reported that SSRIs result in misreading of the DNA code by inhibiting DNA binding by AP-2 (30).

In one study, 74 male patients (group 1) who were receiving treatment for depression (citalopram, escitalopram, fluoxetine, paroxetine, or sertraline) and were known to be previously fertile were compared with 44 healthy fertile adults (group 2) who were not undergoing depression therapy. In that work, the sperm counts were found to be 61.2 ± 11.4 million and 186.2 ± 31.4 million in groups 1 and 2, respectively, while the rates of motile sperm were determined to be 48.2% ± 4.6% and 66.2% ± 4.4%, respectively. These differences were found to be statistically significant (31). In the same study, the group receiving treatment was shown to have significantly poorer sperm morphology. It was also observed that the deterioration of semen parameters was directly proportional to the duration of drug use. In a large study that evaluated 530 infertile male patients, SSRI usage alone was shown to be associated with motility disorders among several factors affecting infertility such as age, smoking, and body mass index (32). The effect of SSRIs on semen parameters has been evaluated in experimental studies. In a study by Kumar et al., fluoxetine, sertraline, fluvoxamine, and citalopram were demonstrated to negatively affect semen parameters and showed a spermicidal effect (33). They proposed that the SSRIs bound to sulfhydryl groups in the sperm membrane and impaired ATP synthesis in the sperm by interacting with phospholipids. Similarly, in our study, the Johnsen scores were decreased in all groups compared with the control group.

As reported previously, a deficiency that occurs in Sertoli or Leydig cells will be indicated by an FSH, testosterone, or LH surge (3). SSRIs can affect reproductive hormones. In one study, rats were administered fluoxetine orally for 60 days, which led to decreased testicular, epididymal, and prostate volumes and reduced sperm counts and motility. Significantly decreased serum testosterone and FSH levels were also detected at the end of the investigation (34). The same study also showed reduced pregnancy rates in rats administered fluoxetine compared with the control group. Our study showed that serum FSH levels were decreased in the fluoxetine group, escitalopram group, and paroxetine group compared with the control group, whereas they were increased in the sertraline group. Several mechanisms have been proposed to explain the reduction in hormone levels, including serotonin inhibition of LH binding to Leydig cells (35). The previously mentioned study by Tanrikut et al., which included 35 healthy male volunteers who received paroxetine for 5 weeks, also demonstrated reduced levels of serum testosterone and estradiol (30). Notably, however, the FSH, LH, and prolactin levels were all normal. Other studies have also reported that SSRIs do not affect serum hormone levels (36).

Oxidative stress is associated with an increased rate of cellular damage induced by oxygen and oxygen-derived oxidants, commonly known as reactive oxygen species (ROS) (37). The major targets of ROS are membrane lipids, in a process known as lipid peroxidation. Oxidative stress is a pathophysiologic process that is common in a number of disease states (37). It is also acknowledged that testicular tissues and spermatozoa are very sensitive to ROS attack and lipid peroxidation. The susceptibility of testicular tissues to oxidation is attributed to the high polyunsaturated fatty acid content of sperm membranes (1). The effect of drugs on semen parameters has been reported in rat models and clinical studies (38). Serum MDA levels are considered to indicate lipid peroxidation and oxidative stress. In this study, however, there was no statistical relationship between SSRIs and oxidative stress.

In conclusion, according to data obtained in clinical and experimental studies, the presence of serotonin receptors in the vas deferens, epididymis, testis, Sertoli and Leydig cells, and sperm cells support the hypothesis that SSRIs are likely to worsen semen parameters and affect fertility.

Our study demonstrated that spermatogenesis was affected in all groups, but the most prominent effect was observed in the paroxetine group. To elucidate the exact mechanism by which SSRIs and serotonin affect sperm, more experimental studies and larger randomized trials are needed.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Jungwirth A, Diemer T, Dohle GR, Giwercman A, Kopa Z, Tournaye H. EAU guidelines on male infertility. Eur Urol. 2013:7.
2. Mascarenhas MN, Flaxman SR, Boerma T, Vanderpoel S, Stevens GA: National, regional, and global trends in infertility prevalence since 1990: a systematic analysis of 277 health surveys. PLoS Med. 2012; 9:e1001356.
3. Sofikitis N, Giotitsas N, Tsounapi P, Baltogiannis D, Giannakis D, Pardalidis N. Hormonal regulation of spermatogenesis and spermiogenesis. J Steroid Biochem Mol Biol. 2008; 109 :323-30.
4. Dohle GR, Colpi GM, Hargreave TB, Papp GK, Jungwirth A, Weidner W; et al.: EAU guidelines on male infertility. Eur Urol. 2005; 48: 703-11.
5. Cabler S, Agarwal A, Flint M, du Plessis SS: Obesity: modern man's fertility nemesis. Asian J Androl. 2010; 12: 480-9.
6. Hougaard KS, Hannerz H, Feveile H, Bonde JP: Increased incidence of infertility treatment among women working in the plastics industry. Reprod Toxicol. 2009; 27: 186-9.
7. Rendtorff R, Hohmann C, Reinmuth S, Müller A, Dittrich R, Beyer M, et al.: Hormone and Sperm Analyses after Chemo- and Radiotherapy in Childhood and Adolescence. Klin Padiatr. 2010 ; 222: 145-9.
8. Dohle GR: Male infertility in cancer patients: Review of the literature. Int J Urol. 2010; 17: 327-31.
9. Sheehan DV, Mao CG: Paroxetine treatment of generalized anxiety disorder. Psychopharmacol Bull. 2003; 37 (Suppl) 1:64-75.
10. Carrasco JL, Sandner C: Clinical effects of pharmacological variations in selective serotonin reuptake inhibitors: an overview. Int J Clin Pract. 2005; 59: 1428-34.
11. Pirraglia PA, Stafford RS, Singer DE: Trends in Prescribing of Selective Serotonin Reuptake Inhibitors and Other Newer Antidepressant Agents in Adult PrimaryCare. Prim Care Companion J Clin Psychiatry. 2003; 5: 153-7.
12. Hellstrom WJ: Update on treatments for premature ejaculation. Int J Clin Pract. 2011; 65: 16-26.
13. Brambilla P, Cipriani A, Hotopf M, Barbui C: Side-effect profile of fluoxetine in comparison with other SSRIs, tricyclic and newer antidepressants: a meta-analysis ofclinical trial data. Pharmacopsychiatry. 2005; 38: 69-77.
14. Bilge S, Bozkurt A, Bas DB, Aksoz E, Savli E, Ilkaya F, Kesim Y: Chronic treatment with fluoxetine and sertraline prevents forced swimming test-induced hypercontractility of rat detrusormuscle. Pharmacol Rep. 2008; 60: 872-9.
15. Snoeren EM, Refsgaard LK, Waldinger MD, Olivier B, Oosting RS: Chronic paroxetine treatment does not affect sexual behavior in hormonally sub-primed female rats despite 5-HT$_1$(A) receptor desensitization. J Sex Med. 2011; 8: 976-88.
16. Hui J, Zhang Z, Liu S, Xi G, Zhang X, Teng G, et al.: Adolescent escitalopram administration modifies neurochemical alterations in the hippocampus of maternally separated rats. Eur Neuropsychopharmacol. 2010; 20: 875-83.
17. Esterbauer H, Cheeseman KH: Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. Methods Enzymol. 1990; 186: 407-21.
18. Hay DW, Wadsworth RM: The contractile effects of 5-hydroxytryptamine on the rat isolated vas deferens.Br J Pharmacol. 1982; 77: 605-13.
19. Collin O, Damber JE, Bergh A: 5-Hydroxytryptamine--a local regulator of testicular blood flow and vasomotion in rats. J Reprod Fertil. 1996 ; 106: 17-22.
20. Jiménez-Trejo F, Tapia-Rodríguez M, Queiroz DB, Padilla P, Avellar MC, Manzano PR, et al.: Serotonin concentration, synthesis, cell origin, and targets in the rat caput epididymis during sexual maturation andvariations associated with adult mating status: morphological and biochemical studies. J Androl. 2007; 28: 136-49.
21. Syed V, Gomez E, Hecht NB: Messenger ribonucleic acids encoding a serotonin receptor and a novel gene are induced in Sertoli cells by a secretedfactor(s) from male rat meiotic germ cells. Endocrinology. 1999; 140: 5754-60.
22. Fujinoki M: Serotonin-enhanced hyperactivation of hamster sperm. Reproduction. 2011; 142: 255-66.
23. Gonzales GF, Garcia-Hjarles M, Velasquez G: Hyperprolactinaemia and hyperserotoninaemia: their relationship to seminal quality. Andrologia. 1992; 24: 95-100.
24. Parisi E, De Prisco P, Capasso A, del Prete M: Serotonin and sperm motility. Cell Biol Int Rep. 1984; 8: 95.
25. Simpson GM, Blair JH, Iqbal J, Iqbal F: A preliminary study of trimiprimine in chronic schizophrenia. Curr Ther Res Clin Exp. 1966; 8: 225-31.
26. Kurland AA, Pinto A, Destounis N, Babikow PW: Effects of trimeprimine (surmontil) on spermatogenesis and mood in normal volunteers. Curr Ther Res Clin Exp. 1970; 12: 186-91.
27. Ortiz Á, Espino J, Bejarano I, Lozano GM, Monllor F, García JF, et al.: The correlation between urinary 5-hydroxyindoleacetic acid and sperm quality in infertile men and rotating shift workers. Reprod Biol Endocrinol. 2010 8; 8:138.

28. Tanrikut C, Schlegel PN: Antidepressant-associated changes in semen parameters. Urology. 2007; 69: 185.e5-7.
29. Rowland D, McMahon CG, Abdo C, Chen J, Jannini E, Waldinger MD, et al.: Disorders of orgasm and ejaculation in men. J Sex Med. 2010; 7: 1668-86.
30. Tanrikut C, Feldman AS, Altemus M, Paduch DA, Schlegel PN: Adverse effect of paroxetine on sperm. Fertil Steril. 20100; 94: 1021-6.
31. Safarinejad MR: Sperm DNA damage and semen quality impairment after treatment with selective serotonin reuptake inhibitors detectedusing semen analysis and sperm chromatin structure assay. J Urol. 2008; 180: 2124-8.
32. Relwani R, Berger D, Santoro N, Hickmon C, Nihsen M, Zapantis A, et al.: Semen parameters are unrelated to BMI but vary with SSRI use and prior urological surgery. Reprod Sci. 2011; 18: 391-7.
33. Kumar VS, Sharma VL, Tiwari P, Singh D, Maikhuri JP, Gupta G, et al.: The spermicidal and antitrichomonas activities of SSRI antidepressants. Bioorg Med Chem Lett. 2006; 16: 2509-12.
34. Bataineh HN, Daradka T: Effects of long-term use of fluoxetine on fertility parameters in adult male rats. Neuro Endocrinol Lett. 2007; 28: 321-5.
35. Das TK, Mazumder R, Biswas NM: Effect of intraventricular injection of 5,6-dihydroxytryptamine on spermatogenesis and plasma testosterone levels in the rat. J Endocrinol. 1985; 106: 395-400.
36. Bell S, Shipman M, Bystritsky A, Haifley T: Fluoxetine treatment and testosterone levels. Ann Clin Psychiatry. 2006; 18: 19-22.
37. Tinkel J, Hassanain H, Khouri SJ: Cardiovascular antioxidant therapy: a review of supplements, pharmacotherapies, and mechanisms. Cardiol Rev. 2012; 20: 77-83.
38. Morakinyo AO, Iranloye BO, Daramola AO, Adegoke OA: Antifertility effect of calcium channel blockers on male rats: association with oxidative stress. Adv Med Sci. 2011; 56: 95-105.

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ORIGINAL ARTICLE

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The effects of carvedilol on ischemia–reperfusion injury in the rat testis

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ABSTRACT

Objective: To analyze the oxidative damage and histopathological alterations caused by ischemia-reperfusion (I/R) injury and ameliorative effects of carvedilol (CVD) in the rat testis.

Materials and Methods: Twenty-one male rats were randomized into 3 groups as follows: Group I (n = 7); control (sham) group, Group II (n = 7); I/R group, in which I/R injury was performed by torsing the left testis 720º clockwise for 2 hours and detorsing for 2 hours. Group III (n = 7); CVD treatment group; in addition to I/R process, one-dose of CVD was administered (2mg/kg, i.p) 30 min. before detorsion. Levels of antioxidant enzymes, superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) and levels of malondialdehyde (MDA) and protein carbonyl (PC) were determined in testicular tissues and serum of rats. Testicular tissues were also examined histopathologically and Johnsen scores were determined.

Results: Activities of SOD and GSH-Px in serum and testicular tissues were increased by I/R, but administration of CVD decreased these levels ($p < 0.001$ and $p = 0.001$). Significantly increased MDA levels in serum and testicular tissues were decreased by CVD treatment ($p < 0.001$ and $p = 0.001$). Concerning PC levels in serum and testicular tissues, there was no statistically significant difference between the groups ($p = 0.989$ and $p = 0.428$). There was not a statistically significant difference in terms of mean Johnsen scores between the groups ($p = 0.161$).

Conclusions: Administration of CVD decreased oxidative damage biochemically in the rat testis caused by I/R injury, but histopathologically no change was observed between all of the groups.

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Key words:
Carvedilol [Supplementary Concept]; Reperfusion; Ischemia; Testis; Oxidative Stress; Antioxidants

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INTRODUCTION

Ischemia-reperfusion (I/R) injury is a deleterious clinical entity in the organism that occurs when blood circulation is restored after an episode of acute ischemia. In this type of injury the blood supply of the tissue is interrupted initially which leads to damage of metabolically active tissues, but the restoration of blood flow to the tissues, which initiates paradoxic cascade of events, leads to further cellular and tissue damage eventually (1). There is increasing evidence that the oxidative stress (OS), which is associated with the over-production of reactive oxygen species (ROS), constitutes the basic pathophysiological process of I/R injury (1,2).

In clinical settings, testicular torsion (TT) is a typical I/R injury of the testicular tissues, which is one of the most serious urologic emergencies encountered mostly in newborn and adolescent males (3). TT causes testicular injury leading to potential serious sequela of subfertility, so immediate diagnosis and intervention is mandatory (3,4). Although the main pathological mechanism of the testicular injury following TT has not been completely understood, the I/R established during torsion and detorsion and OS generated by these events has been implicated as the main factors in cellular and tissue damage (4,5). In addition to showing the activation of several antioxidant defense mechanisms to avoid the tisssue damage due to ROS, which included the production of endogenous antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), the alterations in the serum and tissue levels of lipid peroxidation product malondialdehyde (MDA) and protein denaturation product protein carbonyl (PC) have also been been analyzed in related studies to delineate the damaging effects of ROS (1-5).

In numerous previous studies it has been clearly shown that the administration of exogenous antioxidants or ROS scavenger agents have prevented or minimized the oxidative injury in testicular tissues due to TT in rats (3-9). Carvedilol [1-[carbazolyl-(4)-oxy]-3-[(2-methoxyphenoxyethl)amino]-2-propanol] is a third-generation vasodilator agent which is used in the treatment of hypertension, ischemic heart disease and congestive heart failure (10,11). It selectively blocks α1-receptors and non-selectively antagonizes β1 and β2-adrenoceptors (1,11). Carvedilol (CVD) and some of its metabolites also display antioxidant activity and this antioxidant characteristics of CVD have been shown in previous in vitro studies and animal models (10-14). In this experimental study, it was aimed to assess the potential antioxidant effects of CVD in a rat model of testicular I/R injury. For this purpose, the biochemical and pathological effects of I/R and CVD in testicular tissues and serum of rats have been investigated. To our knowledge, this is the first study in the English literature which investigated the antioxidant effects of CVD against I/R in the rat testicular tissues.

MATERIALS AND METHODS

Animals

The study was conducted on 21 male adult Wistar-albino rats, 5-6 months old and weighing between 270-330g. The protocol of the study was approved by the local Ethics Committee of Gaziosmanpasa University School of Medicine (2011-HADYEK-018), in which the animal care and experimental procedures were executed in compliance with the principles of the NIH Guide for the Care and Use of Laboratory Animals (NIH publication no. 85-23, revised 1985). The rats were housed in cages (3 animals/cage) under temperature controlled standard conditions with free access to standard rodent food and water.

Experimental design

The animals were anaesthetized with intraperitoneal ketamine injection (50mg/kg). All of the surgical procedures were performed under sterile conditions through standart left inguinoscrotal incisions. After entrance to the scrotum, tunica vaginalis was opened and the left testis was delivered to the surgical field. The left testis was rotated 720º in a clockwise direction and maintained torsed by fixing it to the scrotum by 4/0 polyglactin suture for 2 hours with the closure of inguinoscrotal incision. Afterwards the spermatic cord was detorsed and the testis was reperfused for additional 2 hours. At the end of the ischemia and reperfusion period (4 hours) left orchiectomy was performed, 3-4mL of blood was drawn from the inferior vena cava of each rat and the animals were sacrificed by decapitation.

The rats were randomly divided into three experimental groups as follows; Group 1 (n = 7), sham operated control group (sham), the rats in this group underwent excision of the left testis after sham operation. Group 2, (n = 7), ischemia-reperfusion group (I/R); this group of animals served as the injurious control group and underwent torsion/detorsion procedures as explained before. Group 3, (n = 7), comprised the CVD treatment group (I/R+CVD) in addition to I/R process the animals in this group received CVD (2mg/kg, i.p.) 30 min. before detorsion. Carvedilol tablets (Dilatrend®, 6.25mg, Roche, Turkey) were dissolved in

normal saline as explained in a previous study and administered intraperitoneally (10). The harvested testicular tissues were longitudionally bisected. The halves of the testicular tissues were immediately stored at -80°C pending for biochemical analysis and the remainder halves were fixed in 10% formalin for histopathological examination.

Biochemical assays

All of the testicular tissues were washed three times in cold isotonic saline (0.9% [v/w]) solution and wet tissue weights were obtained. The tissues were then homogenized in ice-cold Tris-HCl buffer solution (pH 7.4, 0.2mmoL/L and 50/39.9 [v/v]), within a homogenizer (Ultra Turrax Type T25-B; IKA Labortechnic, Staufen, Germany) for 2 min at 11200 x g. The homogenate was centrifuged at 3500 x g for 60 min. and a supernatant was obtained. The levels of GSH-Px were determined in the supernatant, and MDA and PC levels were studied in the homogenate. For a further extraction procedure, the supernatant was extracted in ethanol/chloroform mixture (5/3, v/v). After a second centrifugation at 3500 x g (20 min.), the clear upper layer (the ethanol phase) was taken and used for SOD activity determination. All procedures were performed at 4°C and icepacks were used to maintain the temperature during the homogenization procedure. The principle of the SOD activity determination method was based on the inhibition of nitroblue tetrasolium reduction by the xanthine-xanthine oxidase system as a superoxide radical generator. One unit of SOD was defined as the enzyme activity causing 50% inhibition in the nitroblue tetrazolium reduction rate. The SOD activity was expressed as units per mg tissue protein (µ/mg prot). GSH-Px was measured by the enzymatic reaction which was initiated by addition of H_2O_2 to the reaction mixture containing reduced glutathione, NADPH and glutathione reductase and the change in the absorbance at 340nm was monitored by spectrophotometer. The activity of the enzyme was given in µ/mg prot. The MDA levels in testicular tissues were analyzed by a method based on the reaction with thiobarbituric acid at 90-100°C. In the thiobarbituric acid test reaction, MDA or MDA-like substances and thiobarbituric acid react together to produce a pink pigment with an absorption maximum of 532nm. The results were expressed as nanomol per gram wet tissue (nmoL/g wet tissue) calculated by using a standard graphics, which was prepared with serial dilutions of standard 1,1,3,3-tetramethoxypropane. The PC contents were determined spectrophotometrically (GBC Cintra 10 E UV/Visible spectrophotometry, Melbourne, Australia) with the reaction of the carbonyl group with 2,4-dinitrophenylhydrazine to form 2,4-dinitrophenylhydrazone. The results were given as nanomoles of protein carbonyl per milligram of protein.

Histopathological evaluation

The testicular tissues were fixed in 10% neutral formaldehyde solution. The tissues were processed for paraffin embedding and 5µm thick paraffin sections were obtained and stained with hematoxylin and eosin staining for light microscopic analysis. The same pathologist who was unaware of the experimental procedures examined the samples histopathologically. The testes were examined for the presence of coagulation type necrosis and the morphological changes in the nucleus of the germ cells. Coagulation type necrosis was classified into four grades as follows: Grade 0 showed an absence of coagulative necrosis in the seminiferous tubules, Grade 1 slight coagulation, < 25% of the seminiferous tubules containing evidence of necrosis, Grade 2 indicated moderate degree of coagulation with ≥ 25% of the tubules containing variable degrees of necrosis, Grade 3 indicated severe coagulation with ≥ 75 % of the tubules demonstrated necrosis. A total of twenty tubular sections for each testicular tissue were evaluated and to assess spermatogenesis mean Johnsen score was calculated according to the Modified Johnsen Scoring system explained elsewhwere (Table-1) (15).

The findings of histopathological evaluation are shown in Figures 1 to 3. The testes of rats in Group 1 (control group) indicated the presence of normal testicular structure and seminiferous tubular morphology with full spermatogenesis (Figure-1). The coagulative necrosis (CN) seen in different grades in all groups and the distortion of tubules are presented in Figure-2. Furthermore, degeneration in the germ cells characterized

Table 1 - Histological criteria and the modified Johnsen score system for assessment of spermatogenesis.

Score	Histologic properties
10	Full spermatogenesis
9	Slightly impaired spermatogenesis, many late spermatids, disorganized epithelium
8	Less than five spermatozoa per tubule, few late spermatids
7	No spermatozoa, no late spermatids, many early spermatids
6	No spermatozoa, no late spermatids, few early spermatids
5	No spermatozoa or spermatids, many spermatocytes
4	No spermatozoa or spermatids, few spermatocytes
3	Spermatogonia only
2	No germinal cells, Sertoli cells only
1	No seminiferous epithelium

Figure 1 - Histological sections of rat testes stained with hematoxylin and eosin. Full spermatogenesis is seen in control group (H-E, x400).

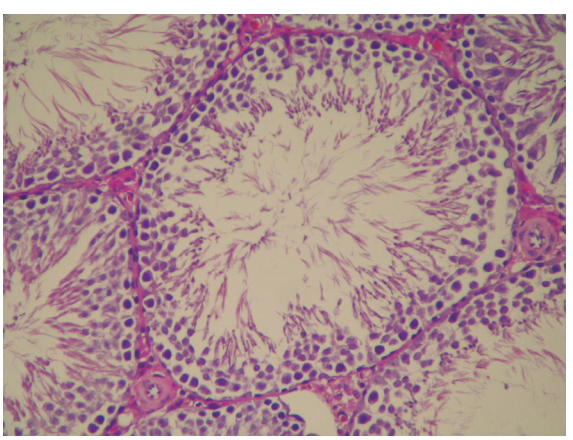
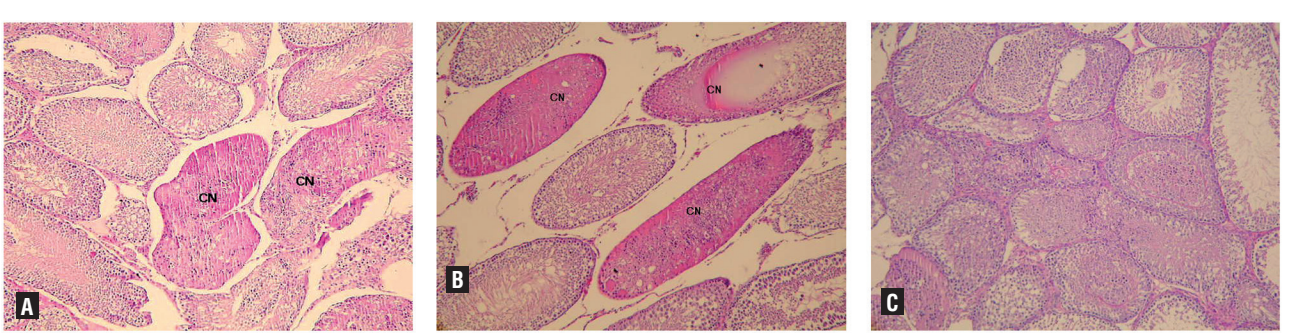


Figure 2 - Coagulative necrosis (CN) in the seminiferous tubules. a) Grade 1 coagulative necrosis, a few seminiferous tubules containing evidence of necrosis; b) Grade 2 coagulative necrosis; c) Grade 3 coagulative necrosis with loss of seminiferous tubule epithelium, edema and sloughed germinal cells (H-E, x200).

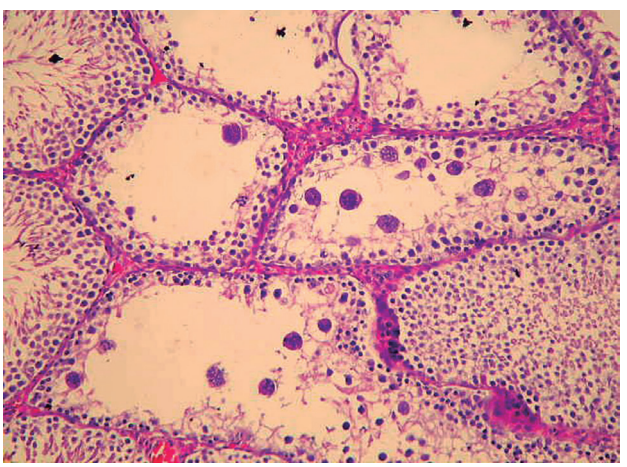


by giant cells and vesicular nuclear changes were seen in I/R group rats (Figure-3).

Statistical Analysis

Kruskall-Wallis tests were used to compare the biochemical and histopathological parameters among groups. When Kruskall-Wallis test results were significant, Bonferroni adjusted Mann-Whitney U test was used in the paired comparison. The continuous variables are presented as the mean ± standard deviation and range (min-max values). Categorical variables were compared by Chi-Square test. Categorical variables are presented as counts and percentages. A p-value < 0.05 was considered significant. Analyses were performed by using commercial software (IBM SPSS Statistics 20, SPSS inc., an IBM Co., Somers, NY).

Figure 3 - Giant cells and vesicular nuclear changes in the germ cells after I/R (H-E, x400).



RESULTS

The results of serum SOD, MDA, GSH-PX and PC values in all groups are presented in Table-2. The serum MDA level increased in the I/R group in comparison to sham group but single dose CVD administration reduced serum MDA levels significantly ($p < 0.001$). However, there was no significant difference between groups in terms of PC levels ($p = 0.989$). Similarly, SOD and GSH-Px activities were also increased in the I/R group when compared to control group, but CVD treatment reduced SOD and

Table 2 - The serum antioxidant activities and levels of MDA and PC in all groups and statistical comparisons.

	Group 1	Group 2	Group 3	P
	(SHAM) (n = 7)	(I/R) (n = 7)	(IR+CVD) (n = 7)	
SOD (U/mL)	12.87 ± 0.31 (12.42 - 13.3)	16.78 ± 0.64 (15.7 - 17.52)[a]	11.73 ± 0.65 (11.05 - 12,72)[a,b]	< 0.001
MDA (µmol/L)	1.62 ± 0.28 (1.28 - 2.07)	3.24 ± 0.62 (2.46 - 3.99)[a]	1.17 ± 0.1 (1.05 - 1.32)[a,b]	< 0.001
GSH-Px (U/mL)	1.97 ± 0.48 (1.37-2.55)	3.85 ± 0.92 (1.98 - 4.68)[a]	1.9 ± 0.33 (1.42 - 2.46)[b]	0.006
PC (nmoL/mL)	403.79 ± 44.27 (353.45 - 470.18)	400.83 ± 46.83 (340.36 - 465.82)	426.23 ± 94.29 (360-597.82)	0.989

Data are shown as mean ± standard deviation and range (min-max).
[a]: There was statistically significant difference from group 1.
[b]: There was statistically significant difference from group 2.
SOD = Superoxide dismutase; **MDA =** Malondialdehyde; **GSH-Px =** Glutathione peroxidase; **PC =** Protein carbonyl.

GSH-Px activities in comparison to the I/R group ($p < 0.001$ and $p = 0.006$, respectively).

The results and the analysis of testicular tissue MDA, PC, SOD and GSH-Px values in all groups are presented in Table-3. The tissue levels of MDA increased in the I/R group in comparison to sham-operated group and a single dose CVD treatment ameliorated MDA levels in testicular tissues significantly ($p = 0.001$). Statistically significant difference was not detected between groups in terms of PC levels ($p = 0.428$). SOD and GSH-Px activities increased in the I/R group but one dose of CVD injection caused decreased SOD and GSH-Px activities in comparison to I/R group as well ($p < 0.001$ and $p = 0.001$, respectively).

At histopathological examination of the testicular tissues, there was no statistically significant difference between all of the groups in terms of mean Johnsen scores and nuclear changes in germ cells ($p = 0.161$ and $p = 0.115$, respectively). Altough I/R caused increase in grade 1 coagulation necrosis in testicular tissue significantly, CVD treatment did not provide a decline in the proportion of grade 1 coagulative necrosis in comparison to I/R group ($p = 0.018$) (Table-4).

DISCUSSION

Although it is treated appropriately within the recommended time, TT may still result in testicular damage and infertility. It has previously been shown that the mammalian testes are highly sensitive to the oxidative insult and the most important component of post-torsion testicular damage is oxidative injury (2-9,16,17). The pathogenetic mechanism of I/R injury has been mostly attributed to the overgeneration of ROS (4,7,8,10,11,18). The ROS have destructive effects on various cellular components in the organism leading to increased microvascular permeability, interstitial edema, loss of membrane integrity, impaired vasoregulation, inflammatory cell infiltration, parenchymal cells dysfunction and eventually necrosis (2,5,8,15).

During the ischemic phase of I/R process a hypoxic condition develops in the testicular tissues due to disruption of the blood flow. Consequently, hypoxic conditions prevail and tissue ATP production decreases because of limited oxygen available in the tissue. Calcium influx into the intracellular compartment increases, leading to conversion of hypoxanthine deoxygenase to xanthine

Table 3 - The antioxidant enzyme activities and levels of MDA and PC in testicular tissues of all groups and statistical comparisons.

	Group 1	Group 2	Group 3	p
	(SHAM) (n = 7)	(I/R) (n = 7)	(I/R+CVD) (n = 7)	
SOD	0.04 ± 0.01	0.14 ± 0.01	0.07±0.02	< 0.001
(U/mg protein)	(0.03-0.05)	(0.12-0.15)[a]	(0.04-0.09)[b]	
MDA	52.88 ± 20.63	221.95 ± 38.13	88.73 ± 44.36	0.001
(nmoL/g wet tissue)	(18.75-81.63)	(192.18-299.34)[a]	(27.54-158.33)[b]	
GSH-Px	3.67 ± 1.41	10.56 ± 2.97	3.58 ± 0.71	0.001
(U/g protein)	(1,54-5,31)	(7.41-15.01)[a]	(2.51-4.49) [b]	
PC	55.38 ± 23.16	61.67 ± 30.47	76.78 ± 34.52	0.428
(nmoL/mg prot)	(31.79-95.42)	(27.9-117.17)	(35.15-144.09)	

Data are shown as mean ± standard deviation and range (min-max).
[a]: There was statistically significant difference from group 1.
[b]: There was statistically significant difference from group 2.
SOD = Superoxide dismutase; **MDA =** Malondialdehyde; **GSH-Px =** Glutathione peroxidase; **PC =** Protein carbonyl.

Table 4 - Johnsen scores and other histologic features in the testicular tissues of rats.

	Group 1	Group 2	Group 3	p
	(SHAM) (n = 7)	(I/R) (n = 7)	(IR+CVD) (n = 7)	
Johnsen Score	9.37 ± 0.37	8.77 ± 0.71	8.83 ± 0.62	0.161
	(8.95 - 9.9)	(7.35 - 9.3)	(7.9 - 9.8)	
Grade of coagulative necrosis				0.018*
0	5 (71.4)	0	0	
1	2 (28.6)	5 (71.4)	4 (57.1)	
2	0	2 (28.6)	2 (28.6)	
3	0	0	1 (14.3)	
Nuclear changes in germ cells (+)	0	1 (14.3)	3 (42.9)	0.115

Data are shown as mean ± standard deviation (min-max) and n (%).
*: There was statistically significant difference between group 1 and other groups.

oxidase, which is a superoxide generator enzyme (2,7,8,17,18). Additionally, ischemic state stimulates the chemotactic factors and leads to migration of polymorphonuclear leukocytes to the ischemic region, which also generates superoxide radicals after reperfusion (17,18). Restoration of the blood flow reverses this ischemic state but paradoxically the harmful insult to the tissue increases. Oxygen becomes abundant during the first 60-90 minutes of reperfusion which promotes the toxic burst of free oxygen radicals to invade neutrophils, macrophages and residual parenchymal cells in the affected tissues (18,19). Consequently, the enzymatic antioxidant defense system including SOD and GSH-Px react to scavenge the deleterious effects of free radicals to protect tissues from I/R injury (1,3,4). The clinical implications of these biochemical events can be summarized as follows: at the beginning torsion causes damage of the testicular tissues due to ischemic process. After rescue or detorsion procedure of the testis this damage is aggravated by reperfusion injury (20-22). In the early phases of tissue destruction the production of excessive amounts of ROS reacts with membrane lipids and results in lipid peroxidation, eventually leading to loss of cellular components of the tissue (21,22).

Biochemical markers and parameters are more sensitive in the acute phase of such injuries. In other words, the changes in biochemical parameters occur earlier in comparison to morphologic changes, so for the evaluation of testicular damage after unilateral TT biochemical analysis would be more informative than the histologic and morphologic studies in the acute phase of I/R models (23). The aim of this study was to investigate the early biochemical changes in the testicular tissues of rats subjected to I/R injury and determine the efficacy of CVD administration on this deleterious condition, which is an antihypertensive, vasodilator agent but also has potent antioxidant activity (1,10,12,13). The changes in the tissue antioxidant enzyme activities and tissue levels of MDA and PC in the I/R group showed evidence of I/R injury in the torted rat testis. According to the statistical analysis of the biochemical results the prominent beneficial effects of CVD treatment could also be seen.

In the present study, it was observed that the tissue levels of MDA, which is a lipid destruction product, was significantly increased in serum and testicular tissues of I/R group rats. The elevation of MDA levels supported I/R injury on testicular tissues. However, the levels of MDA returned closer to sham group upon CVD administration.

The changes in the tissue levels of PC could not be argued in the same way of MDA, because varying types of changes were determined in serum and the testicular tissue levels of PC in the I/R group. According to our results, CVD acted as a prooxidative agent in testicular tissues. In other words, CVD increased protein oxidation in testicular tissues. But these changes in PC levels and comparisons between the groups with regard to PC levels were insignificant.

The main treatment strategy in the prevention of the harmful effects of oxidative stress was the use of antioxidants in previous studies. In this context, to date various drugs and chemicals have been used to protect testes against I/R injury, such as N-acetyl cysteine, zofenopril, caffeic acid phenethyl ester (CAPE), erdosteine, L-carnitine, melatonin, edaravone, dopamine, vit C, trimetazidine and Ginkgo biloba (EGb 761) (2,4,5,7,8,17,23-25). As a non-selective β_1, β_2 and α_1-blocker agent, the mechanisms of antioxidant action of CVD has not been fully understood, but the greatest antioxidant activity has been attributed to its main metabolites, which are the hydroxylated compounds SB 211475, BM 910228 and SB 209995 (26). The suppressor activity of CVD on superoxide activity in neutrophils and endothelial protecting function in I/R injuries in liver, lung, skeletal muscle, renal and heart tissues has also been reported (1,10,13). According to these studies, CVD also exerted anti-ROS and endogenous antioxidant preserving functions, which led to protective properties against OS and successive I/R injury (1,11,13,26).

CVD is a highly lipophilic compound that is rendering a high distribution volume and extensively bounding to high lipid containing tissues. This molecular positioning of the compound within the lipid bilayer and ability to pass through all biological membranes, to enter cells and their subcellular compartments gave CVD the tendency to donate electrons easily to scavenge the ROS (11,26). These anti-ROS activities may be clinically relevant in post-ischemic conditions, such as testis after TT, which seemed to be protective and may have additional therapeutic value in such I/R injuries by free radical scavenging properties (11,26). This present study, which aimed to evaluate the short term protective effects of CVD on I/R injury of the rat testis, has shown that rats treated with CVD before detorsion process sustained improved oxidative parameters. The antioxidant enzymes acted against the ROS effects: in other words they executed ROS scavenging activity by the help of an antioxidant agent CVD. The chosen intraperitoneally administered dosage of CVD followed the previous studies related with I/R injury and CVD administration to avoid OS (1,10,12).

In this study, the histopathological changes associated with TT injury in the acute phase were mild. The statistical analysis of the investigated parameters, especially mean Johnsen scores, yielded non-significant differences between all of the groups. This may be attributed to the prementioned issue that morphologic changes occur later in I/R injury in comparison to biochemical alterations. Consequently, not to investigate the lately seen pathological changes in the rat testis due to TT in another group may be the limitation of this study. Additional studies are required to examine late histopathologic effects of I/R injury in the rat testis and chronic usage of CVD treatment.

CONCLUSIONS

The data obtained by the biochemical and pathological examinations of the testicular tissues of rats supported that TT caused I/R injury in the rat testis as evidenced by the alterations in oxidative parameters both in serum and testicular tissues. The results also demonstrated that treatment with CVD attenuated the I/R induced testicular damage through activation of antioxidant mechanisms. These results may provide a potential therapeutic value in the treatment of post-ischemic testicular damage and improve the fertility potential of the patients who had testicular torsion and detorsion event in the clinical base.

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CONFLICT OF INTEREST

None declared.

REFERENCES

1. Akbas H, Ozden M, Kanko M, Maral H, Bulbul S, Yavuz S, et al.: Protective antioxidant effects of carvedilol in a rat model of ischaemia-reperfusion injury. J Int Med Res. 2005; 33: 528-36.
2. Cay A, Alver A, Küçük M, Işik O, Eminağaoğlu MS, Karahan SC, et al.: The effects of N-acetylcysteine on antioxidant enzyme activities in experimental testicular torsion. J Surg Res. 2006; 131: 199-203.
3. Unsal A, Devrim E, Guven C, Eroglu M, Durak I, Bozoklu A,et al.: Propofol attenuates reperfusion injury after testicular torsion and detorsion. World J Urol. 2004; 22: 461-5.
4. Altunoluk B, Söylemez H, Bakan V, Ciralik H, Tolun FI: Protective effects of zofenopril on testicular torsion and detorsion injury in rats. Urol J. 2011; 8: 313-9.
5. Uz E, Söğüt S, Sahin S, Var A, Ozyurt H, Güleç M, Akyol O: The protective role of caffeic acid phenethyl ester (CAPE) on testicular tissue after testicular torsion and detorsion. World J Urol. 2002; 20: 264-70.
6. Can C, Töre F, Tunçel N, Uysal O, Gürer F, Ak D, Tunçel M: Protective effect of vasoactive intestinal peptide on testicular torsion-detorsion injury: association with heparin-containingmast cells. Urology. 2004; 63: 195-200.
7. Koc A, Narci A, Duru M, Gergerlioglu HS, Akaydin Y, Sogut S: The protective role of erdosteine on testicular tissue after testicular torsion and detorsion. Mol Cell Biochem. 2005; 280: 193-9.
8. Dokmeci D, Inan M, Basaran UN, Yalcin O, Aydogdu N, Turan FN, Uz YH: Protective effect of L-carnitine on testicular ischaemia-reperfusion injury in rats. Cell Biochem Funct. 2007; 25: 611-8.
9. Yurtçu M, Abasiyanik A, Avunduk MC, Muhtaroğlu S: Effects of melatonin on spermatogenesis and testicular ischemia-reperfusion injury after unilateral testicular torsion-detorsion. J Pediatr Surg. 2008; 43: 1873-8.
10. Singh D, Chander V, Chopra K: Carvedilol attenuates ischemia-reperfusion-induced oxidative renal injury in rats. Fundam Clin Pharmacol. 2004; 18: 627-34.
11. Hayashi T, De Velasco MA, Saitou Y, Nose K, Nishioka T, Ishii T, et al.: Carvedilol protects tubular epithelial cells from ischemia-reperfusion injury by inhibiting oxidative stress. Int J Urol. 2010; 17: 989-95.
12. Hayashi T, Saitou Y, Nose K, Nishioka T, Ishii T, Uemura H: Efficacy of carvedilol for ischemia/reperfusion-induced oxidative renal injury in rats. Transplant Proc. 2008; 40: 2139-41.
13. Yue TL, Ma XL, Gu JL, Ruffolo RR Jr, Feuerstein GZ: Carvedilol inhibits activation of stress-activated protein kinase and reduces reperfusion injury in perfused rabbit heart. Eur J Pharmacol. 1998; 345: 61-5.
14. Yasar A, Erdemir F, Parlaktas BS, Atilgan D, Koseoglu RD, Saylan O, et al.: The effect of carvedilol on serum and tissue oxidative stress parameters in partial ureteral obstruction induced rat model. Kaohsiung J Med Sci. 2013; 29: 19-25.
15. Dieckmann KP, Linke J, Pichlmeier U, Kulejewski M, Loy V; German Testicular Cancer Study Group: Spermatogenesis in the contralateral testis of patients with testicular germ cell cancer: histological evaluation of testicularbiopsies and a comparison with healthy males. BJU Int. 2007; 99: 1079-85.
16. Elshaari FA, Elfagih RI, Sheriff DS, Barassi IF: Oxidative and antioxidative defense system in testicular torsion/detorsion. Indian J Urol. 2011; 27: 479-84.
17. Tamamura M, Saito M, Kinoshita Y, Shimizu S, Satoh I, Shomori K, et al.: Protective effect of edaravone, a free-radical scavenger, on ischaemia-reperfusion injury in the rat testis. BJU Int. 2010; 105: 870-6.
18. Wei SM, Yan ZZ, Zhou J: Beneficial effect of taurine on testicular ischemia-reperfusion injury in rats. Urology. 2007; 70: 1237-42.
19. Ozturk H, Ozturk H, Gideroglu K, Terzi H, Bugdayci G: Montelukast protects against testes ischemia/reperfusion injury in rats. Can Urol Assoc J. 2010; 4: 174-9.
20. Filho DW, Torres MA, Bordin AL, Crezcynski-Pasa TB, Boveris A: Spermatic cord torsion, reactive oxygen and nitrogen species and ischemia-reperfusion injury. Mol Aspects Med. 2004; 25: 199-210.
21. Jeong SJ, Choi WS, Chung JS, Baek M, Hong SK, Choi H: Preventive effects of cyclosporine a combined with prednisolone and melatonin on contralateral testicular damage afteripsilateral torsion-detorsion in pubertal and adult rats. J Urol. 2010; 184: 790-6.
22. Yíldíz H, Durmus AS, Simşek H, Yaman M: Protective effect of sildenafil citrate on contralateral testis injury after unilateral testicular torsion/detorsion. Clinics (Sao Paulo). 2011; 66: 137-42.
23. Azizollahi S, Babaei H, Derakhshanfar A, Oloumi MM: Effects of co-administration of dopamine and vitamin C on ischaemia-reperfusion injury after experimental testiculartorsion-detorsion in rats. Andrologia. 2011; 43: 100-5.
24. Unal D, Karatas OF, Savas M, Yeni E, Keser BS, Verit A, et al.: Protective effects of trimetazidine on testicular ischemia-reperfusion injury in rats. Urol Int. 2007; 78: 356-62.
25. Kanter M: Protective effects of Ginkgo biloba (EGb 761) on testicular torsion/detorsion-induced ischemia-reperfusion injury in rats. Exp Mol Pathol. 2011; 91: 708-13.
26. Lysko PG, Webb CL, Gu JL, Ohlstein EH, Ruffolo RR Jr, Yue TL: A comparison of carvedilol and metoprolol antioxidant activities in vitro. J Cardiovasc Pharmacol. 2000; 36: 277-81.

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The Occurrence of Primary Hepatic Adenoma in Deceased Donor Renal Transplant Recipient

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ABSTRACT

Main findings: We reported a case of new-onset, multi-focal hepatic adenoma in an 18 year-old man with no classic risk factors occurring forty months after a renal transplant from a cadaver donor. Histopathology of the adenoma was examined and genotype and phenotype were also analyzed. Histopathologic examination of the adenoma showed no malignancy. Genotype and phenotype analysis revealed no HNF1α or β-catenin gene mutations and no inflammatory infiltration. The patient was well and disease-free postoperatively.
Case hypothesis: Hepatic adenoma occurs mostly in those taking oral contraceptives or androgenic-anabolic steroids or in those with hereditary diseases. Hepatic adenoma in a renal transplant recipient is rare and has only been reported in one case with glycogen storage disease type Ia. Immunosuppressive treatment might have contributed to the development of the neoplasm.
Promising future implications: Although malignant change occurs most often in β-catenin gene mutation hepatic adenoma, surgical resection of the adenoma in a patient under immunosuppressive therapy should be considered in order to avoid the possibility of malignant transformation or hemorrhagic rupture.

ARTICLE INFO

Key words:
Adenoma; Liver Cell; Kidney Transplantation; Immunosuppressive Agents

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INTRODUCTION

The occurrence of hepatic adenoma is rare. Only a single case of hepatic adenoma in a renal transplant recipient has previously been reported (1), and the patient involved suffered from glycogen storage disease type Ia, a previously reported risk factor. We here describe a case of new onset, multi-focal hepatic adenoma in a renal transplant recipient who had no classical risk factors.

Case hypothesis and rational

A man suffered from an idiopathic, afebrile, rapid progression of renal insufficiency with initial presentation of oliguria at the age of 18. After three years of hemodialysis, the patient underwent a successful renal transplant from a cadaver donor. Immunosuppressant therapy consisted of prednisolone (Predonin®), mycophenolic acid (Cellcept®), sirolimus (Rapammune®) and tacrolimus (Prograft®). No induction therapy was used. For the transplant follow-up, the patient underwent monthly laboratory studies including whole blood and renal function tests. Post-transplant creatinine levels were within normal limits. Forty months after transplantation, two hepatic tumors not seen in pre-operative imaging studies were found incidentally during regular ultrasonography:

a 5cm hetero-echoic mass at the left lateral segment and a 2cm hypo-echoic mass in the right lobe. MRI showed one 23.4mm nodule at Segment 3 with hypointensity on T1W and hyperintensity on T2W image. Two nodules at Segments 6 and 7 showed isointensity on the T1W and hyperintensity on the T2W image. Serum alpha-fetoprotein was 2.50ng/mL, that is, within normal limits. The patient was otherwise healthy and had no known predisposing hereditary disease such as glycogen storage disease, and did not take any medications prior to the renal transplant. Viral hepatitis tests were negative for hepatitis B and C. Ultrasonography-guided biopsy showed a low-grade dysplastic nodule. Due to suspected malignancy, the patient underwent segmentectomy of S6 and S7 and partial segmentectomy of S3. Grossly, the tumor was yellow in color with focal hemorrhage. No capsule was seen. Microscopically the tumor had blank-looking nuclei and was arranged in trabeculae. The non-tumorous part was unremarkable. The pathologic diagnosis was hepatic adenoma without evidence of malignancy. Genotype analysis showed no mutations in either HNF1α or β-catenin. Histopathology showed no evidence of inflammatory infiltrate (Figure-1). At the present time, thirty six months after surgery, the patient is well and disease-free.

The IL-6 pathway plays an important role in regulation of the inflammatory status of hepatic adenoma. The deletion of 560-571 of exon-6 of the IL6ST gene that encodes the signaling co--receptor gp130 of IL-6 may lead to a persistent inflammation in the absence of IL-6 (2). Based on this concept, the somatic DNA sequences in exon-6 of the IL6ST gene were identified in our study. Genomic DNA in tumor tissue was purified by QIAamp DNA Mini Kit (Qiagen). The genomic DNA was amplified IL6ST exon 6 by touchdown polymerase chain reaction with DNA templates from the patient. The purified PCR products were directly sequenced using Applied Biosystems 3730 DNA Analyzer. DNA sequencing was performed using primers provided in Table-1. The sequences

Figure 1 - Immunohistochemistry analyses (200X). (A) Hematoxylin and eosin (H&E) stain; (B) L-FABP immunostaining. Cytoplasmic and nuclear L-FABP expression was present in the tumor; (C) β-catenin immunostaining. β-catenin–inactivated normal membranous staining of hepatocytes was similar in the tumor and non-tumor liver; (D) SAA immunostaining. SAA staining was absent in the liver specimen. These results indicate that the patient's HA belonged to the no mutation, no inflammation HA subtype.

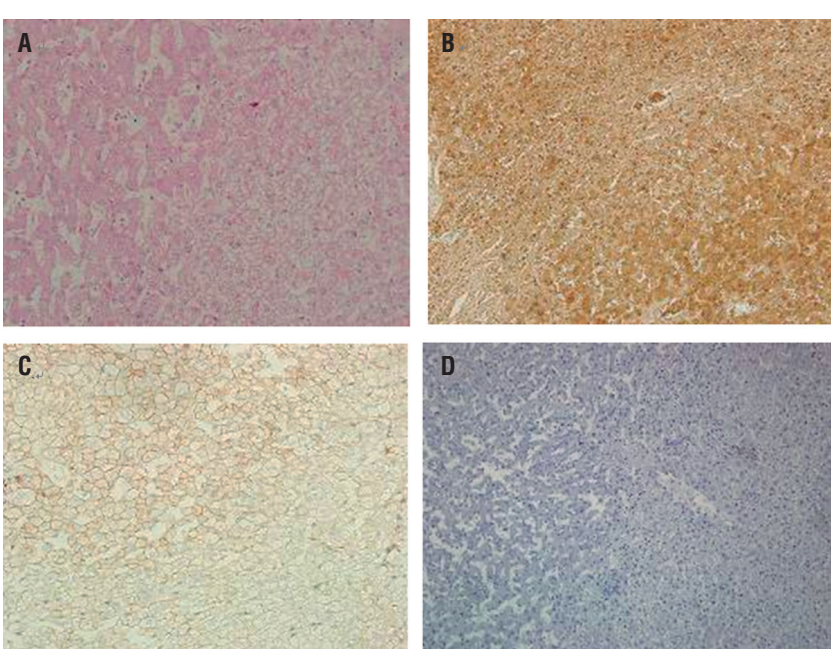


Table 1 - Sequence primer 5'->3'.

IL6ST EXON 6 genomic DNA amplification and sequencing
TAGTGACCAGCAGTTATATTGCAA
CGACTACAGTGTCAAATAAACTCTCA

of HA of our case represented the IL6ST-wide-type sequence without any mutation (Figure-2).

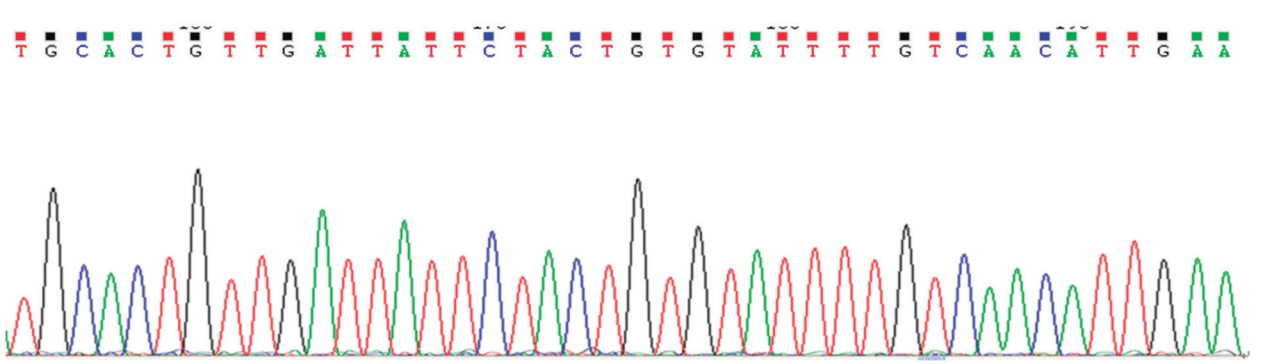
Discussion and future perspectives

Hepatic adenoma (HA) is a rare, benign neoplasm with an estimated incidence of < 5 cases per million persons, but a higher incidence of 1-3 per 100,000 young women with a history of oral contraceptive use (1-3). Patients diagnosed with HA are typically younger women with long-standing oral contraceptive use. Other possible etiologies for HA include androgenic-anabolic steroid use, congenital diseases including glycogen storage disease type I, III and IV, Klinefelter's syndrome, and familial adenomatous polyposis, and some drugs including clomiphene or danazol. The potential for malignant change is extremely low. Only a single case of hepatic adenoma in a renal transplant recipient who suffered from glycogen storage disease type Ia has been previously reported (4). The current case, to our knowledge, is the first report of primary HA developing in a cadaveric donor renal transplant male recipient with a previous healthy liver and no known risk factors.

In our case, no hepatic lesions were visible in the pre-transplant abdominal CT. In the follow-up period, immunosuppressants including prednisolone, mycophenolic acid, sirolimus, and tacrolimus were administered, and the patient tolerated these drugs well. No previous report has demonstrated any correlation between HA and the specific immunosuppressants taken by our patient. In renal transplant recipients, adenomas occur rarely, and the vast majority appears in nephrogenic systems (5) and are related to the use of azathioprine (6). Three cases of post-transplant HA have been reported (7-9) that were attributed to the use of cyclosporine (7). Immunosuppressive effects increase the incidence of malignancy in transplant patients (10). The effect of immunosuppressants on benign adenomas development has not been well studied.

The typical characteristic of HA on ultrasonography is a well-demarcated hyper echoic lesion that tends to heterogenous echogenicity if central necrosis or hemorrhage is present. MRI has advantages in distinguishing between different types of hepatic neoplasms; however the findings for HA vary, and some difficulties exist in distinguishing between HA, focal nodular hyperplasia and well--differentiated hepatocellular carcinoma (11). And the efficacy of percutaneous liver biopsy is limited

Figure 2 - The somatic DNA sequences in exon-6 of the IL6ST gene of our case represented the wide type sequence without any mutation.



with a preoperative accuracy about 50% (11,12). Definite diagnosis relies on surgical resection and histopathology.

The incidence of hemorrhage increases in proportion to the tumor size, especially when the size is above 3-4cm³. Usually rupture of the HA presents with acute abdominal pain and instability of vital signs, and this presentation might be misinterpreted in an immunocompromised patient (13). It has been suggested that the higher incidence of malignancy seen in transplant patients is due to long-term immunosuppressive therapy (14). Therefore surgical resection for a hepatic tumor of undetermined nature in an immunocompromised patient such as our transplant patient is a reasonable option.

HA can be classified into four groups: Group 1, hepatocyte nuclear factor 1 alpha mutations (HNF1α); Group 2, β-catenine mutations; Group 3, presence of inflammatory infiltrate; Group 4, absence of inflammatory infiltrate (15). Of these groups, β-catenine-activated HA has the highest correlation with hepatocellular carcinoma.

The absence of liver-type fatty acid binding protein (L-FABP) indicates HNF1α mutations and can be used to classify Group 1. A combination of GLUL overexpression and nuclear β-catenin staining indicates β-catenin-activating mutations and can be used to classify Group 2. Positive serum amyloid A protein (SAA2) staining can be used to classify Group 3, the inflammatory filtrate group (16). In our patient, HNF1α, β-catenin, and SAA immunostaining were all absent in the liver specimen and the immunostaining analysis showed both cytoplasmic and nuclear expression of L-FABP. Therefore, these results showed that our case belongs to Group 4 (no mutations, no inflammatory infiltrate), a group that represents only 5-10% of all HA cases. The clinical relationship between Group 4 HA and immunosuppressants use is unknown at present and should be studied further.

In the current case, we have reported the occurrence of new onset, multi-focal HA forty months following a renal transplant from a cadaver donor in a young man free from classic risk factors. The lack of previously reported risk factors for development of the adenoma leaves the suspicion that the immunosuppressive treatment given to the patient contributed to its development. Surgical resection of the adenoma seemed advisable because of the possibility of hemorrhagic rupture and, in an immunosuppressed patient, of malignant transformation.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Deneve JL, Pawlik TM, Cunningham S, Clary B, Reddy S, Scoggins CR, et al.: Liver cell adenoma: a multicenter analysis of risk factors for rupture and malignancy. Ann Surg Oncol. 2009; 16: 640-8.
2. Rebouissou S, Amessou M, Couchy G, Poussin K, Imbeaud S, Pilati C, et al.: Frequent in-frame somatic deletions activate gp130 in inflammatory hepatocellular tumours. Nature. 2009; 457: 200-4.
3. Rooks JB, Ory HW, Ishak KG, Strauss LT, Greenspan JR, Hill AP, et al.: Epidemiology of hepatocellular adenoma. The role of oral contraceptive use. JAMA. 1979; 242: 644-8.
4. Gossmann J, Scheuermann EH, Frilling A, Geiger H, Dietrich CF: Multiple adenomas and hepatocellular carcinoma in a renal transplant patient with glycogen storage disease type 1a (vonGierke disease). Transplantation. 2001; 72: 343-4.
5. Banyai-Falger S, Maier U, Susani M, Wiener H, Watschinger B, Hörl WH, Banyai M: High incidence of nephrogenic adenoma of the bladder after renal transplantation. Transplantation. 1998; 65: 511-4.
6. Fournier G, Menut P, Moal MC, Hardy E, Volant A, Mangin P: Nephrogenic adenoma of the bladder in renal transplant recipients: a report of 9 cases with assessment of deoxyribonucleicacid ploidy and long-term followup. J Urol. 1996; 156: 41-4.
7. Yarak S, Floriano M, Decico JE, Konishi CT, Michalany NS, Enokihara MY, et al.: First report of a fibro-adenoma in axillary mammary tissue of a renal transplant patient taking cyclosporin. Int J Dermatol. 2007; 46: 407-9.
8. Sakaguchi T, Nakamura S, Suzuki S, Konno H, Fujita K, Suzuki K, et al.: Intracystic hemorrhage of pancreatic serous cystadenoma after renal transplantation: report of a case. Surg Today. 2000; 30: 667-9.
9. Belldegrun A, Servadio C, Nissenkorn I, Liberman UA: Late appearance of parathyroid adenoma in a renal transplant recipient with multiple primary malignancies. J Urol. 1982; 127: 533-4.
10. Buell JF, Papaconstantinou HT, Skalow B, Hanaway MJ, Alloway RR, Woodle ES: De novo colorectal cancer: five-year survival is markedly lower in transplant recipients compared with the generalpopulation. Transplant Proc. 2005; 37: 960-1.

11. Charny CK, Jarnagin WR, Schwartz LH, Frommeyer HS, DeMatteo RP, Fong Y, et al.: Management of 155 patients with benign liver tumours. Br J Surg. 2001; 88: 808-13.
12. van der Windt DJ, Kok NF, Hussain SM, Zondervan PE, Alwayn IP, de Man RA, et al.: Case-orientated approach to the management of hepatocellular adenoma. Br J Surg. 2006; 93: 1495-502.
13. Benjamin ER, Jim J, Kim TJ, Meals C, Gritsch HA, Tillou A, et al.: Acute care surgery after renal transplantation. Am Surg. 2009; 75: 882-6.
14. Birkeland SA, Storm HH, Lamm LU, Barlow L, Blohmé I, Forsberg B, et al.: Cancer risk after renal transplantation in the Nordic countries, 1964-1986. Int J Cancer. 1995; 60: 183-9.
15. Zucman-Rossi J, Jeannot E, Nhieu JT, Scoazec JY, Guettier C, Rebouissou S, et al.: Genotype-phenotype correlation in hepatocellular adenoma: new classification and relationship with HCC. Hepatology. 2006; 43: 515-24.
16. Bioulac-Sage P, Rebouissou S, Thomas C, Blanc JF, Saric J, Sa Cunha A, et al.: Hepatocellular adenoma subtype classification using molecular markers and immunohistochemistry. Hepatology. 2007; 46: 740-8.

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VIDEO SECTION

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Retroperitoneoscopic pyelolithotomy: A minimally invasive alternative for the management of large renal pelvic stone

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ABSTRACT

Introduction: Large stones in renal pelvis can be treated with percutaneous nephrolithotomy (PCNL) or pyelolithotomy (either by open or laparoscopic techniques). PCNL is difficult in undilated system. For pyelolithotomy, laparoscopy is more preferable over the open surgery. Surgeons are more familiar with the tansperitoneoscopic anatomy than retroperitoneoscopic one, but retroperitoneoscopic approach can be attempted if we anticipate the problems in the transperitoneal route.
Case: A fifty years old gentleman presented to us with the complaint of dull aching right flank pain. On ultrasonographic examination, he was found to have a large stone in renal pelvis with minimal hydronephrosis and thickened omentum on right side. Xray KUB showed a large radio-opaque shadow in renal area. We did the CECT-Urogram of the patient to know the detailed anatomy, which showed a stone of 5.3 x 3.7 cm in right extra-rena pelvis without hydronephrosis and a large focal area of marked fat stranding in omentum on the right side in mid and lower abdomen with swirling of fat stranding on the superior aspect suggestive of omental infarction and torsion.
Due to undilated caliceal system, we preferred laparoscopic surgery over the PCNL in this patient. As whole of the omental tissue was stuck on right side we decided to proceed with transperitoneoscopic route instead of retroperitoneoscopic one. The DJ stent was inserted preoperatively.The surgery was performed in the flank position with three ports, one 10mm port just antero-inferior to tip of 12th rib for camera and two 5mm working ports, one at anterior axillary line and other at renal angle. We created the retroperitoneal space with the customized balloon, made with the glove-fingure.
Results: The operative time was 1 hour 40 minutes, and there were no intra or post-operative complications. The stone was removed in toto. Patient was orally allowed on first postoperative day and foleys was removed on second day. patient was discharged on day 2.
DJ stent was removed after 15 days. At two months follow-up the patient was asymptomatic.
Conclusion: The retroperitoneoscopic pyelolithotomy is a good alternative for removal of large stone in renal pelvis, with the added advantages of no peritoneal contamination and a quick recovery of bowel function.

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EDITORIAL COMMENT

The video submitted by Drs. Chipde and Agrawal nicely depicts a retroperitoneal, laparoscopic solution for removal of a large renal pelvic stone. This patient's case was further complicated by omental infarction and torsion, making a transperitoneal approach very difficult. In 2013 it is rare to find a case of large kidney stones that cannot be treated with percutaneous nephrolithotomy. The authors mention that the lack of hydronephrosis was a factor in their decision to proceed via a laparoscopic approach. These issues can usually be overcome by various retrograde transurethral techniques Cystoscopy with placement of an open-ended ureteral catheter followed by retrograde ureteropylography while the patient is in the prone position can nicely delineate a non-dilated collecting system and can facilitate percutaneous access (1).

There is a small subset of patients that likely can benefit from laparoscopic stone surgery. They are patients with large kidney stones and associated pathology that requires correction, such as a ureteropelvic junction obstruction or ureteral stricture. In patients with relative contraindications to a transperitoneal approach, the retroperitoneal approach is useful.

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VIDEO SECTION

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Robotic nephrolithotomy and pyelolithotomy with utilization of the robotic ultrasound probe

Khurshid R. Ghani, Quoc-Dien Trinh, Wooju Jeong, Ariella Friedman, Yegapan Lakshmanan, Mani Menon, Jack S. Elder

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ABSTRACT

Introduction: The treatment of large renal stones in children can be challenging often requiring combination therapy and multiple procedures. The purpose of this video is to describe our technique of robotic nephrolithotomy and pyelolithotomy for complex renal stone disease in children, and to demonstrate the utility of the robotic ultrasound probe to aid with stone localization.
Materials and Methods: Robotic nephrolithotomy/pyelolithotomy was carried out in four consecutive patients. A robotic ultrasound probe (Hitachi-Aloka, Tokyo, Japan) under console surgeon control was used in all cases.
Results: Two patients underwent robotic pyelolithotomy, one patient underwent robotic nephrolithotomy, whilst the fourth patient underwent robotic pyelolithotomy and nephrolithotomy along with Y-V pyeloplasty for concurrent ureteropelvic junction obstruction. Mean operative time, blood loss and hospital stay was 216 minutes, 37.5 mL and 2 days, respectively. The robotic ultrasound probe aided identification of calculi within the kidney in all cases. For nephrolithotomy it was helpful in planning the incision for nephrotomy. After nephrotomy or pyelotomy, stones were removed using a combination of robotic Maryland forceps, fenestrated grasper or Prograsp. Antegrade nephroscopy introduced through a laparoscopic port was used in all patients for confirmation of residual stone status. Two patients did not require a ureteral stent in the post-operative period. One patient had a minor complication (Clavien Grade 2 - dislodged malecot catheter). All patients were stone free at last follow-up.
Conclusions: Robotic nephrolithotomy and pyelolithotomy with utilization of the robotic ultrasound probe offers a one-stop solution for complex renal stones with excellent stone-free rates.

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EDITORIAL COMMENT

The video by Ghani et al. nicely demonstrates the techniques of robotic pyelolithotomy and nephrolithotomy in children. The video highlights the use of intraoperative ultrasonography using a specially designed probe that can be manipulated by the operating surgeon. Newer probes allow the surgeon to directly control the probe with standard robotic instruments (1). Intraoperative ultrasonography has proven useful during partial nephrectomy for tumor identification to facilitate complete resection (2,3). It is useful in parenchymal incision planning. It is especially helpful in difficult cases such as the completely intraparenchymal tumor (4).

REFERENCES

1. Yakoubi R, Autorino R, Laydner H, Guillotreau J, White MA, Hillyer S, et al. Initial laboratory experience with a novel ultrasound probe for standard and single-port robotic kidney surgery: increasing console surgeon autonomy and minimizing instrument clashing. Int J Med Robot. 2012; 8: 201-5.
2. Gill IS, Desai MM, Kaouk JH, Meraney AM, Murphy DP, Sung GT, et al.: Laparoscopic partial nephrectomy for renal tumor: duplicating open surgical techniques. J Urol. 2002; 167 (2 Pt 1): 469-7; discussion 475-6.
3. Fazio LM, Downey D, Nguan CY, Karnik V, Al-Omar M, Kwan K, et al.: Intraoperative laparoscopic renal ultrasonography: use in advanced laparoscopic renal surgery. Urology. 2006; 68: 723-7.
4. Chung BI, Lee UJ, Kamoi K, Canes DA, Aron M, Gill IS: Laparoscopic partial nephrectomy for completely intraparenchymal tumors. J Urol. 2011; 186: 2182-7.

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VIDEO SECTION

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Robotic Extramucosal Excision of Bladder Wall Leiomyoma

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ABSTRACT

Introduction: Multiple case reports and reviews have been described in the literature for bladder wall leiomyoma resection via different approaches. The minimally invasive partial cystectomy remains the most widely accepted technique; however, case reports for enucleation of bladder wall leiomyoma have also been described. The purpose of this video is to demonstrate the robotic extramucosal excision of a bladder wall leiomyoma, without cystotomy, but with complete removal of the muscular layer.
Materials and Methods: A 35-year old male present with lower urinary tract symptoms and imaging showed bladder wall mass with histopathology showed leiomyoma. The patient consented for mass excision with the possibility of a partial cystectomy. The patient was placed in the supine, 30-degree Trendelenburg position during the procedure. A total of 4 ports were inserted. A 3-arm da Vinci robotic surgical system was docked, and the arms were connected. Extramucosal excision was accomplished without cystotomy and muscle approximation was achieved by 2 0 Vicryle.
Result: The operative time was 90 minutes, blood loss of approximately 50mL and the patient was discharged after 72 hours with no immediate complications and a 6 months follow-up showed no recurrence.
Conclusion: Such a technique results in complete excision of the tumor, without cystotomy, and also maintains an intact mucosa. These steps, in addition to decreasing the risk of local recurrence, also shorten the period of postoperative catheterization and hospitalization.

ARTICLE INFO

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EDITORIAL COMMENT

Al-Othman and colleagues from Riyadh Saudi Arabia present an elegant video demonstrating the robotic excision of a bladder leiomyoma. This is a rare, but good example of how the robot can be used to perform what in essence is a partial cystectomy. The visualization was perfect, the dissection meticulous and the reconstruction was beyond reproach.

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