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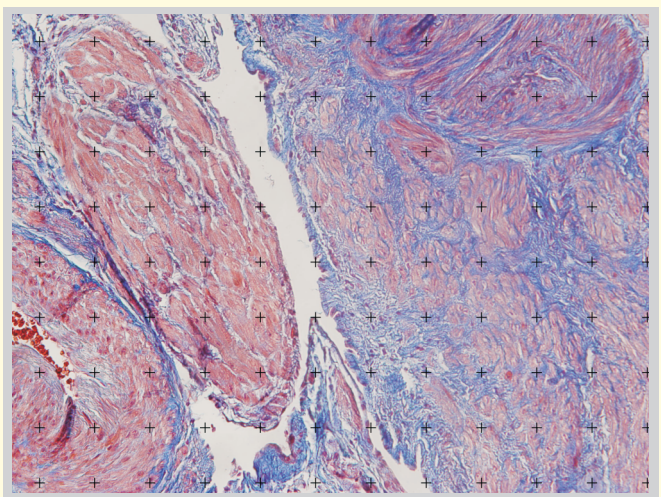

Photomicrography of a control urethra showing the morphometric analysis. Quantification of smooth muscular cells using the software Image J Test grid. Masson's trichrome X200.

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DISCLAIMER

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EDITOR'S COMMENT

The september-octuber 2012 issue of the International Braz J Urol presents original contributions and editorials from many different countries such as Brazil, USA, China, Republic of Korea, Spain, republic of Ireland, etc., and as usual the editor´s comment highlights some papers.

Dr. Hamada and collegues from Glickman Urological and Kidney Institute, Cleveland Clinic, Ohio, USA and Androfert, Center for Male Reproduction (SCE), Campinas, São Paulo, Brazil, performed on page 576 a elegant review about the diagnosis and management of unexplained infertility. Unexplained male infertility (UMI) is a diagnosis reserved for men in whom routine semen analyses results are within normal values and physical as well as endocrine abnormalities were ruled out. The authors proposed an interesting algorithm for the clinical management of men with UMI and make a great review about the diagnosis of UMI. The authors concluded that proper understanding of the in vivo process of human fertilization and sperm egg interaction in vitro is the key to envisage the sperm functional alterations with tremendous influence on diagnosis and treatment of male subfertility.

Doctor Bahia and collegues from state university from Rio de Janeiro, Brazil, performed on page 595 a review showing the cost-effectiveness analysis of medical treatment of benign prostatic hyperplasia (BPH) under Brazilian public health system perspective (Unified Health System). The data obtained from the panel showed that the most common drugs used in the treatment of BPH were finasteride, doxazosine and a combination of both. The most frequent surgery performed was transurethral resection of the prostate. This study suggests that the treatment of BPH with finasteride is cost-effective compared to placebo in the scenario of the Brazilian public health system. Combined therapy (doxazosine + finasteride), although lowering more efficiently and rapidly lower urinary tract symptoms, increases significantly the treatment costs.

Doctor Kyu Oh and collegues from Gachon University Gil Hospital, Incheon, Republic of Korea performed on page 611 a study about the relationships between 2nd to 4th digit ratio (digit ratio) and prostate cancer detection rate and biopsy findings, including Gleason score in 770 men aged 40 years or older that presented with lower urinary tract symptoms (LUTS). The authors concluded that a lower digit ratio is related to an increased detection rate of prostate cancer, a high percentage of core cancer volume and a high Gleason score.

Doctor Liu and collegues from the West China Hospital, Sichuan University, China performed on page 627 an interesting study about the accuracy of multidetector computed tomography (MDCT) in the preoperative staging of renal cell carcinoma (RCC). They retrospectively reviewed the clinical and pathological records of 312 patients with RCC who underwent staging MDCT before surgery. They concluded that MDCT with a dynamic contrast protocol is able to delineate RCC with high accuracy. However, a great portion of tumors were overstaged by MDCT because of overestimation of tumor size and poor visualization of infiltration of the perinephric fat. In addition, nodal metastatic lesion evaluation relies on node size only and remains a difficult task.

EDITOR'S COMMENT

Doctor Gameiro and collegues from São Paulo State University (UNESP), Botucatu and Health Sciences Center (ECM), Paraná State University, Londrina, Brazil performed on page 661 a study about the pelvic floor muscle (PFM) strength in women with stress urinary incontinence (SUI) and urge urinary incontinence (UUI) in 51 women. They concluded that pelvic floor muscle weakness was significantly higher in women with UUI when compared to SUI.

Doctor Carvalho and collegues from Urogenital Research Unit – State Universtiy from Rio de Janeiro, Brazil, performed on page 674 a interesting basic research about morphologic alterations in the proximal and distal urethral edges from patients submitted to end-to-end bulbar urethroplasty. The authors performed a stereological and biochemical analysis of the uretral edges in 12 patients. The authors concluded that after excision of the stenotic segment to a caliber of 28Fr, the exposed and macroscopically normal urethral edges may present altered amounts of elastic fibers and SMC, but are free from fibrotic tissue. When excising the peri-stenotic tissue, the surgeon should be more careful in the proximal end, which is the most altered.

Luciano A. Favorito MD, PhD

Associated Editor
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REVIEW ARTICLE

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Unexplained Male infertility: Diagnosis and Management

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ABSTRACT

Unexplained male infertility is a diagnosis reserved for men in whom routine semen analyses results are within normal values and physical as well as endocrine abnormalities were ruled out. In addition to erectile problems and coital factors, immunologic causes and sperm dysfunction may contribute to such condition. New etiologies of unexplained male infertility include low level leukocytospermia and mitochondrial DNA polymerase gene polymorphism. Contemporary andrology may reveal cellular and sub-cellular sperm dysfunctions which may explain subfertility in such cases, thus aiding the clinician to direct the further work-up, diagnosis and counseling of the infertile male. The objective of this article is to highlight the concept of unexplained male infertility and focuses on the diagnosis and treatment of this condition in the era of modern andrology and assisted reproductive techniques. Extensive literature review was performed using the search engines: Pubmed, Science-direct, Ovid and Scopus.

ARTICLE INFO

Key words:
Male infertility; Diagnosis, Semen analysis; Oxidative stress; DNA damage; Reproductive Techniques, Assisted

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INTRODUCTION

Infertility remains both prevalent and problematic among couples worldwide. It is clinically defined as failure of a couple to conceive after one year of regular sexual intercourse. An estimated 4-17% of couples seek medical treatment in order to rectify their infertility, and it is reasonable to assume that there are many more cases of infertility that are unreported (1). It has been shown that the male factor is solely and partially implicated in 20-50% of the cases of infertility (2). However, despite advances in technologies and diagnostic methods in the field of andrology, there remains a significant subset of these subfertile men who are classified as having unexplained male infertility (UMI). Men are categorized as having UMI when they are infertile despite having normal semen analysis, normal history and physical examination and when female factor infertility has been ruled out (3). The average incidence of UMI is approximately 15%, although reports of UMI in study populations have ranged from 6% to 37% (4-6). Possible factors that might explain the difficulties to conceive in UMI include the presence of antisperm antibodies, sperm DNA damage, elevated levels of reactive oxygen species (ROS), and sperm dysfunction. A further possibility to consider is unexplained female factor infertility and coital factors such as inappropriate timing of intercourse (not within the female fertile window), erectile dysfunction or anejaculation. It is therefore important that both a thorough initial assessment

is performed, including a detailed sexual history and gynecological examination, as well as advanced investigations that test for autoimmune infertility and sperm defects. The objective of this article is to highlight the concept of unexplained male infertility and focus on the diagnosis and treatment of this condition in the era of modern andrology and assisted reproductive techniques.

The importance and limitations of routine semen analysis in unexplained infertility

Currently, routine semen analysis remains the backbone of the evaluation of the male factor infertility, besides detailed medical history and thorough physical examination (7). Such practice is based on the fact that the semen parameters such as sperm concentration, motility, and morphology have been shown to be significantly related to conception. In addition, being a cost-effective and non-invasive test has lead to the widespread use of semen analysis in the initial evaluation of infertile men (8). Nonetheless, the criteria for normal semen parameters vary according to which edition of the WHO laboratory manual for the examination and processing of human semen is used (9).

In 2010, the World Health Organization (WHO) has established new reference values for human semen characteristics which are markedly lower than those previously reported (10). Approximately 2,000 men from eight countries whose partners had a time-to-pregnancy of ≤ 12 months were chosen as individuals to provide reference distributions for semen parameters. Despite using controlled studies involving couples with known time to pregnancy to establish the new limits, reference studies were limited with regard to the population analyzed and the methods used for semen evaluation. The utilization of the new WHO manual reference values into clinical practice will likely result in a re-classification of many of the infertile couples. Specifically, those couples previously classified as having male-factor infertility with sperm parameters above the new reference limits but below the old values will now be diagnosed as having unexplained or female-factor infertility (9). It is unclear at this time, whether this re-classification will result in a more cost-effective evaluation of the infertile couple or in a delay in the male factor evaluation with subsequent delay in the definitive diagnosis and management of the infertile couple.

The 95% reference interval for semen characteristics of recent fathers, included in the newest WHO manual, have been generated in line with clinical chemistry standards and the 5th centile was proposed for the lower limit of semen characteristics. Although reference values are useful for comparison with values obtained from the patient being assessed, it is important not only to compare the patient results with the lower reference limit but also with the 50th percentile, which represents the value beneath which 50% of the reference population of 'fertile' men falls. This strategy may be more realistic and can help in understanding a patient's seminal profile in relation to a given reference group (9).

It is therefore important to acknowledge the limitations of semen analysis results in predicting the health and functional capacity of the male reproductive organs and cells. Normal semen analysis does not guarantee the fertilization potential of sperm, and studies have shown significant overlap in semen parameter values between fertile and infertile men (11). This overlap could be due in part to the marked biological variability in semen samples characteristics, even those taken only a few days apart from the same individual (2). Furthermore, semen analysis does not provide information regarding defects in sperm function. Many key aspects of the fertilization process such as transport of the sperm to the oocyte, sperm interaction with the cervical mucus, and sperm interaction with the oocyte cannot be assessed by conventional semen analysis. For this reason, it has been suggested that sperm function tests should be included in the semen analysis of individuals seeking fertility evaluation (3). Lastly, the male evaluation regarding fertility must go far beyond counting spermatozoa and assessing motility and morphology. It has to be complemented with a proper clinical examination, a comprehensive history taking, and relevant endocrine, genetic, and/or other investigations.

Thus, it is imperative to conclude that further tests are certainly required beyond se-

men analysis for evaluating subfertile men. The time has come for technological developments in the field of andrology to bring robust and cost-effective clinically useful sperm function tests to fix the shortcomings of the routine semen analysis. The term omics encompass the study of genes (genomics), transcript (transcriptomics), proteins (proteomics) and metabolites (metabolomics) (12). These technologies allow for the identification and quantification of cellular components in a spatiotemporal fashion. What researchers once envisioned is now a reality; omics now allows for a transformation from once only genomic analysis to proteomic analysis. This approach offers an opportunity to investigate the relationship between an organism's genotype and resulting phenotype. Sperm proteomics, for instance, is the identification and functional study of sperm proteins. It is based on the separation of proteins to generate a sample suitable for mass spectrometry and subsequent protein identification. Currently it has led to the identification and cataloging of thousands of sperm proteins. Ultimately, the goal is to apply sperm proteomics not only as a research method, but also as a clinical and diagnostic tool in the field of male infertility. The spermatozoon is an excellent target for proteomics because the functional transformation of these cells during their journey from the seminiferous tubules to the surface of the oocyte takes place in the complete absence of contemporaneous gene transcription (12). Development and clinical application of novel sperm function tests, including the 'omics' technology, may improve precision and reliability to the diagnosis of male subfertility (13).

Clinical evaluation of the subfertile male

It is important that the initial assessment of subfertile male patients is rigorous and detailed in order to rule out any evident cause of infertility before delving deeper into evaluating the potential etiologies of unexplained male infertility. The initial workup should first include an exhaustive recording of the patient's medical history and physical examination. For men with normal semen analysis particular emphasis should be applied upon history of previous fertility, duration of infertility, history of frequent miscarriages, congenital abnormalities in the previous pregnancies and medications. Detailed coital history from both partners discloses several problems such as erectile dysfunction, anejaculation, inappropriate coital technique, infrequent intercourse and inappropriate timing of the intercourse (7,14,15).

Next, a physical examination of the patient should be performed to rule out other potential sources of infertility. Again for infertile men with normal semen analyses, attention should be paid towards penile abnormalities such as a hypospadiac urethral meatus or severe chordee which may lead to deposition of sperm into the vaginal cavity at an insufficient proximity to the cervix. The testes and epididymis and spermatic cord should be carefully palpated in order to rule out the presence of potential sources of oxidative stress culminating in sperm dysfunction such as epididymitis, epididymo-orchitis, and varicocele (16,17).

Following medical history and physical examination, semen analysis is the first laboratory test that will be run in the initial workup. Seminal fluid collected from the patient following 2-5 days of abstinence should be assessed with regard to volume, sperm concentration, motility, and morphology in comparison to WHO defined cutoff values for fertile men. At least two samples should be analyzed although the ideal interval between analyzes has not been defined yet. Although fertilization potential decreases as semen parameters decrease in quality, it is difficult to designate patients as fertile or subfertile based on semen analysis alone (2). However, semen analysis in conjunction with previous evaluations in the initial workup may contribute to the diagnosis of infertility or prognosis of fertility potential (18).

An endocrine evaluation in infertile men with normal semen parameters can hardly be of any significance (19). A thorough evaluation of the female partner by gynecologist should also take place in order to rule out implication of the female factor in the unexplained couple's infertility.

Etiologies

Immune infertility

Spermatogenesis does not occur until the onset of puberty and sperm are kept separated

from the immune system by the blood-testis barrier. When the blood-testis barrier is breached for any reason and sperm antigens come into contact with the immune system they will be treated as foreign agents resulting in antisperm antibody (ASA) formation (20).

While previous trauma, infection and obstruction have been implicated as clear etiologies for ASA formation, many cases of immune infertility have not had these events (20-22). Antisperm antibody formation has been reported in 42% of men with unexplained infertility, 10.7% of men undergoing infertility evaluations, 10% of men in couples undergoing IVF treatment but only in 2% of fertile men (23-26).

Immunoglobulin classes A (IgA) and G (IgG) are the functionally significant antibodies with respect to male infertility as IgM have high molecular weight and cannot penetrate the blood testis barrier. These antibodies bind to the sperm and reduce fertilization capability. Clark et al., demonstrated a 27% fertilization rate when ≥ 80% of sperm contained sperm-bound IgA and IgG, while fertilization rate of 72% was seen when ≤ 80% of sperm had sperm-bound ASA (27). It is not clear whether the location of the sperm-bound, whether sperm head or tail, ASA is significant, as there are conflicting reports assessing the value of localization and its relation to fertilization capacity (28).

ASA have the capability to disrupt several phases of the multi-step fertilization process. Complement in female cervical mucus can bind to antibodies and cause lysis of the sperm cell, reducing motility and inhibiting the ability of sperm to penetrate cervical mucus (28-30). Evidence suggests ASA cause sperm to have lower rates of spontaneous and induced acrosome reaction compared to sperm in ASA absent serum (31,32). Certain ASA have also been shown to inhibit spontaneous sperm capacitation reaction, and there is evidence to suggest that ASA can interfere with recognition of sperm binding sites on the zona pellucida (28).

Sperm agglutination or clumping is the only well-known semen abnormality that is correlated with the presence of ASA. Such phenomenon is time-dependent and only rarely involves a large proportion of motile spermatozoa soon after liquefaction even when all ejaculated spermatozoa are antibody coated (33,34). Moreover, semen contains several substances that inhibit the activation seminal complement system which is required by the immobilizing and apoptogenic ASA (35,36). Therefore, finding of normal semen parameters in men with immune infertility is a common event.

Reactive oxygen species

Reactive oxygen species (ROS) are unstable oxygen derived molecules that are formed as byproducts of oxidative metabolism. These metabolites include free radicals and non-free radical molecules. Hydroxyl ions and superoxide are examples of free radicals, whereas hydrogen peroxide and lipid peroxide are examples of non-radical species [36]. Reactive nitrogen species are also included in the ROS category; some examples include nitrous oxide, nitroxyl ion, and peroxynitrite (37,38).

In human semen, the primary producers of ROS are leukocytes and immature spermatozoa (39). In spermatozoa ROS are generated through two ways: NADPH oxidase system at the level of the sperm plasma membrane and the NADH-dependent oxido-reductase system at the mitochondrial level (40). Conditions that provoke inflammatory cells accumulation in the genital tract such as infections or lead to production of immature sperm such as varicocele, lifestyle factors that stimulate sperm to generate excess of ROS such as smoking are all implicated (17,41,43).

Polymorphonuclear leukocytes and macrophages represent approximately 50-60% and 20-30% of all seminal leukocytes, respectively (44). These leukocytes can be activated by infection and inflammation, in which case they are capable of producing 100 times greater amounts of ROS than inactivated leukocytes (45,46). In addition, even low level leukocytospermia (below 1 million of white blood cells per 1 milliliter of semen) has been recently discovered to be harmful, and therapy of such low levels of leukocytes may result in improvement in pregnancy rates (43,47).

There is strong evidence that smoking is linked to increase in ROS. Many of the 4,000 com-

pounds in tobacco smoke are either reactive oxygen or nitrogen species. A study of smoking and non-smoking infertile men showed a 48% greater seminal leukocyte concentration and a 107% increase in ROS levels among smokers (41,42). Alcohol abuse and exposure to radiation and toxic chemicals can also increase seminal ROS (48).

ROS are physiologically essential in the fertilization process by aiding in sperm acrosome reaction, hyperactivation, motility and capacitation (49). However, greatly elevated levels of ROS can overwhelm the body's natural anti-oxidant defense and cause damage, a condition known as oxidative stress (OS). Studies have shown that elevated ROS levels can be found in 40-80% of infertile men (50). Additionally, elevated ROS levels can be found in up to 11-78.5% of infertile patients with normal semen parameters (51-53). Lipids such as the polyunsaturated fatty acids present in the sperm plasma membrane are the most chemically susceptible macromolecule to OS (54). Damage to the plasma membrane leads to impaired sperm function such as a decrease in motility and failure to undergo sperm-oocyte fusion (55,56).

Genetic defects

Genetic damage in sperm can occur at several levels, all of which have the potential to cause infertility in men. Sperm chromosomal abnormalities are most often seen in men with decreased sperm cell count (oligozoospermia), decreased motility (asthenozoospermia), or high percentage of morphologically abnormal sperm (teratozoospermia) (57). Several reports have shown the rates of disomy for autosomes and sex chromosomes are 0.11% and 0.44% for normozoospermic infertile men, and the rate of diploidy is 0.3-1% (58,59). The likelihood of sex chromosomal abnormalities are 15 times greater in infertile men than in the general population, while autosomal abnormalities occur with six times greater frequency (60,61).

Gene mutations and polymorphism have been also recognized in infertile men with normal spermiograms. CatSper gene 1 mutation, which will be described later under hyperactivation defects, and CAG repeat polymorphism in the gene coding for polymerase gamma (POLG) are examples for such gene abnormalities. Polymerase gamma is the catalytic subunit of the enzyme mitochondrial DNA polymerase that is responsible for synthesis and repair of mitochondrial DNA. Mitochondrial DNA encodes several mitochondrial proteins that are important in generation of energy and ROS. PLOG gene polymorphism is discovered in infertile men with normal spermiogram. The sperm from these men have lower oocyte penetration ability and fertilization rates (62).

Recently, Garrido et al. conducted microarray analysis on sperm mRNAs in sperm samples of normospermic infertile men versus fertile controls and showed differential expression of hundreds of genes between the two study groups (63). Moreover, when determining genes that are ten times or more differentially expressed, three genes are overexpressed, whereas 136 genes are underexpressed in infertile normospermic men vs. fertile controls. This study provides clear evidence of genetic contribution to UMI, however, analytic functional data of these differentially expressed genes and their products are incompletely understood and further studies are certainly needed to examine their roles.

Sperm DNA damage is a broad term that accounts for many defects in the DNA structure including: single or double DNA strand breaks, base deletion or modification, inter-strand or intra-strand cross-linkage, and DNA-protein cross-linkage (64). Post-meiotically initiated abortive apoptosis, unresolved strand breaks during spermiogenesis, and oxidative stress have all been implicated as potential sources of this damage (65). Additional factors associated with altered DNA integrity include advanced paternal age, inadequate diet, drug abuse, tobacco use, environmental factors such as pesticide exposure or air pollution, varicocele, systemic diseases, and genital inflammation (66). Studies have linked DNA damage with infertility, showing greater DNA damage in the sperm of infertile men than of fertile men (67,68). In fact, it has been suggested that sperm DNA fragmentation is one of the chief causes of reduced fertility potential. DNA damage is reported in 5-8% of infertile normozoospermic men (66,69). In a recent controlled study on small

number of infertile normozoospermic individuals (n = 28), sperm DNA integrity defects measured by sperm chromatin structure assay was reported to be 89.2% (53).

High percentages of DNA damage in spermatozoa have a negative impact on a man's ability to achieve pregnancy naturally, and are correlated with spontaneous pregnancy loss (70,71). It has also been shown that high rates of DNA damage in spermatozoa are correlated to failure of fertilization in intrauterine insemination (IUI) (72) and conventional in vitro fertilization IVF (72-74) but not with ICSI (72,74,75). Although sperm DNA integrity emerges as a specific marker of male fertility potential, clinical utility of such test may be undermined by lack of consensus on a standardized method of measurement and universal cut off value.

Fertilization defects

Fertilization of the oocyte involves multiple complex and intricate processes. Defects in any one of these processes can hinder the sperm fusion with the oocyte and formation of the zygote. One of the first steps of fertilization is capacitation. Sperm capacitation occurs naturally during travel through the female reproductive tract, while in vitro capacitation can be induced by removal of seminal plasma and subsequent addition of corresponding culture medium (76). Several changes are observed in the sperm that undergo capacitation, including changes in membrane composition, membrane potential, intracellular pH and calcium levels, and changes in the protein phosphorylation (57). Hortas et al. (77) examined in particular the activity of sperm protein phosphorylation in relation to capacitation. The study observed low expression of D-mannose receptors in 6 of 15 subjects with unexplained infertility, revealing a failure of normal physiological capacitation development. These various alterations are induced by efflux of cholesterol as well as shifts in ion channel and transport activities (78). Capacitation increases the capacity of the sperm to fuse with the oocyte membrane and prepares the cell to undergo hyperactivation as well as acrosome reaction (79).

Sperm ability to undergo proper hyperactivation (HA) has been shown to relate to its fertilization potential (80). The sperm motion pattern changes from progressive to motility characterized by lateral head displacement, high curvilinear velocity and large amplitudal flagellar waves (81). This style of motility is termed 'hyperactivation motility' and essential in the fertilization process, as it allows the sperm to travel through the cervical mucus and cumulus oophorus and penetrate the dense zona-pellucida (11). Mackenna et al. (82) observed that the ability of follicular fluid to induce sperm hyperactivation was significantly lower in men with unexplained infertility compared to fertile men.

The exact physiological mechanism for hyperactivation is thought to be an increased intracellular calcium entry through sperm calcium channels known as CatSper1-4 (83-85). CatSper1-4 is located in the sperm principle piece, along with various other voltage gated proton pumps important to the initiation of hyperactivation (83). Avenarius et al. discovered that male patients with mutated CatSper1gene are infertile with poor HA response despite their normal sperm count, morphology and even their initial motility (86). Interestingly, there are two known CatSper2 gene related mutations in humans that cause male infertility, termed CatSper-related non-syndromic male infertility and deafness-infertility syndrome (87). However, both syndromes are associated with gross semen abnormalities. Further investigation is needed to disclose the genetic and molecular nature of fertilization in patients with defective HA response and unexplained infertility. Moreover, minor mutations in human CatSper genes are yet to be deciphered in males with unexplained infertility.

Sperm binding to the zona pellucida is essential in the process of fertilization and is an important precursor to the acrosome reaction. There are specific receptor-ligand interaction between the sperm cell and the zona glycoprotein layer of the oocyte that allows for mutual recognition and subsequent binding (57). The ZP3 protein on the oocyte is the chief protein involved in sperm zona pellucida binding (88). The exact identity of the ZP3 receptor on the sperm has not yet been identified, although studies have concluded the most likely candidate is beta-1, 4-galactosyltransferase

I (GalT) (88,89). During ovulation the zona pellucida is surrounded by cumulus oophorus consisting of cumulus cells embedded in an extracellular matrix primarily composed of hyaluronic acid. The protein sperm adhesion molecule 1 (SPAM1) is sperm plasma membrane molecule which is capable of hyaluronidase activity to traverse through the cumulus oophorus and bind to the zona pellucida (90). Defects in ZP binding are reported in 15% of infertile men with normal semen (91-93).

Sperm binding to the zona pellucida triggers the release of hydrolyzing enzymes known as the acrosome reaction (AR). AR is essential for fertilization both because it allows the sperm cell to penetrate the zona pellucida and exposes the site of the sperm that fuses with the oocyte plasma membrane (57). Acrosin is the specific enzyme that is released. Physiological inducers of the AR are the protein ZP3 and progesterone, which is found in high concentrations in follicular fluid and cumulus (94-96). These compounds are agonists which stimulate intracellular calcium concentration, producing a shift of internal pH and stimulating the exocytotic process (94). The timing of AR is very important, as sperm that undergo AR prior coming in contact with the ZP binding are unable to either bind to or penetrate the ZP (91). One study showed that defective zona pellucida induced-acrosomal reaction (ZPIAR) was found in 25% of normozoospermic subfertile men (97).

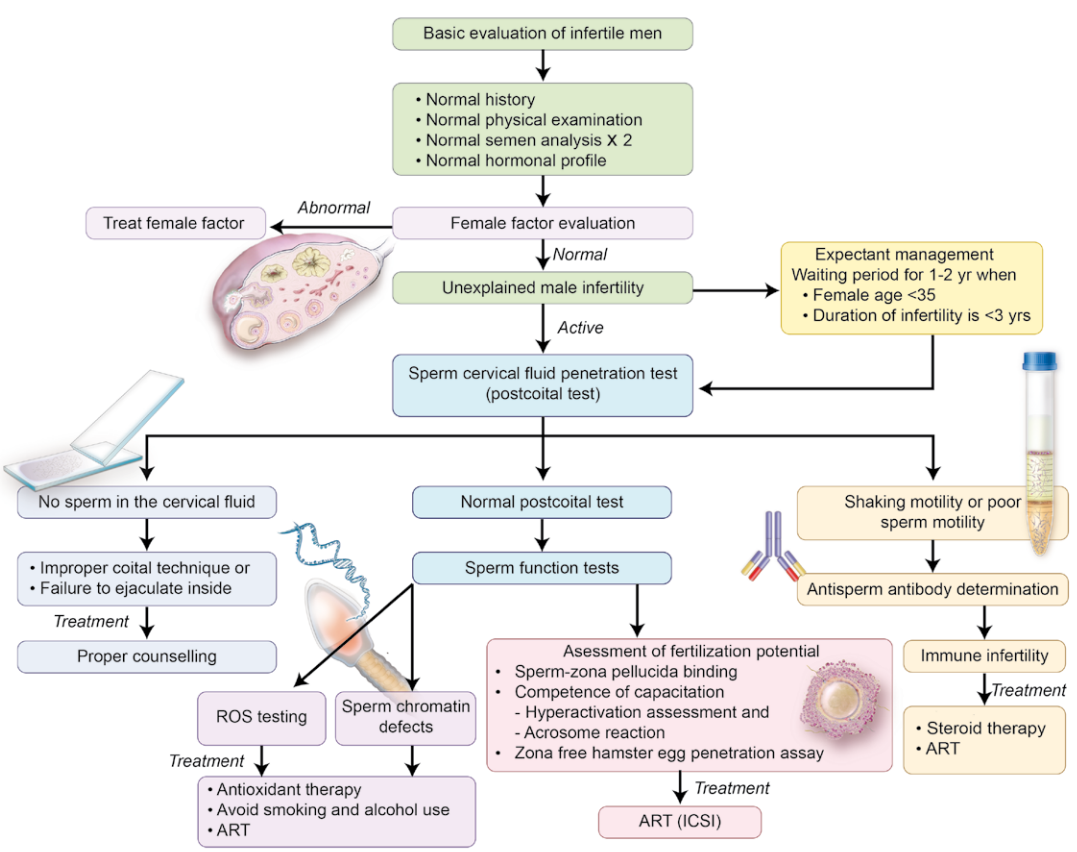
Management

A proposed algorithm for the clinical management of men with UMI is depicted in Figure-1.

Expectant management

Once thorough initial assessment is conducted and immediately treatable causes of infertility, as well as presence of a female factor have been ruled out, the clinician then must de-

Figure 1 - A proposed algorithm for the clinical management of men with unexplained male infertility.



cide how to proceed in managing men with unexplained infertility. It should be kept in mind that the reported chance of conception per cycle of a sexually active so-called 'fertile' couple is 15-20% and the cumulative chance of pregnancy is approximately 85% per year (98-100). Therefore, that leaves 15-20% of couples who would be defined as having infertility and only a subset of those will fit in the unexplained category. Studies have shown that spontaneous conception occurs in the majority of couples with unexplained infertility in the next 1-2 years if they continue to have fertility-focused intercourse (101,102). One study reported that over 50% of such couples will have a live birth within 36 months after failing to achieve pregnancy in the first year (102). A landmark study by Hull et al. found a cumulative pregnancy rate of 50-80% over three years as a function of female age, and 30-80% as a function of infertility duration (100). Other studies report spontaneous pregnancy rate of 60% within 2 years of being classified as infertile (103). However, as Hull et al. report showed, these rates can vary with female age and duration of infertility. Female partner age greater than 35 or infertility duration of more than 3 years are associated with a significant decrease in spontaneous pregnancy rate. After more than 3 years of infertility, the chance of spontaneous pregnancy drops 2% every year after the age of 25. Additionally, monthly fecundity rates in couples with unexplained infertility and female partner age over 35 are only 1-3% (100,103,104).

These results lead to several conclusions in determining how the clinician should proceed with the management of men with unexplained infertility. Young couples with short-term infertility and female partner age under 35, particularly under 30, may be withheld from treatment and expectantly followed, as the spontaneous pregnancy rate in these couples is very high. This is combined with the fact that advanced investigations and treatment are costly, and that natural means of conception is most desirable amongst these couples. On the other hand, couples with infertility lasting more than 3 years or with female age greater than 35 should be immediately referred to an active management plan that includes advanced investigations and possible ART treatment options.

Interventional management

Once a clinician deems active intervention for a couple with unexplained male infertility, he or she is then faced with the prospect of identifying the precise cause of infertility. This is a crucial step, as it allows the most appropriate treatment option to then be considered. A multitude of tests are available which can assess the various potential defects causing infertility. Many of the tests are extremely specific, examining only one aspect of the multi-step fertilization process. Therefore, in the interest of the cost and time, as well as overall benefit to the patient, it is necessary to first narrow down the potential causes of the man's unexplained infertility. The post-coital test (PCT), when is appropriately performed, can be a valid initial indicator of what aspect of fertilization should be first evaluated and treated.

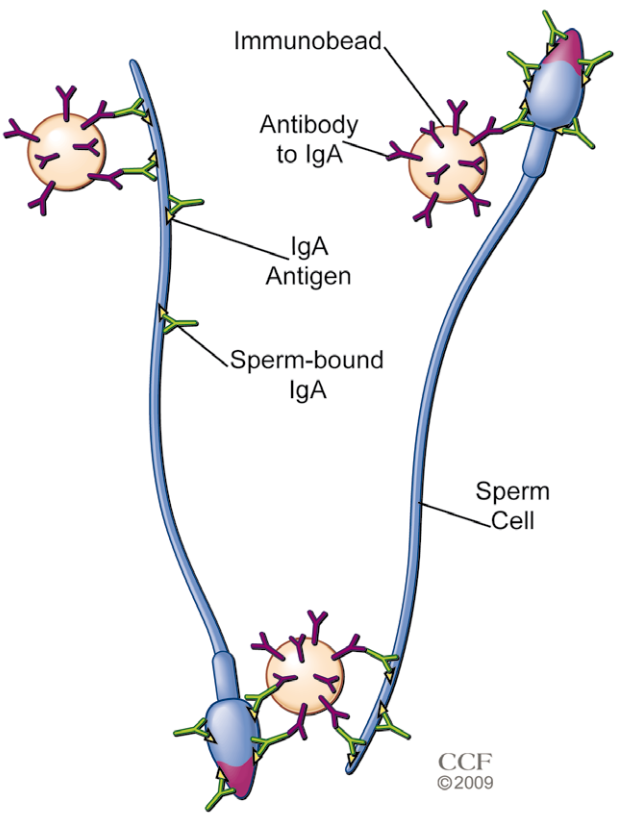
The PCT provides an assessment of the quantity and quality of cervical mucus, sperm-mucus interactions and the presence of antisperm antibodies (101,102). The test involves microscopic examination of extracted endocervical mucus, which should be conducted in the pre-ovulatory phase and 8-12 h after intercourse. However, controversy remains surrounding the predictive power of the PCT for conception (101,102). Glazener et al. (105) determined that, although the PCT test is a poor predictor of pregnancy in couples with unexplained fertility for duration of greater than 3 years, it is nonetheless a good initial assessment of sperm function, provided that care is taken to eliminate negative results caused by poor mucus quality. First, the PCT test must be performed near the time of ovulation, otherwise the cervical mucus is hostile to sperm and an abnormal result will occur. Second, adequate waiting period (8-12 hours) should be given for PCT before reading the results. The reason is that detection of certain abnormalities such as ASA requires this period for activation of complement system in the cervical mucus to exert their effects on sperm. A properly timed PCT test can present one of three results: 1) no sperm is present in the cervical fluid, 2) the PCT test is normal or 3) adequate number

of sperm are seen but of poor motility or shaking motion (Figure-1). In cases where no sperm are observed in the cervical fluid particular emphasis should be applied on improper coital techniques, abnormal penile curvature or anejaculation while poor sperm motility or shaking is suggestive of the presence of ASA. It should be noted, however, that results of the PCT are subject to considerable intra- and interobserver variability (106). Unfortunately, routine use of this test in clinical practice has led to the widespread use of ART for couples with a negative PCT. It is important to stress that a negative PCT result is not suitable to indicate a treatment modality, and the use and interpretation of this test should be well balanced (107).

Subsequently, more specific tests should be performed by the clinician in order to confirm that ASA are present in significant quantities and these ASA are interfering with sperm function. The most effective test available for detecting the presence of ASA is the direct immunobead test (IBT) (Figure-2). The World Health Organization considers the patient with ASA positive if greater than 50% of sperm are ASA bound (108). Direct mixed antiglobulin reaction (MAR) tests are very similar to the IBT. The Sperm MAR test, for example, uses latex particles, instead of immunobeads, that are coated with antihuman IgG.

Several approaches are used to treat ASA as a cause of male infertility such as steroid therapy and assisted reproductive techniques. Corticosteroid treatment suppresses the immune system, and thereby decreases ASA production. The effectiveness of this treatment is otherwise questionable. One study showed that of infertile men with ASA treated with steroids for 3 cycles, only 20% showed a decrease in sperm-bound ASA (109). Another study showed the steroids had little effect of steroids on sperm-bound IgA in infertile men, and furthermore revealed no significant difference in pregnancy outcomes between the treated group and a placebo group (110). This lack of definitive benefits of corticosteroids, coupled with the risks that steroid use has on human body such as Cushing's syndrome, bone osteonecrosis, fluid and electrolyte imbalance discourage clinicians from use of this modality of treatment. Another possible treatment option for managing

Figure 2 - Immunobead test. Spermatozoa are mixed with beads that have been coated with IgG class-specific secondary antibodies. Reprinted from Int J Urol. 2010; 17: 839-47. Samplaski, et al. New generation of diagnostic tests for infertility: review of specialized semen tests, with permission from publisher (John Wiley and Sons) (127).



patients with ASA is to use assisted reproductive techniques such as intrauterine insemination (IUI) and in vitro fertilization (IVF). Prior removal of ASAs that are already bound to the sperm by methods such as sperm washing and IgA protease treatment, yields limited success in several studies. Agarwal et al. (111) found that among 45 couples who underwent IUI and sperm washing treatment for two years, 15 couples achieved pregnancy. Kutteh et al. (112) used IgA protease treatment and reported an 80% decrease in ASA bound to the sperm but the author did not show the advantages of such finding on improvement of sperm function and fertilizing potential. The most successful method of treatment for male patients with ASA is intracytoplasmic sperm injec-

tion (ICSI) as it bypasses the otherwise necessary fertilization mechanisms that can potentially be affected by ASA. One study showed that couples who had a poor fertilization rate during IVF (6%) showed a dramatic increase in fertilization rate with ICSI treatment (79%) which was comparable to an ASA negative group (68%) (113). In another study, Esteves et al. reported that the outcome of ICSI in men with autoimmune infertility was not influenced by the percentage of ASA-bound spermatozoa (114).

Normal PCT results can direct further work-up towards unraveling other potential causes of unexplained male infertility cases such as oxidative stress, DNA damage and fertilization defects.

Oxidative Stress

Currently, the most common way to measure ROS is by chemiluminescence assay which indirectly measures seminal ROS levels. It records the intensity of light produced from the reaction of the luminol probe with the ROS in relative light units (RLU). Chemiluminescence measures both intra and extracellular ROS. To ensure accurate readings, semen samples should contain sperm concentration 1 x 106/mL or greater and be analyzed within the first hour of collection. Flow cytometry can also be used to measure intracellular sperm ROS; however this is a much more expensive tool and thus is not as practical for widespread clinical use.

There are several treatment possibilities for excess ROS. The patient should be immediately advised to avoid tobacco use as abstinence from tobacco use could help lower seminal ROS levels (42). Lifestyle modifications such as losing weight for obese men, eating of fruits and vegetables are also helpful. Moreover, recent reports support the use of antioxidants for treatment of oxidative stress related male infertility. Antioxidants serve to prevent excessive ROS formation and subsequent damage by interrupting free radical chain reactions and forming non-harmful non-radical end product. Some clinical trials were able to demonstrate beneficial effects of antioxidant therapy in cases of male fertility in terms of improving semen parameters, pregnancy rates and sperm DNA fragmentation index (measure for DNA integrity defects) (115). Useful antioxidants include vitamin E, Vitamin C, Coenzyme Q-10, selenium, zinc, lycopene and carnitine. A recent Cochrane meta-analysis on the use of oral antioxidants in male infertility found that these agents significantly improved pregnancy rates and live births and decreased sperm DNA damage (116). The evidence suggests that antioxidant supplementation in subfertile males may improve the outcomes of live birth and pregnancy rate for subfertile couples undergoing fertility treatment. However, large clinical trials are still necessary to identify the superiority of one antioxidant over the other in different subpopulations of infertile males, as well as other important aspects such as dose and duration of therapy. Lastly, Hamada et al. reported that even low level leukocytospermia (important source of ROS) may be harmful and prescribing doxycycline 200 mg twice daily for three weeks results in significant improvement in pregnancy rates (47).

Chromosomal abnormalities and DNA damage

Several methods exist for the detection and evaluation of sperm chromosomal and DNA abnormalities such as sperm Karyotype and fluorescence in situ hybridization (FISH). FISH is not only very highly sensitive and specific method, but it also allows for the study of much greater numbers of spermatozoa, therefore increasing both the accuracy and efficiency of the process of detecting sperm chromosomal aneuploidy rates in infertile men. It should be noted however that sperm chromosomal abnormalities are exceedingly rare in patients with UMI.

On the other hand, assessment of sperm DNA integrity has greater importance for about 10% of men with normal semen analysis. These men may harbor single or double-stranded DNA fragmentation (66,69). Various tests exist that allow for detection and evaluation of sperm DNA damage in spermatozoa. These tests can be categorized as either direct or indirect measurements of DNA damage. Comet assay, also known as single cell gel electrophoresis, is a sensitive technique that measures DNA damage directly. Terminal deoxy-nucleotidyl transferase-mediated

deoxyuridine triphosphate (dUTP) nick end-labeling (TUNEL) assay is another sensitive and specific method for measuring of sperm DNA damage. Unlike Comet, TUNEL is able to detect both single and double strand breaks simultaneously (117). However, TUNEL only reveals the number of cells with DNA damage in a population, while Comet is able to quantify the degree of DNA damage in each cell (118). The sperm chromatin structure assay (SCSA) is an indirect method of assessing DNA damage by measuring sperm chromatin integrity. In SCSA, sperm DNA is exposed to acridine orange which binds to DNA (67). This acridine orange fluoresces green when it is attached to normal double stranded DNA, and red when bound to single stranded fragmented DNA. The ratio of green to red fluorescence is determined in what is known as the DNA fragmentation index (DFI). The advantages of SCSA are that it is relatively simple and quick procedure and the DFI has been identified as a useful predictor of pregnancy (118).

There is some debate, however, regarding what the cut off values should be in categorizing men as fertile or infertile based on DNA damage, as well as what tests should be used. Chohan et al. (119) observed that results of various tests correlated well with each other. DFI as measured by SCSA seems to currently be the most established indicator of DNA damage. Several studies report a cut off value greater than 30% in DFI has shown to be associated with significant decreases in IVF fertilization as well as clinical pregnancy rates, with dramatically higher rates of success below the cut off (72,120,121). Esteves et al. (7) asserted that TUNEL was the best method for observing DNA fragmentation rates, citing the test's ability to precisely identify all existing breaks in sperm DNA. Cut off values for TUNEL are a subject of debate among researchers. One study demonstrated that when greater than 12% of DNA was fragmented, as assessed by TUNEL, IUI success rates were 0% (122). Another study claimed a cutoff rate of 19.25% DNA fragmentation differentiated fertile from infertile men (123). The desire to define established cut off values for TUNEL stems from the fact that it is less technically demanding than SCSA, and can provide more specific information as well (123).

ICSI is the primary treatment option for patients with a rate of DNA damage above the established cut off value for the corresponding test (124,125). Fragmentation of spermatozoal DNA is linked with defects in various fertilization processes, and thus fertility is restricted by natural barriers in IVF and IUI treatments. ICSI, however, bypasses those natural barriers, allowing for direct fertilization of the oocyte. The data seems to support this theory, as studies have found no correlation between high rate of DNA damage and fertilization rate in ICSI treatment (72,74,126). Patients should be advised, however, that the effects of spermatozoa with DNA damage being used for fertilization are still controversial, and further testing is required to assess potential long term effects.

Fertilization defects

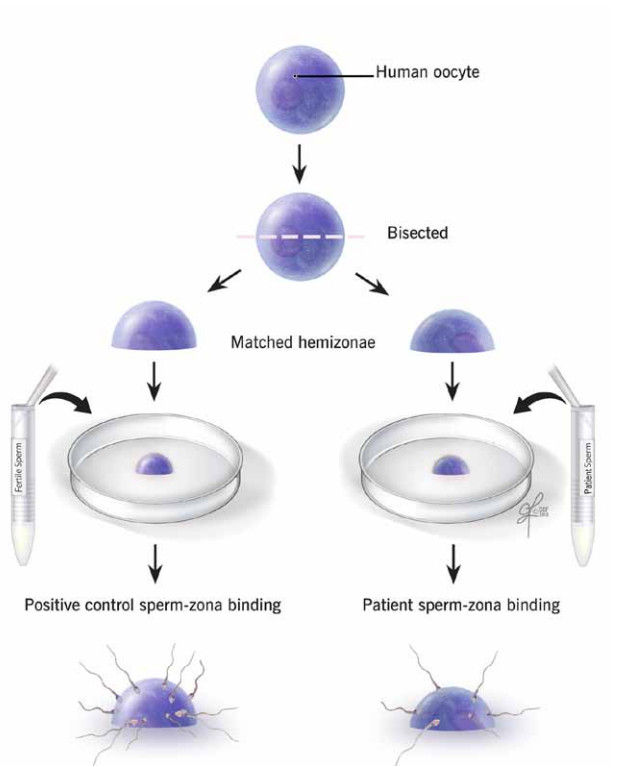
Sperm function tests for fertilization potential are particularly useful in discovering the cause of a man's otherwise unexplained infertility, and it has often been found that the infertile male patient yields poor functional test results in the face of otherwise normal semen analysis parameters. The sperm penetration assay (SPA), also known as the zona-free hamster oocyte penetration assay is one of the common tests used to measure a spermatozoa's ability to undergo capacitation, AR, fusion and penetration through the oocyte plasma membrane, and finally decondensation within the cytoplasm of the oocyte (127). The SPA uses hamster oocytes whose ZP has been removed to allow for cross species fertilization. The human sperm is incubated with the zona-free hamster oocytes and the percentage of ova penetrated, as well as the number of sperm penetrations per ovum are measured (8). Normal values for fertile men are 10-30% ova penetrated (8). One study revealed that 34.1% of patients with UMI scored less than 10% ova penetrated (128). The SPA is highly sensitive as it has been shown to be positively correlated with IVF fertilization rates as well as achievement of pregnancy in patients with UMI, and practical, as it uses readily available hamster oocytes rather than human ones (69,129,130).

There are several additional tests which assess specific defects in fertilization processes.

Sperm-ZP binding and AR are two particularly important functions that can be examined. The hemizona assay evaluates sperm ability to bind to the ZP. Zona pellucida from a human oocyte is isolated and divided in half. One half is incubated with sperm from a fertile donor, while the other half is incubated with the patient's sperm. The number of sperm bound to the ZP in each group is counted following incubation, and a ratio of patient to fertile is calculated (Figure-3). A ratio of less than 30% is considered abnormal, and the patient would be determined to possess a defect in sperm ability to bind to the ZP. Poor ZP binding rates are associated with high rates of IVF failure (131). The availability of human oocytes is limited, and thus this test should only be performed if multiple failed IVF attempts have occurred (132).

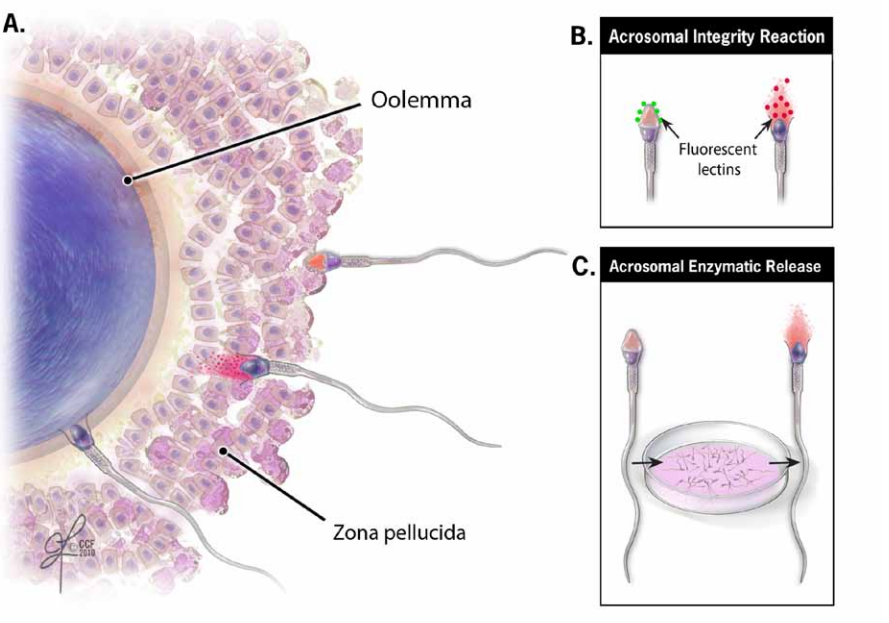
Once the sperm has bound to the ZP, AR must occur in order to penetrate the zona pellucida and fertilize the oocyte. Tests exist that can independently assess both the integrity of the acrosomal cap as well as the functional ability of the sperm to release the acrosomal enzymes. Fluorescently labeled plant lectins such as pisum sativum agglutinin (PSA) binds to chemicals in the acrosomal matrix. However, PSA cannot penetrate intact acrosomal membranes, and thus can only identify acrosome reacted sperm. Conversely, peanut agglutinin (PNA) binds to outer acrosomal membranes and thus can indicate sperm cell which remains acrosome-intact. These lectin tests do not differentiate between acrosome reacted sperm and morphologically abnormal sperm with damage to the plasma membrane, therefore cell viability assessment and removal of damaged sperm should be conducted to ensure accurate results (133). The competence of acrosomal enzymatic release in sperm can be directly assessed. Sperm can be prompted to undergo AR in the presence of an artificial inducing agent, ionophore A23187, or natural physiological inducers, progesterone or ZP3 (57,127) (Figure-4). The percent of reacted sperm can be counted, and studies show that semen samples containing 5-30% reacted sperm have higher fertility potential (134). Again, these are very specialized tests, and are recommended only in the event of multiple IVF failure (57).

Figure 3 - Hemizona assay. The zona pellucida is isolated and divided in half. One half is incubated with fertile donor sperm (positive control) and the other half is incubated with patient sperm. The ratio of fertile to donor binding is measured. Reprinted from Int J Urol. 2010; 17: 839-47. Samplaski, et al. New generation of diagnostic tests for infertility: review of specialized semen tests, with permission from publisher (John Wiley and Sons) (127).



Analysis of sperm hyperactivation motility is also critical to determine fertilization potential. Hyperactivation parameters include curvilinear velocity and lateral head displacement of the sperm. These parameters are difficult to assess manually, both because they are difficult to quantify accurately by observation and also because the heightened movement speed of the sperm which means they often leave the microscope's field of view. In order to accurately evaluate hyperactivation, computer-assisted sperm motility assessment (CAMA) is used which assesses the sperm hyperactivation parameters (135,136). CAMA measurements have been positively correlated with IVF fertilization rates (137); however, it has been shown that assessment of sperm motion

Figure 4 - (a) Normal physiology: proteolytic enzymes in the acrosome digest through the zona pellucida, allowing for sperm--oolemma fusion. (b) Assessing acrosomal integrity: Different fluorescent lectins are applied to label either the outer membrane or acrosomal contents. (c) Assessing acrosomal enzymatic release: Enzymatic release is induced and the proportion of reacted spermatozoa is assessed. Reprinted from Int J Urol. 2010; 17: 839-47. Samplaski, et al. New generation of diagnostic tests for infertility: review of specialized semen tests, with permission from publisher (John Wiley and Sons) (127).



characteristics alone cannot be a reliable predictor of fertilization outcome (138).

Intracytoplasmic sperm injection has emerged as the essential therapeutic modality for sperm fertilization defects. In ICSI, a single spermatozoon is injected directly into the oocyte cytoplasm, bypassing many of the natural steps involved in fertilization such as capacitation, hyperactivation, ZP binding, and AR. Higher rates of pregnancy are obtained by ICSI compared to both standard IVF as well as IUI (139).

Extensive evaluation of male factor infertility in the era of ICSI - An expert opinion

The recent innovation of sophisticated diagnostic testing, achieved in the field of Andrology, has improved our understanding of sperm defects in male infertility. Couples facing unexplained infertility are characterized by being childless despite presence of normal semen parameters and normal female partner evaluation. Although detailed history taking and physical examination are always crucial to disclose erectile dysfunction or infrequent intercourse, more novel expensive tests are required to scrutinize hidden sperm functional defects. ICSI may help solve the problem of unexplained male infertility and bypass all the natural barriers that a dysfunctional sperm must overcome to induce fertilization. However, such therapy is not without risks and complications. The successful pregnancy achieved by using a dysfunctional sperm carries a risk of transmission of the same infertility traits to the male offspring.

Furthermore, the paternal part of the embryonic genome is actively expressed at the four- to eight-cell stage in human embryos. Therefore, sperm DNA strand breaks that can not be repaired by the oocyte DNA repair system may adversely affect the later stages of embryonic development. Aitken and Krausz recognized that sperm DNA damage is promutagenic and can give rise

to mutations after fertilization (140). Mutations sustained at the very early stage of embryonic development will be fixed in the germline and may give rise to the induction of infertility, childhood cancer and higher risk of imprinting diseases in the offspring (141). So far, however, short term follow-up studies of children born after ICSI compared with children born after conventional IVF have not been conclusive regarding the risks of congenital malformations, imprinting diseases and health problems in general. Long term studies on the risks and complications of ICSI on the produced offspring are critically required.

Taking this risk into account mandates frequent conduction of sperm function testing to elucidate the basic sperm molecular defects which should be rectified by utilizing molecular targeted therapies before using of the dysfunctional sperm in ICSI. Although these types of therapies are still under investigations, exploring the presence and frequency of metabolic targets may help specifically direct the therapeutic research plans on correcting these metabolic alterations. In addition, identifying certain abnormalities in these tests e.g. sperm DNA integrity defects may help in pre-ICSI counseling of the couples about the advantages as well as the possible failures and complications of ART procedure.

CONCLUSIONS

Normal semen analysis results, as routinely assessed, do not guarantee fecundity. This premise is important for all clinicians involved in the management of the subfertile men. Currently, one of the chief objectives of male infertility research is to invent a diagnostic test that efficiently correlates with sperm fertilizing potential. Proper understanding of the in vivo process of human fertilization and sperm egg interaction in vitro is the key to envisage the sperm functional alterations with tremendous influence on diagnosis and treatment of male subfertility.

CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Cost-effectiveness analysis of medical treatment of benign prostatic hyperplasia in the Brazilian public health system

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ABSTRACT

Objective: To perform a cost-effectiveness analysis of medical treatment of benign prostatic hyperplasia (BPH) under Brazilian public health system perspective (Unified Health System - "Sistema Único de Saúde (SUS)").

Material and Methods: A revision of the literature of the medical treatment of BPH using alpha-blockers, 5-alpha-reductase inhibitors and combinations was carried out. A panel of specialists defined the use of public health resources during episodes of acute urinary retention (AUR), the treatment and the evolution of these patients in public hospitals. A model of economic analysis(Markov) predicted the number of episodes of AUR and surgeries (open prostatectomy and transurethral resection of the prostate) related to BPH according to stages of evolution of the disease. Brazilian currency was converted to American dollars according to the theory of Purchasing Power Parity (PPP 2010: US$ 1 = R$ 1.70).

Results: The use of finasteride reduced 59.6% of AUR episodes and 57.9% the need of surgery compared to placebo, in a period of six years and taking into account a treatment discontinuity rate of 34%. The mean cost of treatment was R$ 764.11 (US$449.78) and R$ 579.57 (US$ 340.92) per patient in the finasteride and placebo groups, respectively. The incremental cost-effectiveness ratio (ICERs) was R$ 4.130 (US$ 2.429) per episode of AUR avoided and R$ 2.735 (US$ 1.609) per episode of surgery avoided. The comparison of finasteride + doxazosine to placebo showed a reduction of 75.7% of AUR episodes and 66.8% of surgeries in a 4 year time horizon, with a ICERs of R$ 21.191 (US$ 12.918) per AUR episodes avoided and R$ 11.980 (US$ 7.047) per surgery avoided. In the sensitivity analysis the adhesion rate to treatment and the cost of finasteride were the main variables that influenced the results.

Conclusions: These findings suggest that the treatment of BPH with finasteride is cost-effective compared to placebo in the Brazilian public health system perspective.

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INTRODUCTION

Benign Prostatic Hyperplasia (BPH) is one of the most common diseases in men, being considered part of the physiological process of aging. BPH prevalence among men of 70 years old is about 40%, according to population studies (1-3). However, there is a great variation of prevalence in the literature, ranging from 13% to 46%, according to diagnostic criteria, region studied and age of sample (4). Regarding to BPH incidence, the data are still more scarce (5-7). A cohort study conducted in Holland showed a general incidence of 15 per 1000 men/year, with an evident correlation between incidence and age (4). BPH is a chronic and progressive condition that, if inappropriately

treated, may progress to acute urinary retention (AUR), recurrent urinary infection, hydronephrosis and eventually renal failure. However, clinical presentation is widely variable as well as individual progression of the disease (8). While some men present lower urinary tract symptoms (LUTS) and significant reduction of quality of life, even in the absence of significant prostatic growth, others with an important prostatic growth may be asymptomatic or present mild symptoms. The presence of mild to moderate LUTS and urinary flow less than 15 mL/s probably represent BPH diagnosis and treatment options should be discussed with the patient. Several options are available, from watchful waiting, medical treatment to surgery. The objective is to reduce the intensity of symptoms, normalize the dynamics of lower urinary tract and prevent eventual complications (9). Cost-effectiveness analysis of medical treatment of BPH in the public health system of Brazil (Unified Health System – "Sistema Único de Saúde – SUS") presents useful information for those involved in the development of health policies and financial resources allocation. Benign Prostatic Hyperplasia (BPH) is one of the most common diseases in men, being considered part of the physiological process of aging. BPH prevalence among men of 70 years old is about 40%, according to population studies (1-3). However, there is a great variation of prevalence in the literature, ranging from 13% to 46%, according to diagnostic criteria, region studied and age of sample (4). Regarding to BPH incidence, the data are still more scarce (5-7). A cohort study conducted in Holland showed a general incidence of 15 per 1000 men/year, with an evident correlation between incidence and age (4). BPH is a chronic and progressive condition that, if inappropriately treated, may progress to acute urinary retention (AUR), recurrent urinary infection, hydronephrosis and eventually renal failure. However, clinical presentation is widely variable as well as individual progression of the disease (8). While some men present lower urinary tract symptoms (LUTS) and significant reduction of quality of life, even in the absence of significant prostatic growth, others with an important prostatic growth may be asymptomatic or present mild symptoms. The presence of mild to moderate LUTS and urinary flow less than 15 mL/s probably represent BPH diagnosis and treatment options should be discussed with the patient. Several options are available, from watchful waiting, medical treatment to surgery. The objective is to reduce the intensity of symptoms, normalize the dynamics of lower urinary tract and prevent eventual complications (9). Cost-effectiveness analysis of medical treatment of BPH in the public health system of Brazil (Unified Health System – "Sistema Único de Saúde – SUS") presents useful information for those involved in the development of health policies and financial resources allocation.

MATERIALS AND METHOS

Systematic Review of Literature

The following databases were used to find clinical randomized studies and systematic reviews: Cochrane Database of Systematic Reviews (CDSR); Cochrane Clinical Trials; Database of Abstracts of Reviews of Effectiveness (DARE); Health Technology Assessment (HTA) Database; NHS Economic Evaluations Database (NHS EED); EMBASE; LILACS; MEDLINE via Pubmed.

The following key words were used to search MEDLINE via Pubmed: α-Blockers: #1: "Doxazosin"[Mesh] #2: "tamsulosin "[Substance Name] #3: "terazosin "[Substance Name] #4: "alfuzosin"[Substance Name]; 5 α-reductase inhibitors: #1: "Finasteride"[Mesh] #2: "dutasteride "[Substance Name]; Benign Prostatic Hyperplasia: #1: "Prostatic Hyperplasia"[Mesh].

Data were obtained independently by two reviewers using a standardized sheet. A third reviewer solved the discrepancies between the two main reviewers.

Treatment of BPH in the Brazilian Public Health System

Due to the lack of data regarding BPH treatment in Brazil, a panel of specialists was conducted, in order to provide data to the economical model. The results were used in addition to the literature data and the current available consensus. The panel was constituted by 5 urologists from Rio de Janeiro City (Hospital Municipal

Souza Aguiar and Hospital Federal de Bonsucesso) and two from São Paulo (Hospital das Clínicas).

The specialists were instructed to fill out a questionnaire of 66 objective questions. The alternatives tried to identify the most frequent treatments on different stages of and gather information about local treatments, complications and adverse reactions during ambulatory follow-up.

Economic analysis

The studied population was a hypothetical cohort of men over 55 years with BPH treated in public health facilities. The drugs considered for the analyses were finasteride, doxazosine or combinations, based on randomized clinical trials (10-13) and Brazilian health system reality (less costly).

The period of time analyzed of the base case scenario reflected the follow-up period of the clinical trials that evaluated the safety and efficacy of the above drugs. In the sensitivity analysis, this period of time was adapted to life time, since BPH is a chronic disease that demands long-term use of drugs, from the time of diagnosis until death.

Epidemiological data regarding incidence, prevalence and natural history of the disease were collected in international observational studies. The economic analysis considered the need of surgery and occurrence of AUR as outcomes. The risks of these events were obtained in clinical trials during the systematic review of literature.

Some data obtained in the specialist panel were used in the economic model. In that case, the information were submitted to sensitivity analysis. The annual mortality rate used was obtained from the last report of the Instituto Brasileiro de Geografia e Estatística (IBGE) (14).

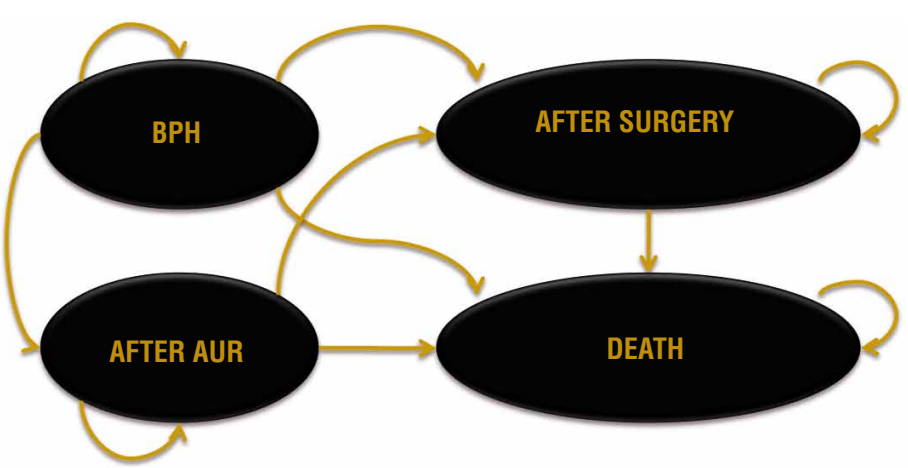
Health resources included drugs, materials, laboratorial and image exams, surgeries and hospitalizations.

The costs of the were obtained from the Price Database of the Ministry of Health (15), which presented the mean price of the drugs bought by public hospitals, according to the quantity purchased.

The unitary costs of laboratorial and image exams, medical visits and physiotherapy were obtained using the software Sigtap Desktop ("Sistema de Gerenciamento da Tabela de Procedimentos, Medicamentos e OPM do SUS" – Management of Procedures and Drugs of SUS). The unitary costs of materials were obtained from the magazine SIMPRO. Prices were related to December 2010 (16).

The current economic analysis included only the direct medical costs. For the estimation of costs and treatments, it was proposed a model that included the estimated number of AUR episodes and surgeries related to BPH, according to the different stages of the disease and based on the Markov states (Figure-1). Microsoft Excel 2007 software was used to analyze the data.

Figure 1: Health states considered in the Markov model.



A discount rate of 5% per year was applied in order to reflect the present cost, varying from 0% to 10%. Sensitivity analysis was performed according to the recommendations of the Brazilian Ministry of Health (17). Brazilian currency was converted to American dollars according to the theory of Purchase parity Power (PPP 2010: US$ 1 = R$ 1.70) (18).

RESULTS

Cost-Effectiveness Analysis

The data obtained from the panel showed that the most common drugs used in the treatment of BPH were finasteride, doxazosine and a combination of both. The most frequent surgery performed was transurethral resection of the prostate.

The comparative results of the available alternatives of treatment were determined by the incremental cost-effectiveness ratio (ICERs), defined as the additional cost of treatment divided by the additional gain in health obtained by two different alternatives of treatment.

This benefit was expressed in terms of AUR episodes and BPH-related surgeries avoided. Considering the following therapeutic options: monotherapy with finasteride X placebo (observation), finasteride + doxazosine X placebo and finasteride + doxazosine X finasteride, the results of efficacy, costs and incremental cost-effectiveness rates are present in Tables 1, 2 and 3, respectively.

Sensitivity Analysis

In order to further analyze the results of the treatment with finasteride compared to placebo, several parameters were evaluated in a univariate sensitivity analysis and are presented in Table-4.

Table 1 - Efficacy and incremental costs of finasteride treatment or placebo in a period of 6 years (per patient).

	Finasteride	Placebo	Incremental
Outcome			
Number of surgeries performed	0.05	0.12	0.07
Number of episodes of acute urinary retention	0.03	0.07	0.04
Costs			
Drug[1]	R$ 235.02	R$ 0.00	R$ 235.02
Follow-up[2]	R$ 479.57	R$ 461.74	R$ 17.83
Surgery	R$ 8.03	R$ 19.89	-R$ 11.86
Acute urinary retention	R$ 37.28	R$ 88.55	-R$ 51.27
Follow-up after surgery	R$ 4.21	R$ 9.28	-R$ 5.08
Infection after AUR*	R$ 0.04	R$ 0.11	-R$ 0.07
Total	R$ 764.11	R$ 579.57	R$ 184.57
ICER	R$ 2.734,74 (US$1.608.67) per surgery avoided		
ICER	R$ 4.130,21 (US$2.429.54) per AUR episode avoided		

[1] Cost only with finasteride;

[2] Cost of follow-up for patients using finasteride or without medical treatment (watchful waiting)

* AUR: acute urinary retention

Table 2 - Efficacy and incremental costs with the combination of doxazosine + finasteride or placebo in a 4 year period (per patient).

	Combination	Placebo	Incremental
Outcome			
Number of surgeries performed	0.01	0.04	0.03
Number of episodes of acute urinary retention	0.00	0.02	0.01
Cost			
Drug[1]	R$ 232.43	R$ 0.00	R$ 232.43
Follow-up[2]	R$ 425.79	R$ 311.25	R$ 114.55
Surgery	R$ 1.25	R$ 5.13	-R$ 3.88
Acute urinary retention	R$ 10.11	R$ 30.46	-R$ 20.35
Follow-up after surgery	R$ 0.92	R$ 2.73	-R$ 1.80
Infection after AUR*	R$ 0.00	R$ 0.02	-R$ 0.02
Total	R$ 670.50	R$ 349.58	R$ 320.93
ICER	R$ 11.979,87 (US$7.046.98) per surgery avoided		
ICER	R$ 21.960,63 (US$12.918.02) per AUR episode avoided		

[1] Cost with doxazosine and finasteride only;

[2] Cost of follow-up of patients using doxazosine + finasteride or patients without treatment (watchful waiting)

*AUR: acute urinary retention

It was observed that the cost of finasteride, the surgery number and the rate of treatment discontinuity were the variables with greater impact in the results (Figure-2).

A probabilistic sensitivity analysis was also performed considering the variations of many parameters at a time, assigning an appropriate distribution of each parameter previously described in Table-4. This analysis was calculated using 1,000 simulations and the results are shown in Figure 3 (A and B).

In a period of 6 years, one patient using finasteride had an additional cost of R$ 184.50 (CI 95% R$ 135.83 - R$ 232.32), a decrease of surgeries of 0.0672 (CI 95% 0.0482 - 0.0862) and a decrease of the AUR episodes of 0.0443 (CI05% 0.0286 - 0.0583).

DISCUSSION

Prospective studies demonstrate a relationship between the incidence of symptomatic BPH and aging (4), black race, inherited factors and obesity (19). Although the Brazilian incidence of BPH is unknown, the population is getting older, has a significant percentage of black people and the incidence of obesity resembles the incidence in developed countries, (14).

These factors may anticipate an increase of men that will need treatment of BPH in years to come. Besides, most Brazilian population depends exclusively on the Public Health System (SUS) for their treatments.

According to obtained data from the panel of specialists, a public patient has limit access to

Table 3 - Efficacy and incremental costs with the combination of doxazosine + finasteride and monotherapy with finasteride in a 4 year period (per patient).

	Combination	Finasteride	Incremental
Outcome			
Number of surgeries performed	0.01	0.02	0.003
Number of episodes of acute urinary retention	0.00	0.01	0.002
Cost			
Drug[1]	R$ 232.43	R$ 178.70	R$ 53.73
Follow-up[2]	R$ 425.79	R$ 364.65	R$ 61.15
Surgery	R$ 1.25	R$ 1.83	-R$ 0.59
Acute urinary retention	R$ 10.11	R$ 12.05	-R$ 1.94
Follow-up after surgery	R$ 0.92	R$ 1.11	-R$ 0.19
Infection after AUR*	R$ 0.00	R$ 0.01	R$ 0.00
Total	R$ 670.50	R$ 558.34	R$ 112.16
ICER	R$ 43.947,88 (US$ 25.851,69) per surgery avoided		
ICER	R$ 50.764,83 (US$ 29.861.66) per AUR episode avoided		

[1] Cost with doxazosine and finasteride only

[2] Cost of follow-up of patients using doxazosine+finasteride or patients with monotherapy with finasteride

*AUR: acute urinary retention

drugs treatment and the percentage of long-term adherence to treatment is low (30% of patients discontinue treatment after 1 year). Costs and adverse reactions, especially sexual complaints, are the main reasons for treatment discontinuation. On the other hand, the panel revealed that when a prostatic surgery is indicated, the patients wait a median of six months for it even with the use of a bladder catheter. These data were obtained in two major Brazilian medical centers probably nationally this reality is still more dramatic.

Although several aspects of physiopathology of BPH are still unknown, many important achievements were observed in the last decades and some risk factors for the worsening of symptoms and AUR were identified through longitudinal studies, as age, prostatic volume, presence of more severe symptoms and higher PSA values (20, 21).

Finasteride diminishes significantly symptoms, the occurrence of AUR episodes and the need of surgery (22). Although there is a stigma about the impact on sexual health, the percentage of these side effects is low (22).

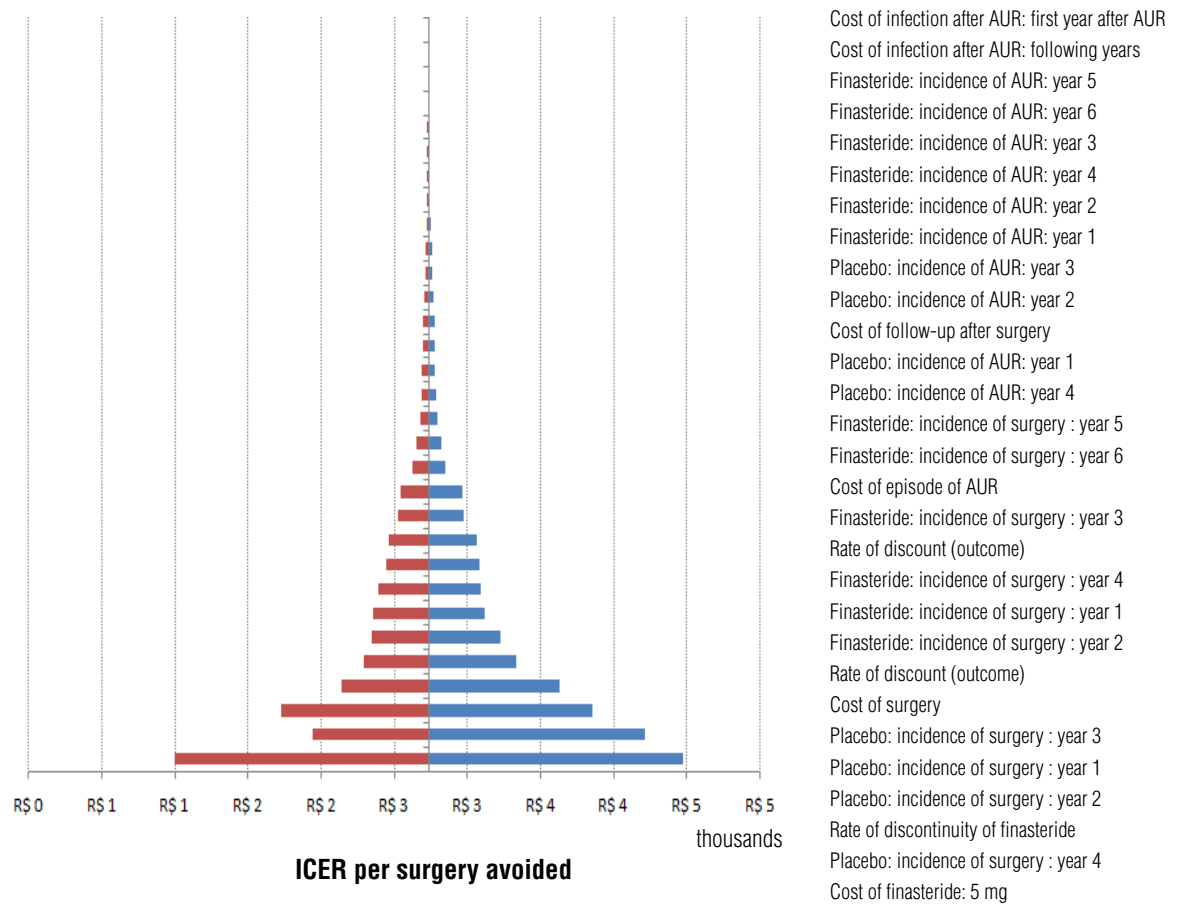
Dutasteride, a more recent 5-alpha-reductase inhibitor, shows comparable efficacy and tolerability (12), although with higher cost. On the other hand, doxazosine (11) and other alpha-blockers are efficient to reduce symptoms (23), delay the need of surgery (24, 25) without avoiding AUR episodes or surgery (26). The combined treatment with alpha-blockers and 5-alpha-reductase inhibitors is more efficient than monotherapy in patients with bigger prostates (11, 12).

Table 4 - Different parameters of sensitivity analysis (finasteride or placebo in a 6 year period).

Parameters	Base Case	Inferior limit	Superior limit
Discount (outcome and cost)	5.0%	0%	10%
Finasteride cost - 5mg	R$ 0.16	R$ 0.08	R$ 0.25
Cost of episode of AUR	R$ 265.49	R$ 132.74	R$ 398.23
Cost of infection after AUR: first year after AUR	R$ 0.24	R$ 0.12	R$ 0.36
Cost of infection after AUR: following years	R$ 0.36	R$ 0.18	R$ 0.54
Cost of surgery	R$ 759.58	R$ 379.79	R$ 1.139.36
Cost of follow-up after surgery	R$ 96.48	R$ 48.24	R$ 144.72
Placebo: Incidence of AUR* - First year 1	2.40%	1.20%	3.60%
Placebo: Incidence of AUR – Year 2	1.86%	0.93%	2.79%
Placebo: Incidence of AUR – Year 3	1.42%	0.71%	2.13%
Placebo: Incidence of AUR – year 4	1.32%	0.66%	1.98%
Finasteride: Incidence of AUR – year 1	0.99%	0.50%	1.49%
Finasteride: Incidence of AUR: year 2	0.74%	0.37%	1.11%
Finasteride: Incidence of AUR: year 3	0.48%	0.24%	0.72%
Finasteride: Incidence of AUR: year 4	0.70%	0.35%	1.05%
Finasteride: Incidence of AUR: year 5	0.08%	0.04%	0.12%
Finasteride: Incidence of AUR: year 6	0.55%	0.28%	0.83%
Placebo: Incidence of Surgery: Year 1	2.46%	1.23%	3.69%
Placebo: Incidence of Surgery: year 2	3.58%	1.79%	5.37%
Placebo: Incidence of Surgery: Year 3	2.32%	1.16%	3.48%
Placebo: Incidence of Surgery: year 4	2.35%	1.18%	3.53%
Finasteride: Incidence of Surgery : year 1	1.19%	0.60%	1.79%
Finasteride: Incidence of Surgery: year 2	1.48%	0.74%	2.22%
Finasteride: Incidence of Surgery: year 3	0.62%	0.31%	0.93%
Finasteride: Incidence of Surgery: year 4	1.49%	0.75%	2.24%
Finasteride: Incidence of Surgery: Year 5	0.40%	0.20%	0.60%
Finasteride: Incidence of Surgery: Year 6	1.02%	0.51%	1.53%
Percentage of Discontinuity of Finasteride (in 4 years)	34.00%	17.00%	51.00%

*AUR: Acute urinary retention

Figure 2 – Tornado graph (finasteride or placebo in a 6 year period).



More recent data suggest that the continuous use of inhibitors of 5-alpha-reductase may lower the incidence of prostate cancer (27, 28). A possible increase of more aggressive tumors demands more studies of the capacity of finasteride to prevent prostate cancer (29).

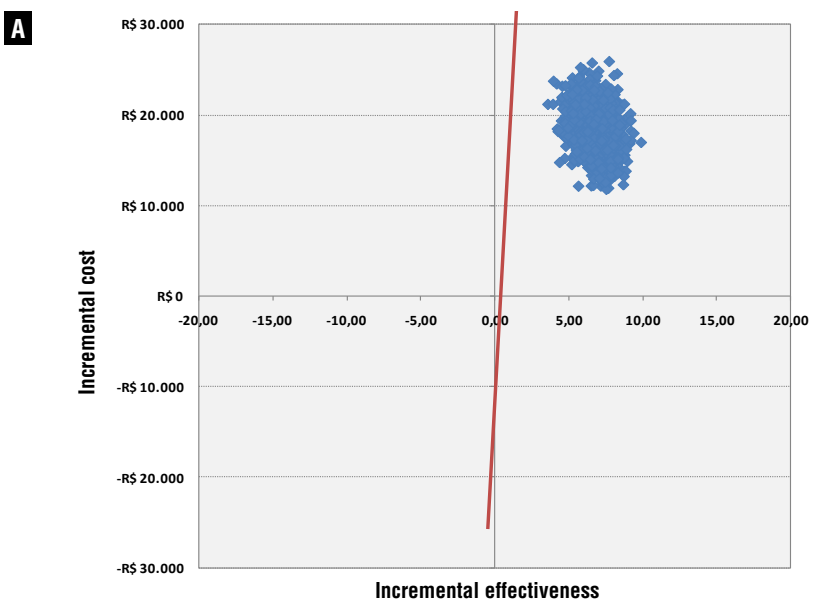
Our study, based on the literature evidence and according to the Brazilian reality, concluded that monotherapy with finasteride for men with BPH over 55 years is a cost-effective therapeutic option. The combination of finasteride and doxazosine, although controlling more rapidly the lower urinary tract symptoms, increases significantly the cost of the treatment and the ICER of outcomes avoided.

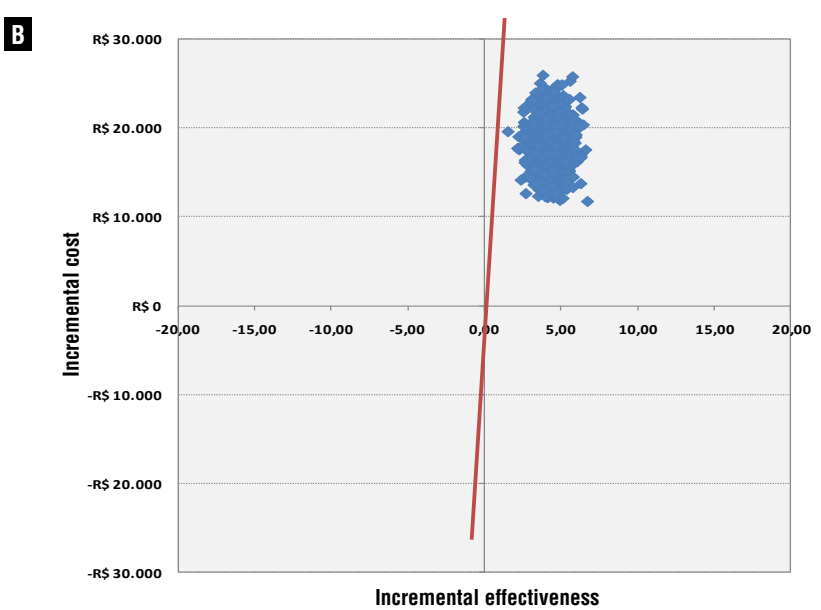
According to the World Health Organization (30), a treatment can be considerate cost-effective if the treatment costs are equivalent to 3 times the gross national product (GNP) per capta (31) (Brazilian GNP/per capta: R$ 19.016 / US$ 11.186).

In view of these facts, the availability of finasteride at public health units, combined with identification of high risk patients would allow an efficient reduction of morbidity associated to BPH, with great impact on quality of life.

This study has several limitations; the decision model analysis was based on efficacy data of randomized clinical trials, not corrected to real world (effectiveness) or to Brazilian people. This was due to the lack of validated methods to correct this distortion and absence of national data. This was one of the reasons why a panel of specialists was performed, that in turn had a limited number of specialists representing only two Brazilian states (southeast region). In order to minimize the impact

Figures 3 A and 3 B): Results of the sensitivity analysis of ICER per surgery (A) and AUR (B) avoided (finasteride or placebo in a 6 year period). The results were evaluated and classified in: Quadrant 1 (incremental efficacy > 0 and incremental cost > 0): Quadrant 2 (incremental efficacy < 0 and incremental cost < 0); Quadrant 3 (incremental efficacy < 0 and incremental cost < 0); Quadrant 4 (incremental efficacy > 0 and incremental cost > 0). For this analysis, it was considered a cohort of 100 patients.





of this limitation on the results, all data were incorporated into a sensitivity analysis. An extrapolation of the analysis for a longer period of time (lifetime was done; however, it is known that this extrapolation lowers the precision of the analysis. Still, the evolution of medical knowledge changes over time and offers new therapeutic and preventive options not considered initially in the model.

Also, it was not possible to determine the costs of the adverse events, the indirect costs and those related to quality of life. We considered these data very important and we figured a balance between the impact of side effects on the adherence and the benefits of the lowering of the symptoms with the use of the drug. New studies in this field will be very relevant and important for the decision-making process.

CONCLUSIONS

This study suggests that the treatment of BPH with finasteride is cost-effective compared to placebo in the scenario of the Brazilian public health system. Combined therapy (doxazosine + finasteride), although lowering more efficiently and rapidly lower urinary tract symptoms, increases significantly the treatment costs.

CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Perioperative platelet inhibition in transurethral interventions: TURP/TURB

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ABSTRACT

Purpose: To determine whether transurethral surgery under platelet inhibition is a feasible procedure. Before transurethral resection of prostate (TURP) or bladder tumours (TURB), the administration of platelet-inhibiting medication is often interrupted due to possible bleeding complications. We studied the performance of TURP and TURB under the current recommendations of the American College of Chest Physicians (ACCP) on perioperative platelet inhibition.

Materials and Methods: Patients assigned for transurethral intervention were preoperatively divided into the following risk groups: low, medium and high cardio- or cerebrovascular risk. In patients with a low-risk profile, acetylsalicylic acid (ASA) was discontinued. Patients of the medium risk group continued taking 100 mg of ASA. Patients of the high-risk group receiving dual platelet inhibition (ASA + clopidogrel) were not treated operatively. In total 346 patients from the low and medium risk groups underwent transurethral intervention.

Results: Forty-two out of 198 TURP were performed under 100 mg of ASA. Without ASA, a significantly shorter length of stay and earlier removal of the transurethral catheter was documented. In the parameters postoperative haemorrhage and operative revision, no significant differences were observed.

Thirty-two out of 148 TURB were performed under 100 mg of ASA. Regarding the length of stay, time until catheter removal, postoperative haemorrhage and operative revision, no significant differences were found under ASA. Only significantly longer continuous irrigation was documented under ASA.

Conclusion: In the case of a verified indication for use of platelet inhibitors, it is possible to avoid discontinuation and the consequent increased risk of thromboembolic incidents in transurethral surgery is admissible.

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Key words:
Transurethral Resection of Prostate; Platelet Aggregation Inhibitors; Thrombosis

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INTRODUCTION

In relation to cardiovascular risks, the administration of platelet inhibitors reduces the incidence of perioperative myocardial infarction or stroke by 1/3 (1). Before surgical interventions such as transurethral resection of prostate (TURP) or bladder tumours (TURB), the administration of these medicines is often discontinued due to possible bleeding complications (2). The perioperative discontinuation of platelet inhibitors without checking the indication or their necessity can lead to potentially fatal arterial thromboses (3-5). We studied the performance of TURP and TURB, taking into account the current recommendations of the American College of Chest

Physicians (ACCP) for perioperative platelet inhibition according to standardized perioperative and postoperative parameters (4).

MATERIALS AND METHODS

All patients were divided before the transurethral interventions according to the criteria listed below (Table-1) into the following risk groups: low, medium and high cardio- or cerebrovascular risk. The average age of the patients was 68.02 years. In patients with a low-risk profile, administration of the current oral platelet-inhibiting medication (ASA) was discontinued. Patients of the medium-risk group continued to take ASA (clopidogrel was

Table 1 - Perioperative platelet inhibition in transurethral resections.

1) Low cardiovascular or cerebrovascular risk:
- no CHD * / PAD*/ atherosclerosis
- no cardiovascular stents
- no medium-grade to high-grade vascular stenoses
- no stroke or TIA in medical history
=> discontinue ASA 100 mg 5 days before surgery until 7 days after the surgery
2) Medium cardiovascular or cerebrovascular risk:
- Prostate volume max. 75 cm^3
- CHD / PAD/ vascular stenosis / atherosclerosis
- cardiac stents: DES* > 12 months, BMS* > 6 weeks
- status post stroke or TIA
- status post myocardial infarction > 12 months
=> do not discontinue ASA 100 mg
3) High cardiovascular or cerebrovascular risk: ASA and clopidogrel
- status post DES* implantation < 12 months or BMS* < 6 weeks
- extracranial and intracranial stent implantation < 6 weeks
- P3 - synthetic bypass
- status post acute stroke < 12 months
- critical vascular stenosis
- status post myocardial infarction < 12 months
=> postponed elective interventions
=> In exceptional cases administration of GPIIb/IIIa inhibitors

*** DES =** drug-eluting stent; BMS = bare metal stent
CHD = Coronary heart disease,
PAD = Peripheral arterial disease,
TIA = Transient ischaemic attacks

preoperatively replaced with ASA). Patients of the high-risk group receiving dual platelet inhibition with ASA and clopidogrel were not treated operatively. The following characteristics were studied: days of continuous irrigation, postoperative haemorrhage, length of stay in hospital, days until catheter removal, revision and occurrence of cardio-cerebral event.

Out of 198 prostate resections, 42 were operated under ASA 100 mg. During continuation of the platelet-inhibiting medication, a maximum preoperative prostate volume of 75 mL was determined by transrectal ultrasonography. TURP was performed by low-pressure resection after applying trocar cystostomy under irrigation with Purisole®. A 24 Charrière resectoscope was used. The resections were performed following the same protocol until a wide view was obtained. After the completion of the intervention, the 20 Charrière haematuria-irrigation catheter was inserted and blocked in the prostatic fossa at 15 mL. After the surgery, artificial irrigation with NaCl solution took place for 24 hours which was adjusted by an experienced nursing staff to individual bleeding situations. After that, the irrigation was finished in an optimal case and the catheter was left for a further 24-hour period.

In the case of TURB, 32 patients out of 148 took ASA 100 mg. All tumors resected were uni- or multilocular tumours (1-5) of the bladder ranging in size from 3 mm to 4 cm. There were no differences between the groups. A 24 Charrière resectoscope was used. Except for re-resections and palliative therapy situations, resection was performed by use of photodynamic diagnostics, after instillation of a fluorescent substance. After that, only catheter drainage took place for 24 hours. Irrigation was used only in the case of progressive haematuria.

The results were documented until the day of discharge and subsequently analysed statistically with the Mann-Whitney U-test and Fisher's exact test for significance. Significance was determined at the p-value ≤ 0.05.

RESULTS

Concerning transurethral resections of the prostate, the average postoperative length of stay of patients who were not on antiplatelet therapy was significantly shorter ($p = 0.001$): 3.86 days without ASA versus 5.72 days with ASA. Catheter removal could be performed on average after 2.21 days without platelet inhibition and after 2.74 days with platelet inhibition ($p = 0.015$). Postoperative haemorrhage, defined by the presence of clot retention, resumption of bladder irrigation or reinsertion of the urinary catheter, occurred without ASA in 13.5% of cases, and with ASA significantly more often ($p = 0.032$) in 34% of cases. Nine out of 156 patients without ASA had to be submitted for a surgical revision. Under ASA, 5 patients out of 42 had to be reoperated on. In one case haemorrhage requiring transfusion occurred. There was no statistically significant difference in the number of surgical revisions ($p = 0.181$). The gland volumes were approximately equal, with a mean value of 41.3 mL vs. 39.6 mL. No cardiovascular events occurred in the total patient population.

In the case of transurethral resections of the urinary bladder, no significant difference ($p = 0.238$) was found in the average postoperative length of stay of patients who were not under platelet inhibition (2.91 days versus 3.34 days under ASA). Catheter removal could be performed on average after 1.27 days without platelet inhibition, and after 1.5 days with platelet inhibition ($p = 0.47$). Postoperative haemorrhage occurred without ASA in 8.6% and with ASA in 18.7% of cases. The difference was not significant ($p = 0.115$).

Four out of 116 patients without platelet inhibition had to be submitted to a surgical revision. In the ASA group, 2 patients out of 32 had to be reoperated on. Again there was no significant difference ($p = 0.61$).

However, under ASA there was significantly longer continuous irrigation: 0.625 days versus 0.267 days without platelet-inhibiting medication. No cardiovascular events occurred in the total patient population.

DISCUSSION

Patients who are submitted to a transurethral intervention are often in an advanced age with corresponding cardiovascular risk factors or diseases. For example, Wasson et al. reported that

in a series of 280 urological patients in the USA with an average age of 66, in 42 patients a coronary heart disease was present (6). These patients often receive antiplatelet medication with ASA and/or clopidogrel (3,7). The administration of such platelet inhibitors reduces by one third the incidence of myocardial infarct as well as stroke in patients with cardiovascular risks or diseases (1). The unverified discontinuation of this medication may lead, however, to life-threatening iatrogenic coagulopathies such as stent thromboses (3-5). Even though the problems of perioperative discontinuation of such medication have frequently been described in the guidelines, there is no guideline for high-risk cardiovascular patients on antiplatelet medication before non-cardiac surgical interventions.

Since intraoperative bleeding during transurethral resection of the prostate or urinary bladder complicates the technical execution of these interventions and postoperative bleeding potentially leads to the necessity of blood transfusions, revisions and longer stay in hospital, the administration of such medication is often discontinued or avoided for fear of bleeding complications. Enver et al. reported in this context that 178 out of 287 urologists in the UK asked their patients to stop taking platelet inhibitors before prostate resection (TURP). Among these 178 urologists, 62% terminated the medication without verifying the indication for these medicines (2).

In the currently available literature, increased bleeding tendencies and blood transfusion rates under aspirin are described in two studies referring to TURP (8,9). A prospective, randomized and placebo-controlled study by Nielsen JD et al. revealed no significant intraoperative blood loss under 150 mg of ASA, but described significantly increased postoperative blood loss under ASA (10). Based on these studies, discontinuation of ASA before the surgical intervention was recommended. Ala-Opas et al. found by comparison of 40 patients under ASA versus 42 patients without oral platelet inhibition no difference in the average blood loss and thus saw no contradictions for TURP under ASA (11).

Our results support the reports of an increased bleeding tendency in transurethral interventions under ASA. However, they also demonstrate that under ASA serious bleeding necessitating revision does not occur significantly more often. In summary, discontinuation and thereby an increased risk of thromboembolic incidents can be avoided if there is a verified indication for a platelet-inhibiting medication.

The investigated groups differ regarding their risk categories. This might have had an impact on long term outcome since the comorbidities of patients taking platelet inhibitors are usually higher. Since our study focused on very specific short term peri- and postoperative results the authors did not expect any relevant bias. However, long term studies with a prospective randomized and double blinded study design should be initiated.

CONCLUSIONS

Based on the results, we can conclude that the performance of transurethral resection under oral platelet inhibition with ASA 100 mg, under strict observance of the criteria listed in Table-1, is justified and therefore viable.

CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Second to fourth digit ratio: its relationship with core cancer volume and Gleason score in prostate biopsy

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ABSTRACT

Objective: To investigate the relationships between 2nd to 4th digit ratio (digit ratio) and prostate cancer detection rate and biopsy findings, including Gleason score.

Materials and Methods: In 770 consecutive men aged 40 years or older that presented with lower urinary tract symptoms (LUTS), right hand 2nd and 4th digit lengths were measured prior to PSA determinations, DRE and transrectal ultrasonography (TRUS). Among these, 166 men with a prostate specific antigen (PSA) level ≥ 3 ng/mL or abnormal digit rectal examination (DRE) prospectively underwent prostate biopsies. The relationship between digit ratio and prostate cancer detection rate and biopsy findings was investigated.

Results: The study subjects were allocated to two groups by digit ratio (group A: digit ratio < 0.95; n = 420; group B: digit ratio ≥ 0.95; n = 350). Despite similar biopsy rates (22.4% vs. 20.6%, p = 0.544), group A had higher cancer detection rate (46.8% (44/94) vs. 23.6% (17/72), p = 0.002; OR = 2.847, 95% CI = 1.445-5.610). When we analyzed 408 positive biopsy cores (group A: digit ratio < 0.95, n = 282; group B: digit ratio ≥ 0.95, n = 126), group A had higher percentage of core cancer volume (46.7% vs. 37.1%, p = 0.005) and more biopsy cores with high Gleason score (sum of Gleason score ≥ 9: 18/282 (6.4%) vs. 1/126 (0.8%), p = 0.010; primary Gleason score = 5: 12/282 (4.3%) vs. 0/126 (0.0%), p = 0.021).

Conclusions: A lower digit ratio is related to an increased detection rate of prostate cancer, a high percentage of core cancer volume and a high Gleason score.

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INTRODUCTION

The ratio of the 2nd to 4th digit length (digit ratio) of the right hand is known to be fixed in utero (1-3), and is sexually dimorphic and lower in men than in women (4-6). The digit ratio is negatively related to prenatal testosterone and positively related to prenatal estrogen concentrations (7).

The digit ratio of the right hand is related to the activity of the androgen receptor (AR) (8). Manning and colleagues demonstrated that the digit ratio of the right-hand is positively correlated with the CAG repeat number of the AR gene, and that individuals with a low digit ratio possess AR alleles with low CAG repeat numbers (8). It has been well established that a low AR CAG repeat number increases the risks of prostate cancer (9,10).

It has recently been suggested that the digit ratio is related to prostate specific antigen (PSA) level and the prostate cancer risk (11).

Based on the above-mentioned evidence, we thought that if digit ratio is related to the prostate cancer risk, digit ratio might be related to the detection rate of prostate biopsies and the

biopsy findings including the indices of tumor volume (i.e., the number of cores involved and the percentage of cores involved) and Gleason score. We investigated the relationship between digit ratio and prostate cancer detection rate and biopsy findings.

MATERIALS AND METHODS

Among the men that presented with lower urinary tract symptoms (LUTS) at a single tertiary academic center, 770 consecutive men aged 40 years or older were prospectively enrolled. All patients in the present study come from a same ethnic Korean group.

Right hand 2nd and 4th digit lengths were measured by an investigator prior to the PSA determinations and digit rectal examination (DRE) and transrectal ultrasonography (TRUS). The digit lengths were measured directly on the ventral surface of the fingers using a digital vernier calliper (6); this measurement has been previously reported to provide a high degree of repeatability (12,13). To minimize measurement errors, the mean values of duplicate measurements were used in the analysis.

Among 770 men, 166 men with a PSA level ≥ 3 ng/mL or abnormal DRE findings underwent a 12 core prostate biopsy as an initial biopsy. Biopsies were performed transrectally using an 18-gauge biopsy needle and a biopsy gun under TRUS guidance to provide the 17 mm long tissue cores.

The study subjects were allocated into two groups by digit ratio. As noted in previous study (11), we chose 0.95 as the cut-off value because the mean and median values of digit ratio of all patients (n = 770) were 0.948 and 0.946.

The cancer detection rates and biopsy findings were analyzed according to digit ratio. Student's t-test and Chi-square test were used to compare the variables of the two study groups, which were divided by digit ratio. To identify the independent predictive factors influencing prostate cancer detection, univariate and multivariate analysis were performed using logistic regression model. The analysis was performed using SPSS 12.0 (SPSS, Chicago, IL), and differences were considered statistically significant when the P values were less than 0.05.

RESULTS

The patients' characteristics are summarized in Table-1. The mean patients' age, testosterone level, prostate volume, PSA level and prostate specific antigen density (PSAD) were 61.4 ± 10.4 yrs (mean ± SD), 453.64 ± 167.45 ng/dL, 34.70 ± 18.04 cc, 3.96 ± 11.98 ng/mL and 0.101 ± 0.312 ng/mL/cc, respectively. The mean 2nd and 4th digit lengths and the mean digit ratio were 7.223 ± 0.467 cm, 7.625 ± 0.483 cm and 0.948 ± 0.043, respectively. Among 770 men, only five men (0.6%) had a family history of prostate cancer (first-degree relative) and 41 men (5.3%) had abnormal DRE findings. Among 770 men, 166 men (21.6%) underwent prostate biopsies and 61 men (7.9%) were found to have

Table 1 - Characteristics of the studied population.

	Mean ± SD
Age (years)	61.4 ± 10.4
2nd digit length (cm)	7.223 ± 0.467
4th digit length (cm)	7.625 ± 0.483
Digit ratio	0.948 ± 0.043
PV (cc)	34.70 ± 18.04
PSA (ng/mL)	3.96 ± 11.98
PSAD (ng/mL/cc)	0.101 ± 0.312
Testosterone (ng/dL)	453.64 ± 167.45
Family history (%)	0.6% (5/770)
Abnormal DRE (%)	5.3% (41/770)
Biopsy (%)	21.6% (166/770)
Cancer (%)	7.9% (61/770)
Cancer detection rate (%)	36.7% (61/166)

Digit ratio = 2nd digit length / 4th digit length; **PV =** prostate volume; **PSA =** prostate specific antigen; **PSAD =** prostate specific antigen density; **DRE =** digital rectal examination.

prostate cancer. Cancer detection rate of prostate biopsy was 36.7% (61/166).

Besides age, PSA and DRE, univariate and multivariate analysis showed that digit ratio was also an independent predictor of prostate cancer detection (Table-2).

Table 2 - Univariate and multivariate analysis using logistic regression model in biopsied patients (N = 166).

		Ca	Non Ca	OR (95% CI)	Univariate p-value	Multivariate p-value
Age (yrs)	≥ 65	51	67	2.893 (1.318-6.348)	0.007	0.013
	< 65	10	38			
PSA (ng/mL)	≥ 6	46	45	4.089 (2.032-8.228)	0.000	0.009
	< 6	15	60			
PV (cc)	≥35	41	74	0.859 (0.435-1.694)	0.660	
	< 35	20	31			
DRE	Abnormal	29	12	7.023 (3.208-15.376)	0.000	0.000
	Normal	32	93			
Digit ratio	< 0.95	44	50	2.847 (1.445-5.610)	0.002	0.003
	≥ 0.95	17	55			

Ca = cancer; **PSA =** prostate specific antigen; **PV =** prostate volume; **DRE =** digital rectal examination; **Digit ratio** = 2nd digit length / 4th digit length

The study subjects were allocated into two groups by digit ratio (group A: digit ratio < 0.95, n = 420; group B: digit ratio ≥ 0.95, n = 350). Despite similar biopsy rates (22.4% vs. 20.6%, p = 0.544), group A had a higher cancer detection rate (46.8% vs. 23.6%, p = 0.002; OR = 2.847, 95% CI = 1.445-5.610). However, no intergroup difference was found for age, serum testosterone level, prostate volume, PSA, DRE findings, biopsy findings and clinical stage (Tables 3 and 4).

We analyzed the 408 positive biopsy cores (group A: digit ratio < 0.95, n = 282; group B: digit ratio ≥ 0.95, n = 126). Table 5 shows the relationships between digit ratio and biopsy findings. Group A had a higher percentage of core cancer volume (46.69 ± 31.73% vs. 37.07 ± 29.43%, p = 0.005) (Table-5). The distributions of the primary Gleason scores of the positive cores were different between the two groups (Table-5). Furthermore, in group A, a significantly greater proportion of cores were found to have Gleason scores ≥ 9 (18/282 (6.4%) vs. 1/126 (0.8%), p = 0.010) and primary Gleason score = 5 (12/282 (4.3%) vs. 0/126 (0.0%), p = 0.021) (Table-5).

When we analyzed the 266 positive biopsy cores with the sum of Gleason scores ≥ 7, the distributions of sum of Gleason scores as well as primary Gleason score were different between the two groups (Table-6).

Table 3 - Comparison of the study variables between the two studied groups.

	Digit ratio < 0.95	Digit ratio ≥ 0.95	p-value
No of total patients	**420**	**350**	
Age (years)	61.7 ± 10.2	61.0 ± 10.6	0.371
Digit ratio	0.919 ± 0.024	0.983 ± 0.033	0.000
PV (cc)	34.45 ± 17.77	34.99 ± 18.37	0.681
PSA (ng/mL)	4.41 ± 12.78	3.41 ± 10.95	0.248
Abnormal DRE	24/420 (5.7%)	17/350 (4.9%)	0.598
Biopsy rate (%)	94/420 (22.4%)	72/350 (20.6%)	0.544
No of biopsy patients	**94**	**72**	
Age (yrs)	68.3 ± 7.9	69.2 ± 9.0	0.492
Digit ratio	0.918 ± 0.024	0.980 ± 0.025	0.000
PV (cc)	48.80 ± 22.51	51.23 ± 26.16	0.522
PSA (ng/mL)	16.34 ± 23.42	12.81 ± 21.80	0.323
Abnormal DRE	24/94 (25.5%)	17/72 (23.6%)	0.778
Cancer detection rate (%)	44/94 (46.8%)	17/72 (23.6%)	0.002

Digit ratio = 2nd digit length / 4th digit length; **PV =** prostate volume; **PSA =** prostate specific antigen; **DRE =** digital rectal examination.

DISCUSSION

In humans, the growth and pattern of digits and the differentiation of gonads are controlled by the homeobox genes HOXA and HOXD (2,13,14). Therefore, gonadal fetal products such as testosterone may influence finger morphology (6,15,16). For example, a high concentration of testosterone, indicating high prenatal testicular activity leads to low digit ratio (17). Recently, Lutchmaya et al. (7) showed that digit ratio is negatively associated with prenatal testosterone levels and it is positively associated with prenatal estrogen levels.

It is well known that testosterone and androgen receptors (AR) play central roles in prostate growth and the development of prostate cancer. The short CAG repeat length of the androgen receptor gene (AR) has been reported to be associated with the aetiologies of prostate cancer (9,10). Manning et al. (8) showed that the right-hand digit ratio is positively correlated with the CAG repeat number of AR.

Recently, Rahman et al. (18) reported that digit ratio is a reasonable marker for evaluation of prostate cancer risk. In their large case-control study, a higher digit ratio is related to more protective effect on prostate cancer risk, in particular, patients with age under 60 years. Considering these studies, it is highly suggestive that digit ratio may be related to prostate cancer.

In other study of the relationship between digit ratio and prostate cancer, Jung et al. (11) proposed that the 2nd to 4th digit ratio (digit ratio) of the right hand is related to PSA

Table 4 - Comparison of the study variables between the two studied groups.

	Digit ratio < 0.95	Digit ratio ≥ 0.95	p-value
No of cancer patients	**44**	**17**	
Age (yrs)	70.4 ± 6.9	72.7 ± 8.6	0.273
Digit ratio	0.921 ± 0.021	0.979 ± 0.018	0.000
PV (cc)	45.32 ± 22.38	46.99 ± 19.62	0.787
PSA (ng/mL)	26.84 ± 30.63	30.75 ± 38.56	0.679
No of positive cores	6.5 ± 3.8	7.4 ± 3.6	0.420
Max core cancer vol (%)	56.83 ± 34.08	59.29 ± 31.92	0.815
Max Sum of GS	7.1 ± 1.0	7.1 ± 1.0	0.849
Max Primary GS	3.6 ± 0.5	3.6 ± 0.5	0.867
Max Secondary GS	3.5 ± 0.7	3.5 ± 0.7	0.880
Clinical stage			
cT1	15	2	0.293
cT2	15	6	
cT3	10	6	
cT4	4	3	

Digit ratio = 2nd digit length / 4th digit length; **PV =** prostate volume; **PSA =** prostate specific antigen; **PSAD =** prostate specific antigen density; **GS =** Gleason score.

level and the presence of prostate cancer. They showed the significant negative relationships between digit ratio and PSA level and the presence of prostate cancer. However, Jung et al. (11) did not find that the prostate biopsy findings were correlated to digit ratio because the number of prostate cancer patients in their study was not sufficient to reveal the relationship of digit ratio to the biopsy findings.

The histologic grade is the most important piece of information obtained from the needle biopsy. The Gleason grading system is the most commonly used classification scheme for the histologic grading of prostate cancer (19,20). Gleason grade has been shown to correlate with the pathologic extent of disease (21-25). The presence of a Gleason pattern 4 or greater or a Gleason sum of 7 or greater is particularly predictive of a poorer prognosis. Numerous multivariate analyses support the assertion that Gleason sum is a strong predictor of the extent of prostate disease (22,23,26-28).

According to our data, only five patients had family history of prostate cancer (first-degree relative). At present, the screening of prostate cancer in Korea is not as widespread as in Western countries (29). Furthermore, among Korean men, prostate cancer accounts for 2.4% and 1.5% of the total cancer cases and deaths, respectively (30,31). Also, age-adjusted incidence and mortality rates of prostate cancer in Korea are much lower than those in most Western nations (32). Therefore, it is unusual that the prostate biopsy patients have family history of prostate cancer.

In the present study, besides age, PSA and DRE, univariate and multivariate analysis using logistic regression model showed that digit ratio is also an independent predictor of prostate can-

Table 5 - Comparison of the positive cores between the two studied groups.

		Digit ratio < 0.95	Digit ratio ≥ 0.95	p-value	OR (95% CI)
No of positive cores		282	126		
% core cancer vol		46.69 ± 31.73	37.07 ± 29.43	0.005	
No of cores	4	8	1	0.140	
with sum of GS	5	3	2		
	6	86	42		
	7	111	55		
	8	56	25		
	9	18	1		
	≤ 8	264	125	0.010	8.523 (1.125-64.562)
	≥ 9	18	1		
No of cores with primary GS	2	8	1	0.042	
	3	153	67		
	4	109	58		
	5	12	0		
	≤ 4	270	126	0.021	1.044 (1.019-1.070)
	5	12	0		
No of cores with secondary GS	2	11	3	0.150	
	3	135	74		
	4	128	48		
	5	8	1		

% = percentage; **Digit ratio =** 2nd digit length / 4th digit length; **GS =** Gleason score.

cer detection (Table-2). Therefore, it can be suggested that digit ratio is associated with prostate cancer risk.

One of the novel findings of this study was that digit ratio is related to the cancer detection rate and the aggressiveness (high percentage of core cancer volume and high Gleason score) of prostate cancer.

To date, only three studies have investigated the relationship between digit ratio and prostate cancer risk (11,18,33). Two studies have reported a strong association between 2D:4D and risk of prostate cancer (11,18). In the other study, although it is weak, an inverse association was observed between 2D:4D and risk of prostate cancer for patients aged < 60 (33). However, these studies did

Table 6 - Comparison of the positive cores with GS ≥ 7 between the two studied groups.

		Digit ratio < 0.95	Digit ratio ≥ 0.95	p-value
No of positive cores		185	81	
% core cancer vol.		57.57 ± 30.24	40.27 ± 29.95	0.000
Sum of GS		7.5 ± 0.7	7.3 ± 0.5	0.028
Primary GS		3.7 ± 0.6	3.7 ± 0.5	0.965
Secondary GS		3.8 ± 0.5	3.6 ± 0.5	0.020
No of cores with sum of GS	7	111	55	0.044
	8	56	25	
	9	18	1	
No of cores with Primary GS	3	64	23	0.025
	4	109	58	
	5	12	0	

% = percentage; Digit ratio = 2nd digit length / 4th digit length; GS = Gleason score.

not reveal the relationship between digit ratio and cancer detection rate and cancer aggressiveness.

In our study, despite similar biopsy rates (22.4% vs. 20.6%, p = 0.544), lower digit ratio group had a higher cancer detection rate than higher digit ratio group (46.8% vs. 23.6%, p = 0.002; OR = 2.847, 95% CI = 1.445-5.610) (Table-3).

Furthermore, when we analyzed the 408 positive biopsy cores (group A: digit ratio < 0.95, n = 282; group B: digit ratio ≥ 0.95, n = 126), lower digit ratio group had a higher percentage of core cancer volume than higher digit ratio group (46.69 ± 31.73% vs. 37.07 ± 29.43%, p = 0.005) (Table-5). Also, a significantly greater proportion of cores were found to have sum of Gleason score ≥ 9 (18/282 (6.4%) vs. 1/126 (0.8%), p = 0.010) and primary Gleason score = 5 (12/282 (4.3%) vs. 0/126 (0.0%), p = 0.021) in lower digit ratio group (Table-5).

In other words, most of the positive cores with sum of Gleason score ≥ 9 or all the positive cores with primary Gleason score = 5 were found in the group with a lower digit ratio rather than in the group with a higher digit ratio. These results suggest that digit ratio may predict the histologic grade as well as the cancer detection rate on prostate biopsy. We think that this is very important and it can be highly suggestive of the relationship between digit ratio and cancer aggressiveness.

According to our data, we have found that the prostate biopsy findings of each cancer patient were not related to digit ratio (Table-4). Actually, the total number of patients who had prostate cancer was 61. So, we think that the number of prostate cancer patients was not sufficient to reveal the relationship of digit ratio to the biopsy findings of the patients. However, when we considered the prostate biopsy findings of each positive core, the prostate biopsy findings of the positive cores were related to digit ratio (Tables 5 and 6). Since the total number of positive cores was 408, we think that this number is sufficient to reveal the relationship of digit ratio to the biopsy findings.

Our results show that the positive cores of the patients with a lower digit ratio have a higher percentage of core cancer volume and that a significantly greater proportion of cores with a lower digit ratio have a high Gleason score. Concluding,

digit ratio may be related to the histologic grading of prostate cancer and the extent of disease.

Digit ratio is reflective of prenatal androgen exposure and the in utero milieu. Our results show that digit ratio is related to prostate cancer, like was shown by the study of Jung et al. (11). This can be one of the evidences that prenatal androgen exposure (the in utero milieu) may be related to the later development of prostate cancer, which was already proposed by Henderson et al. (34) in 1988.

CONCLUSIONS

Our results showed that a lower digit ratio is related to an increasing probability of detection of prostate cancer, a high percentage of core cancer volume and a high Gleason score. These findings mean that patients with a lower digit ratio have a higher chance of developing prostate cancer and they might have more aggressive prostate cancer.

CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Initial Brazilian Experience in the Treatment of Localized Prostate Cancer Using A New Generation Cryotechnology: Feasibility Study

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ABSTRACT

Introduction: The objective of our study is to present the first Brazilian cryoablation experience in the treatment of low and intermediate risk localized prostate cancer using 3rd generation cryoablation and real-time biplanar transrectal ultrasonography.
Materials and Methods: Ten Brazilian patients underwent primary cryoablation for localized prostate cancer between October 2010 and June 2011. All patients consented for whole gland primary cryotherapy. The procedures were performed by 3rd generation cryoablation with the Cryocare System® (Endocare, Irvine, California). Preoperative data collection included patient demographics along with prostate gland size, Gleason score, serum prostate specific antigen, and erectile function status. Operative and post-operative assessment involved estimated blood loss, operative time, complications, serum PSA level, erectile function status, urinary incontinence, biochemical disease free survival (BDFS), and follow-up time.
Results: All patients in the study successfully underwent whole gland cryoablation. The mean of: age, prostate size, PSA level, and Gleason score, was 66.2 years old; 40.7g; 7.8ng/mL; and 6 respectively. All patients were classified as low or moderate D'Amico risk (5 low and 5 moderate). Erectile dysfunction was present in 50% of patients. The estimated blood loss was minimal, operative time was 46.1 minutes. All patients that developed erectile dysfunction post-treatment responded to oral or intracavernosal medications with early penile rehabilitation. All patients maintained urinary continence by the end of a 10 months evaluation period and none had biochemical relapse within the mean follow-up of 13 months (7-15 months).
Conclusion: Our initial experience shows that cryoablation is a minimally invasive option for the treatment of localized prostate cancer. Short term data seems to be promising but longer follow-up is necessary to verify oncological and functional results.

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INTRODUCTION

Cryoablation is an accepted minimally invasive surgical option for the treatment of localized prostate cancer (1). It is estimated that in the United States more than 6,500 cryoablation procedures were performed in 2005, and > 15,000 procedures in 2010 (2).

Currently, primary cryoablation is indicated to all patients that are eligible to other treatment

modalities, including clinically localized disease in younger and low morbidity patients. Due to technical refinements such as improved ultrasonography imaging and routine use of urethral warmers and small gauge needle delivery systems, complication rates have significantly decreased and renewed the interest in this treatment modality (2-5).

Cryoablation triggers cell apoptosis with continuing cellular destructive mechanism and intracellular osmotic injury (6). The indirect mechanisms involve thrombosis, ischemia - reperfusion injury, and programmed cell death. Reports from the US and European academic centers demonstrated that cryosurgery for the treatment of prostate and renal cancer offers good oncological and functional outcomes and is gaining acceptance worldwide (7,8). In Brazil, the Agência Nacional de Vigilância Sanitária (ANVISA) approved the cryoablation technology in 2007 under the following registration codes: 80181930029 and 80181930032. The objective of our study is to present the first Brazilian cryoablation experience in the treatment of low and intermediate risk localized prostate cancer.

MATERIALS AND METHODS

A total of 10 male patients underwent primary cryoablation for localized prostate cancer between October 2010 and June 2011 and included in the study after institutional review board approval; an informed consent was obtained. All patients had biopsy-proven prostate cancer and were classified as low to intermediate risk according to D'Amico risk stratification. Preoperative data collection included patient demographic data, prostate gland size, Gleason score, serum prostate specific antigen (PSA), and potency.

Operative and postoperative assessment included estimated blood loss, operative time, complications, serum PSA, potency, urinary incontinence, biochemical disease free survival (BDFS), and clinical follow-up. Beginning at 3 months urinary incontinence and erectile dysfunction were assessed. Incontinence was defined as uncontrolled leakage of urine through the urethra requiring at least one male incontinence pad. Potency was diagnosed by a self-reported ability to achieve erection for intercourse without any pharmaceutical or vacuum device assistance. Biochemical disease free survival was determined using American Society of Therapeutic Radiation Oncology (ASTRO) criteria of three consecutive rises in PSA level and with the Phoenix criteria of PSA nadir plus 2 ng/mL. Complications were graded using the Clavien-Dindo scale (9). Clavien I-II complications were considered minor while Clavien III-V were classified as major complication. Data collection and statistics used Microsoft Excel (Microsoft Corporation, Redmond, WA). Data were reported as mean ± standard deviation and frequency (percentage of total).

All procedures were performed with Cryocare System® (Endocare, Irvine, California). Cryoablation involved a dual freeze-active thaw cycles using 8-17 gauge cryoprobes with helium and argon gas. The patients were placed in a lithotomy position and cryoablation was performed under general anesthesia. Cryoprobes and thermocouples were placed with assistance of a brachytherapy grid guided by transrectal ultrasound (TRUS) in both the sagittal and the transverse planes. Thermoprobes were placed at the urinary sphincter and Denonvillier's fascia. The urinary sphincter and Denonvilliers thermocouples monitored temperatures close to 0° C to protect the anterior rectal wall and urinary sphincter. Cystoscopy was conducted to confirm correct cryoprobes placement and that the needles did not penetrate into the urethra or bladder. A urethral warming catheter set at 42° C protected the urethra for the entirety of the procedure. A Foley catheter was placed following cryoablation. Patients were discharged within 24hrs. post-cryoablation with the Foley catheter. On postoperative day 5 the catheter was removed. Patients returned for follow-up 1 month after the procedure, and every 3 months thereafter. Serum PSA was collected at every visit. Penile rehabilitation was performed in patients without preoperative erectile dysfunction. These patients were encouraged to use daily Phosphodiesterase 5 inhibitors (PDE 5 i), i.e., Tadalafil 5mg and a vacuum erectile device twice a day.

RESULTS

All patients in the study successfully underwent whole gland cryoablation as primary

treatment for localized prostate adenocarcinoma. Table-1 shows patient demographics and preoperative disease characteristics. Mean age was 66.2 years old. Average prostate size was 40.7 grams. Mean PSA was 7.8 ng/dL and mean Gleason Score was 6. Patients were evenly distributed between low and moderate according D'Amico Score. Half of the patients were defined as potent preoperatively. Mean follow-up for all patients was 13 months (7-15 months). Surgical outcomes are presented in Table-2. Blood loss was minimal in all procedures and operative time was < 60 minutes. Figure-1 depicts changes in PSA level; pre versus post-operative PSA for each patient and the average PSA level (ng/mL). Of note, the highest postoperative PSA (1.44ng/mL) occurred in the patient with the largest prostate (70 grams) whose initial PSA was 5.51ng/mL. There was no biochemical relapse observed during this short-term clinical follow-up. All patients who underwent penile rehabilitation were able to achieve erection for penetration with pharmacological therapy. Two patients required PDE 5 inhibitors alone while the remaining three patients responded to intracavernosal therapy.

No intraoperative complications were observed and all patients were discharged within 24 hours post procedure. Table-3 demonstrates postoperative complications. All complications were

Table 1 - Patient Demographic and Pre-operative Data.

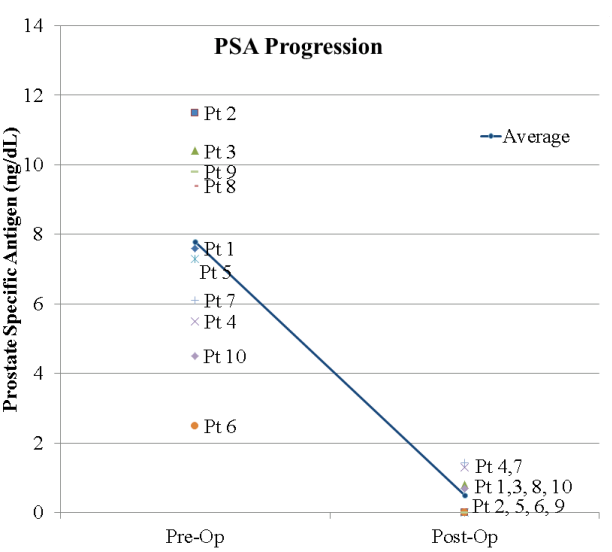
Age (yr)	66.2 ± 10.8
Size (g)	40.7 ± 16.4
PSA (ng/dL)	7.8 ± 2.8
Gleason	
6	6 (60%)
7	4 (40%)
D'Amico	
Low	5 (50.0%)
Moderate	5 (50.0%)
High	0 (0.0%)
Potency	5 (50.0%)

Table 2 - Surgical and Post-Operative Data.

EBL	Minimal
ORT (min)	46.1 ± 3.3
Post-Operative PSA (mg/dL)	0.54 ± 0.64
Potency	
Unresponsive to Meds	0 (0.0%)
Responsive to Oral Meds	2 (40.0%)
Responsive to Oral or ICI Meds	5 (100.0%)
BDFS	10 (100.0%)
Follow-up Time (m)	13.0 ± 4.5

EBL = Estimated Blood Loss; **ORT** = Operative Time; Meds = Medications; **CI** = Intracavernosal Injection; BDFS = Biochemical Disease Free Survival

Figure 1 - Changes in PSA; pre versus post-operative PSA for each patient and the average PSA level (ng/mL).



Clavien Grade I. Hematuria was observed in 3 patients. Two of the patients with hematuria were associated with coagulation issues, i.e., low platelet count and liver dysfunction. Complete resolution of hematuria occurred after medical therapy and clot evacuation. Two patients were diagnosed with urinary retention at 2 weeks postop and required Foley catheterization for an additional week. Both

Table 3 - Complications.

Complications	Clavien Grade	Frequency	Management	Resolution
Dysuria	I	1 (10.0%)	Observation	6 m
Hematuria	I	3 (30.0%)	clot evacuation	1 week
Perineal Pain	I	1 (10.0%)	Observation	-
Scrotal Hematoma	I	1 (10.0%)	scrotal support	1 week
Urinary Retention	I	2 (20.0%)	Foley catheter	1 week

patients voided without issues 3 weeks post-surgery. Scrotal hematoma was noted in one patient who was treated with a scrotal support and analgesic and resolved in 1 week. One patient had dysuria that was managed with reassurance and observation, and completely resolved at 6 months post-op.

DISCUSSION

Cryosurgery for prostate cancer was first applied in 1964 by Gonder et al. using liquid nitrogen (10). The technique encompassed transurethral freezing of the prostate without the proper technology to position the cryoprobes adequately and to monitor the extension of the ice ball. This resulted in severe and frequent complications such as urinary incontinence, urethral sloughing and recto-urethral fistulae. After 1980, Onik et al. refined the technique by using interventional radiological procedures and transrectal ultrasound (11). The TRUS - guided transperineal placement of cryoprobes with real - time monitoring and control of the freezing process has significantly decreased the complications (12,13).

Since the use of urethral warmers, thermocouples in Denonvillier´s fascia and neurovascular bundles and the application of gas - based cryosurgery, complication rates have further decreased (14,15). The introduction of argon gas for freezing and helium gas for thawing permitted a dramatic reduction in the diameter of the cryoprobes. The ultrathin 17 - gauge (1.47 mm) cryoneedles have a very sharp tip and because of the smaller diameter, more needles can be placed. This permits a precise contouring of the ice ball, subsequently resulting in a more effective ablation of the gland (16). These developments have significantly minimized the scrotal swelling and perineal ecchymosis that occurred after the procedure (17). By active instead of passive thawing the procedure can be performed much quicker. Most patients are discharged from the hospital either the same day or the following day after treatment (7).

The use of a urethral - warming catheter decreased the sloughing rate of the urethral mucosa and subsequently the risk of obstructive problems (2,5). Consequently, cryosurgery was recognized by the American Urological Association (AUA) as a therapeutic option for localized prostate cancer in 1996. Cryoablation is recognized as an established minimally invasive procedure for the treatment of localized prostate cancer in both the primary and salvage setting (18-20). The Brazilian Society of Urology initially determined cryoablation an experimental technique. The newest Brazilian recommendation published in 2011 ascertained the 2009 decision that cryoablation is a treatment option for localized prostate cancer, especially those with comorbidities (21).

While new advances in technology for the treatment of prostate cancer can be very costly with little clinical data to support large financial investment, i.e, robotic radical prostatectomy, cryotechnology may be a cost effective treatment modality, saving the institution and the patient significant resources (22). The evolution of cryoablation has further decreased the learning curve for procedure. The percutaneous needle ablation simplifies surgery compared to other therapies such as open, laparoscopic or robotic prostatectomy.

Outcomes from the Cryo On-Line Data registry reported that among the 1.198 consecutive patients who underwent primary cryoablation, 3.6% of patients developed urinary retention and 0.4% rates of recto-urethral fistulas (23). In our study, no recto-urethral fistula was observed. Two patients developed acute urinary retention that resolved after prolonged urethral catheterization.

Biochemical failure after cryoablation of prostate requires re-biopsy of the prostate. Other Brazilian investigators (Hayek et al.) published their recurrence rates with cryoablation in 21 high-risk patients in 2007 defining biochemical failure as PSA > 1.0 ng/mL using an older cryo system. The PSA failure rate was 39%, 52.9%, and 42.8% at 12, 24, and 60 months of follow-up, respectively. In that study 12 (57.2%) patients had biochemical failure, while 7 (58.3%) of these patients had positive prostate re-biopsy. They concluded that prostate cryoablation is a minimally invasive treatment with promising results, resulting in low complication rates for high risk patients with prostate cancer (24). Jones et al. using the COLD registry database reported a 5-year BDFS in 1.198 patients using the Phoenix criteria of 91%, 78%, and 62% for low, moderate, and high risk respectively (23). Cohen et al. demonstrated a 10 year BDFS of 80.6%, 74.2%, and 45.5% for low, moderate, and high risk patients using the Phoenix criteria (25). A randomized trial comparing radiation and cryoablation for patients with localized prostate cancer showed similar results regarding BDFS and overall survival after 3 years of follow-up between the two modalities (26). In our series, we demonstrate the first series of Brazilian patients with low and intermediate risk prostate cancer treated with cryoablative technology. Using ASTRO and Phoenix criteria, our short-term follow-up revealed no biochemical recurrence.

Reported incidence of urinary incontinence varies significantly due to lack of standard definition. Past studies have shown that the incidence of incontinence with modern cryotherapy may range from 1.3% to 7.5%. Jones and Long reported in their series a total incontinence rate of 4.8% with 2.9% of patients requiring pads (24,27). The 0% urinary incontinence rate (no pads) in our present series may be due to a smaller group of patients and a biased population with low risk and healthier medical status. Two patients that presented with mild acute urinary incontinence post-surgery resolved spontaneously and/or with biofeedback therapy within 30 days post-op.

Historically, investigators believed that prostate cryoablation would permanently cause erectile dysfunction in all patients. Impotence rates vary in the literature. Asterling et al. published a prospective evaluation of sexual function in 53 patients who underwent cryosurgery as a primary treatment for prostate cancer with 39% of return of sexual function (28). Additionally, Lambert et al. reported up to 71% return of potency in their study (29). Ellis et al. emphasized the application of penile rehabilitation post cryotherapy to achieve erection for intercourse with or without oral pharmacological agents. In their series, the return of potency rates was 41.5% and 51.3% of patients at 12 months and 48 months post cryo treatment respectively (30). In the only randomized trial comparing cryoablation with external beam radiation for localized prostate cancer, Donnely et al. demonstrated equivalent results between cryoablation and radiation after a follow-up of 3 years (26). Although our study had relatively few patients, 50% of these patients had erectile dysfunction before treatment. The underlying causes for this were not explored with the patients, but more men with localized prostate cancer have reported sexual problems than similarly aged men without prostate cancer in comparison group studies (31,32). Some of these problems appear to be disease-linked, regardless of the type of treatment used (33). In our series, we performed early penile rehabilitation in patients that were potent pre-operatively and desired return of sexual function post-op. Interestingly, all patients that underwent post procedure penile rehabilitation were able to have sexual intercourse with penetration with pharmacological aid.

Technological and procedural modifications of cryoablation have led to a new era of cryotherapy for the treatment of prostate cancer. Indications and complications due to prostate cryoablation evolved offering a new minimally invasive modality to manage localized prostate

cancer with minimal to no required hospital stay, acceptable quality of life and oncological outcome. Worldwide medical practices have adapted new technological advances with caution and especially nowadays they must consider adequacy of treatment including cost-effectiveness, quality of life and oncological outcomes based on scientific evidence for the treatment of localized prostate cancer.

This study has several limitations to be considered. The study is retrospective and has a very small number of patients. Oncological data still requires longer follow-up for a more robust conclusion. We did not use a validated questionnaire to access erectile dysfunction. However, this is an initial experience that demonstrates the successful treatment of localized prostate cancer by 3rd generation cryoablation in Brazil.

CONCLUSIONS

Cryoablation is an accepted minimally invasive surgical option for the treatment of localized prostate cancer worldwide. Our initial results concur with current clinical data available since 10 patients were treated for minor complications. Third generation cryoablation has a low learning curve. Short term data seems to be promising but longer follow-up is necessary to verify oncological and functional results.

CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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The accuracy of multidetector Computed Tomography for preoperative staging of renal cell carcinoma

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ABSTRACT

Purpose: The purpose of this study was to evaluate the accuracy of multidetector computed tomography (MDCT) in the preoperative staging of renal cell carcinoma (RCC).
Materials and Methods: We retrospectively reviewed the clinical and pathological records of 312 patients with RCC who underwent staging MDCT before surgery. Radiographic findings were compared to the findings at surgery and pathological examination. All staging used 2009 updated TNM classification.
Results: The difference in tumor size between radiographic and pathological findings was 0.21cm. In T1a group, the difference was 0.33cm. Agreement between MDCT and histopathological findings was moderate for T staging (Kappa = 0.469), fair for N staging (Kappa = 0.322), and excellent for M staging (Kappa = 0.932). The sensitivity and specificity of MDCT in detecting perinephric fat invasion were 32.26% and 85.87%, in detecting tumor thrombosis were 84% and 100%, in detecting adrenal gland invasion were 60% and 95.79%, in detecting lymph node involvement were 50% and 96.36%, in detecting distant metastasis were 100% and 99.67%, respectively. In regard to stage grouping, 237 of 314 patients were correctly staged by MDCT, with an overall accuracy of 75.48%.
Conclusions: MDCT with a dynamic contrast protocol is able to delineate RCC with high accuracy. However, a great portion of tumors were overstaged by MDCT because of overestimation of tumor size and poor visualization of infiltration of the perinephric fat. In addition, nodal metastatic lesion evaluation relies on node size only and remains a difficult task.

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INTRODUCTION

Renal cell carcinoma (RCC) is the most common primary renal malignant neoplasm in adults. It accounts for approximately 90% of renal tumors and 3.8% of all adult malignancies.1 Approximately 58,240 new cases of RCC and 13,040 deaths are expected to have occurred in the United States in 2010 (1). With the increasing use of cross-sectional imaging modalities, incidence of serendipitously discovered RCC has risen dramatically (2). Of the incidentally discovered tumors, a prominent proportion were considered small (< 4 cm) (2).

Although radical nephrectomy remains the standard treatment for both localized and advanced RCC in patients with a normal contralateral kidney, surgical techniques have evolved over the years. Currently, nephron sparing surgery (NSS) has been proposed in the treatment of patients with localized small tumor (3). Therefore, detailed preoperative information regarding

tumor location, size, organ confinement, presence and extent of tumor thrombus in vena cava, lymph node involvement and visceral metastasis are important for planning of surgical approach and providing accurate prognostic information for patient.

Staging of RCC is the most important factor affecting the prognosis and survival of patients. Currently, the most applied staging system for RCC is TNM classification including the most prominent histopathological features, such as tumor size, tumor extension and tumor thrombus. In preoperative staging of the RCC, imaging modalities are expected to adequately evaluate these parameters. Although a variety of examinations (ultrasound [US], magnetic resonance imaging [MRI], angiography) can be used in the workup of patients with suspected RCC, the preferred method of imaging these patients is dedicated renal computed tomography (CT) (4). Since the introduction of multidetector computed tomography (MDCT) in late 1990s, it has won popularity in preoperative imaging of RCC for its high spatial resolution, high speed of acquisition and imaging reformatting in any plans which can provide excellent anatomical details (5,6).

The aim of the present study was to evaluate the accuracy of MDCT in preoperative staging of RCC, by taking the postoperative histopathological staging as the reference method.

MATERIALS AND METHODS

We retrospectively reviewed the clinical and pathological records of patients with RCC who underwent radical nephrectomy or NSS between January 2008 and June 2010. All patients who had triphasic enhanced MDCT scan done at our institution within two weeks prior to surgery were included. Patient with cystic lesion, preoperative arterial embolization, positive surgical margins or known hereditary disease such as Von Hippel-Lindau and tuberous sclerosis were excluded. Papillary RCC was defined as a tumor with largest diameter larger than 5 mm and those of less than 5 mm considered papillary adenoma were excluded. In patients with multiple unilateral tumors, the largest tumor was included. When tumors were found in bilateral kidneys, both were taken into consideration. Both radiographic and histopathologic staging used 2009 updated TNM classification.

MDCT imaging

All MDCT scans were performed using a 64-slice MDCT scanner (Philips Brilliance, Germany) with a 0.5 second gantry rotation speed, a tube voltage of 120 KV, and a tube current of 250 mAs. In all patients, four phases image were obtained: an unenhanced scan from the thorax to the kidney to identify possible lung metastasis, renal calcification and intratumoural fat; a arterial phase from diaphragm to lower pole to evaluate the renal cortex, renal arteries, and tumor vascularization; a parenchymal phase from diaphragm to lower pole to detect small lesions and assess renal venous drainage; and a excretory delayed phase from lower pole to bladder to evaluate the relationship between the tumor and collecting system.

When performing unenhanced CT scan, a collimation of 5 mm, thickness of 5 mm, a table speed of 5 mm per revolution, and an image reconstruction interval of 5 mm were used. Contrast enhanced scanning was performed using collimation of 5 mm, thickness of 2 mm, reconstruction interval of 1 mm, and a table speed of 5 mm. For each study, 120-200 mL (2 mL/kg) of iodinated contrast agent (Iopromide, Ultravist 320 mgI/mL, Bayer Schering Pharma AG, Guangzhou, China) was injected intravenously at 3 mL/sec through an antecubital vein. Arterial-phase and parenchymal venous phase images were obtained after a 30-second delay and a 60-second delay, respectively. The excretory phase was acquired 5 minutes after the beginning of the injection.

Image evaluation

CT scan examinations were re-reviewed in a purposeful manner, by a single experienced radiologist who was unaware of the histopathological results. Tumor staging included the following parameters: tumor location; tumor diameter (the largest of craniocaudal, anterioposterior, and transverse planes was defined as the radiological size), invasion of perinephric fat (determined

by the presence of small hyperdense strands and nodules surrounding the lesion); involvement of the adrenal gland or satellite lesions within the Gerota's fascia; presence and extent of tumor thrombus, lymph node involvement and visceral metastasis. Renal hilar, paraaortic, and paracaval lymph nodes with short-axis diameter > 1 cm were considered to be positive.

Surgical Results

All histopathological specimens were reviewed by urological pathologists and histological subtype was classified following the 2004 WHO classification of RCCs. All tumors were graded according to the 1982 Fuhrman grading system. Pathological size was defined as the maximal transaxial diameter on specimen.

Statistical Analysis

All radiographic findings were compared with operative and pathological findings. Tumor size was analyzed as a continuously variable and analyzed by either the two-tailed Student t test or one-way analysis of variance, when appropriate. Agreement between the two staging systems was determined using the kappa statistic (0.00-0.20, poor; 0.20-0.40, fair; 0.40-0.60, moderate; 0.60-0.80, good; and 0.80-1.00, excellent). Statistical analysis was performed using SPSS software package version 16.0 (Statistical Package for Social ScienceTM, Chicago, IL, USA) and $p < 0.05$ was considered to be statistical significant.

RESULTS

Surgical findings

312 patients with histopathologically confirmed 314 RCC were included: in two men, bilateral tumors were found. The mean age of all included patients was 54.99 ± 1.41 years old (range, 10-83 year). Patients with both bilateral tumors and another 91 patients underwent NSS and 219 patients were submitted to unilateral radical nephrectomy. Histological characteristic was evaluated in all RCC, revealing the following results: clear cell (n = 285), papillary cell (n = 12), chromophobe (n = 10), unclassified (n = 3), multiple cystic (n = 3), XP11.2 translocation (n = 1). Fuhrman grade I (n=11), grade II (n = 154), grade III (n = 121), grade IV (n = 27). The mean pathological tumor size was 4.92 ± 2.58 cm. The mean size of T1a tumors (n = 158) was 3.14 ± 0.77 cm, of T1b tumors (n = 87) 5.47 ± 0.68cm, of T2a tumors (n = 20) 8.47 ± 0.95 cm, of T2b tumors (n = 8) 11.38 ± 1.09 cm, of T3a tumors (n = 30) 7.56 ± 3.92 cm, of T3b tumors (n = 4) 7.75 ± 2.84cm, of T3c tumors (n = 2) 7.75 ± 1.06 cm, of T4 tumors (n = 5) 8.20 ± 3.09 cm. There were 31 tumors with perinephric fat invasion (9.87%), 25 tumors with renal vein or vena cava thrombosis (7.96%), 5 tumors with adrenal involvement (1.59%). 12 tumors were detected with lymph node invasion (3.82%) (4 N1, 8 N2). Metastatic lesions were found in 7 tumors with three in lung, two in vertebrae or ribs and two in liver.

MDCT findings

The mean radiographic size of all included tumors was 5.13 ± 2.52 cm, 0.21 cm larger than the mean pathological size (P = 0.001). Primary T1 and T2 RCC are defined as tumors limited to the kidney. As pointed out by Catalano (7), presence of a well-defined pseudocapsule is an important finding to predict confined renal tumor (Figure-1). In the T1a group, the increment of tumor size was 0.33 cm ($P < 0.001$). 124 of 314 tumors were staged as T1a (34.59%), 75 tumors as T1b (23.89%), 24 tumors as T2a (7.64%), 3 tumors as T2b (0.96%), 69 tumors as T3a (21.97%), 1 tumors as T3b (0.32%), 1 tumor as T3c (0.32%), 17 tumors as T4 (5.41%). With respect to primary T staging, 196 tumors were correctly staged by MDCT and the overall accuracy was 62.42% (Table-1). In T1a tumors, 45/158 were overstaged; in T1b tumors, 34/87 were overstaged, 10/87 were understaged; in T2a tumors, 7/20 were overstaged, 2/20 were understaged; in T2b tumors, 4/8 were overstaged, 2/8 were understaged; in T3a tumors, 4/30 were overstaged, 5/30 were understaged; in T3b tumors, 3/4 were overstaged; in T3c tumors, 1/2 were understaged; in T4 tumors, 1/5 were understaged.

Evidence of perinephric fat invasion was presented in 50 tumors on image evaluation

Table 1 - Histopathological information of all included tumors.

	No. tumors	Tumor size(cm)
Included tumors	314	4.92 ± 2.58
Histopathological subgroup		
Clear cell	285	
Papillary cell	12	
Chromophobe	10	
Unclassified	3	
Multiple cystic	3	
XP11.2 translocation	1	
Fuhrman grade		
I	11	
II	154	
III	121	
IV	27	
Primary T stage		
T1a	158	3.14 ± 0.77
T1b	87	5.47 ± 0.68
T2a	20	8.47 ± 0.95
T2b	8	11.38 ± 1.09
T3a	30	7.56 ± 3.92
T3b	4	7.75 ± 2.84
T3c	2	7.75 ± 1.06
T4	5	8.20 ± 3.09

(Figure-2) (Table-2); only 10 were confirmed by specimen examination. In the patients with tumor thrombosis, MDCT was able to correctly identify and localize the presence and level of the thrombus in 21 patients. Of which, 13 were of thrombosis in renal vein (Figure-3), 7 in inferior vena cava (Figure-4) and 1 in inferior vena cava and pulmonary vein. Though focal enhancement of venous wall or infiltration of adjacent soft tissue is suggestive of venous wall infiltration, especially in vena cava, MDCT failed to detect another 4 tumors with venous wall invasion (2 in renal vein, 2 in inferior vena cava). Direct invasion of ipsilateral adrenal gland was suspected in 16 patients on MDCT (Table-2) and only 3 had tumor involvement on specimen. Another two patients with ipsilateral adrenal gland invasion were not detected on imaging evaluation.

In the evaluation of lymph node involvement which included renal hilar, paraaortic, or paracaval lymph nodes, 297 tumors were staged

Figure 1 - Confined renal tumor with a well-defined pseudocapsule (arrow).

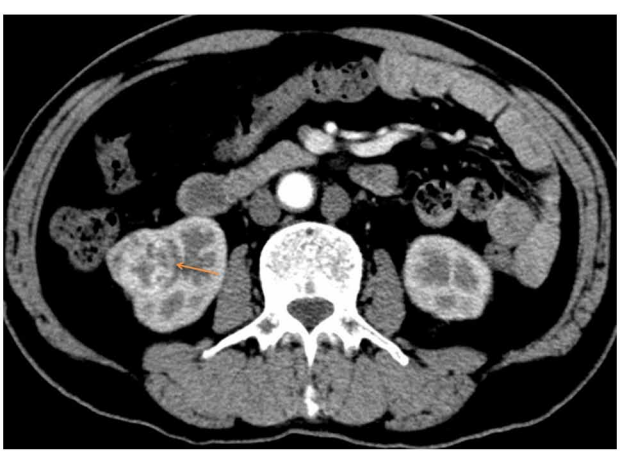


Figure 2 - Renal tumor with prinephric fat invasion, the presence of small hyperdense strands and nodules surrounding the lesion (arrow).

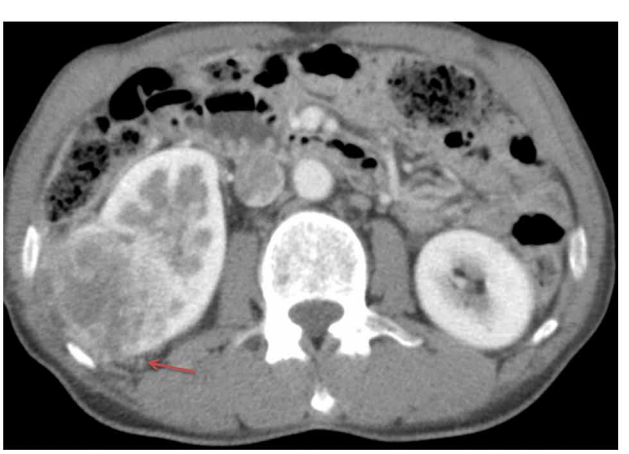


Table 2 - Histopathology and multidetector computed tomography (MDCT) staging of tumors (T).

		MDCT								Total
		T1a	T1b	T2a	T2b	T3a	T3b	T3c	T4	
Histo-pathologic	T1a	113	27	1	0	17	0	0	0	158
	T1b	10	43	9	0	20	0	0	5	87
	T2a	0	2	11	1	6	0	0	0	20
	T2b	0	0	2	2	3	0	0	1	8
	T3a	1	3	1	0	21	0	0	4	30
	T3b	0	0	0	0	0	1	0	3	4
	T3c	0	0	0	0	1	0	1	0	2
	T4	0	0	0	0	1	0	0	4	5
Total		**124**	**75**	**24**	**3**	**69**	**1**	**1**	**17**	**314**

Figure 3 - A) Thrombus in right renal vein (Red arrow); B) Thrombus in right renal vein (Blue arrow).

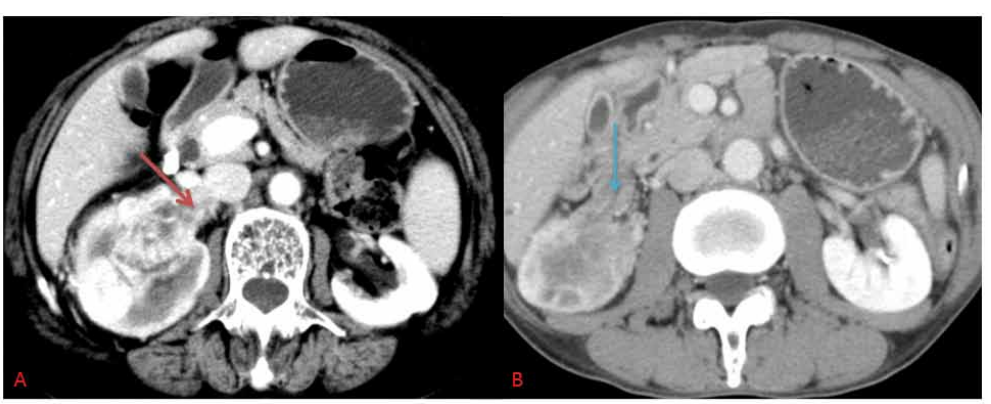
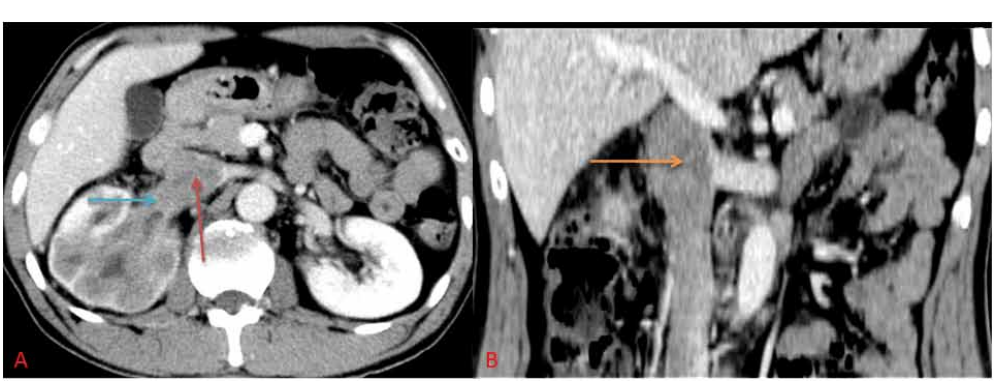


Figure 4 - A 50 year old male with renal cell carcinoma. A) Contrast enhanced axial scan thrombus in right renal vein (Blue arrow) and inferior vena cava (Red arrow). B) 3D reconstruction image. thrombus in interior in inferior vena cava (Orange arrow).



N0, 8 tumors were staged N1 and 9 tumors were staged N2 by MDCT(Table-3). 295 tumors (93.95%) were correctly staged. 11 tumors (3.5%) were overstaged and 8 tumors (2.55%) were understaged. In the 11 tumors with false-positive lymph nodes involvement by MDCT, the nodes were larger than 1 cm in short-axis diameter but were characterized as reactive hyperplasia on pathology. In the 6 tumors with false negative lymph nodes, microfocuses of cancer cell metastasis were identified. Similarly, another two tumors staged N2 were understaged as N1 by CT scan because malignant cell were also found in more than one paraaortic lymph node even with a diameter less than 1 cm. With respect to evaluation of distant metastatic disease, 8 patients were suspected in arterial phase or parachymal phase and all were confirmed by pathological examination (Table-4) (Figure-5).

Statistical findings

Agreement between MDCT and histopathologic findings was moderate for T staging (Kappa = 0.469), fair for N staging (Kappa = 0.322), excellent for M staging (Kappa = 0.932), fair for stage grouping (Kappa = 0.502). 237 of 314 patients were correctly staged by MDCT, with an overall accuracy of 75.48%. The sensitivity and specificity of MDCT in detecting perinephric fat invasion were 32.26% and 85.87%, in detecting venous thrombosis were 84% and 100%, in detecting adrenal gland invasion were 60% and 95.79%, in detecting lymph node involvement were 50% and 96.36%, in detecting distant metastasis were 100% and 99.67%. In stage grouping, 237 of 314 patients were correctly staged by MDCT, with an overall accuracy of 75.48%.

DISCUSSION

Since nephrectomy is still the only curative method in the treatment of RCCs, preoperative evaluation of RCCs is of great importance. MDCT now serves as the most preferable imaging modality in determining tumor location, tumor size, tumor extension, thrombosis, lymph node

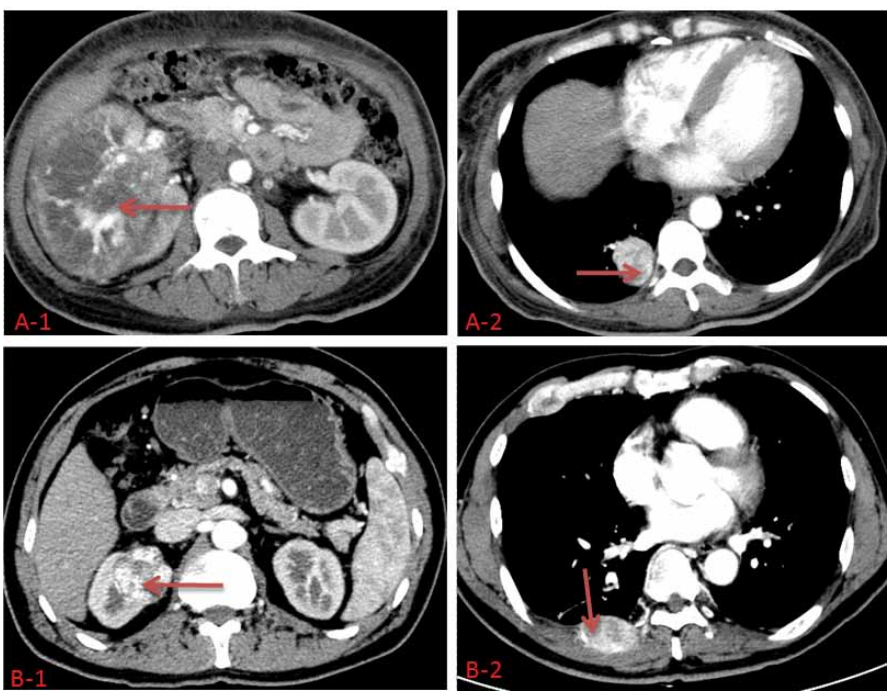
Table 3 - Histopathology and multidetector computed tomography (MDCT) staging of perinephric fat invasion, adrenal invasion and tumor thrombus.

Perinephric fat invasion		MDCT		Total	Adrenal invasion		MDCT		Total	Tumor thrombus		MDCT		Total
		No	Yes				No	Yes				No	Yes	
Histopathology	No	243	40	283	Histopathology	No	296	13	309	Histopathology	No	289	0	289
	Yes	21	10	31		Yes	2	3	5		Yes	4	21	25
Total		**264**	**50**	**314**	**Total**		**298**	**16**	**314**	**Total**		**293**	**21**	**314**

Table 4 - Histopathology and multidetector computed tomography (MDCT) staging of nodal (N) and distant metastasis (M).

Nodal metastasis		MDCT			Total	Distant metastasis		MDCT		Total
		N0	N1	N2				M0	M1	
Histopathology	N0	291	6	5	302	Histopathology	M0	306	1	307
	N1	4	0	0	4		M1	0	7	7
	N2	2	2	4	8					
Total		**297**	**8**	**9**	**314**	**Total**		**306**	**8**	**314**

Figure 5 - A) A 57 year old female with renal cell carcinoma. The left is the kidney tumor (A-1) the right is the lung metastasis (A-2). B) A 52 year old male with renal cell carcinoma. The left is the kidney tumor (B-1), the right is the metastasis in ribs (B-2).



involvement and distant metastasis. Previous studies have demonstrated that the accuracy of MDCT for detection and staging of renal mass is up to 90% (7), however, it is not of limitation.

Tumor size is known as the primary component of the 2009 updated TNM classification and an important prognostic variable for RCC. NSS or partial nephrectomy is now recommended to patients with small localized tumors (< 4 cm) (2), and that preoperative radiographical size estimation is an essential parameter to select the appropriate treatment for RCC. Although CT measurement of the renal tumor size correlates well with the actual size of the tumor, CT scan tends to overestimate the tumor size (8,9). We have found an average overestimation of 0.21 cm on CT scan with significant difference ($p < 0.001$). In T1a group, the overestimation was even greater, of 0.33 cm ($p < 0.001$). This may be the most reasonable explanation to the fact that 28 of 158 (17.72%) T1a tumors were overstaged as T1b and 9 of 87 (10.34%) T1b tumors overstaged as T2a. Similarly, Nazim found 10 of 14 (71.43%) T1a tumors were overstaged as T1b and 14 of 44 (31.82%) T1b tumors were overstaged as T2a (10). Kanofsky also reported that the overestimation in tumor size was enough to upstage the tumor by TNM system in 16% of clear cell RCCs (11). Since a large portion of patients were overstaged preoperatively, these nephron sparing approaches should be considered in patients with tumor size slightly larger than 4 cm on CT scan without any other metastatic sign.

In prior studies, it has been shown that imaging using CT had low accuracy rates for the detection of perinephric tumor extension, as stranding in the perinephric fat is non-specific and can be due to edema, vascular engorgement or previous inflammation (12,13). The presence of enhancing nodules in the perinephric fat is now considered the most reliable finding of perinephric invasion (12). Comparing with the spiral CT used before, MDCT has proved to have higher spatial resolution and better anatomy detail delineation.

Catalano reported that MDCT had 95% accuracy for perinephric fat infiltration with sensitivity of 96% and specificity of 93% (7). However, even with MDCT and three dimensional technology, Hallscheidt and Türkvatan suggested the evaluation of renal tumor extension in to perinephric fat remains a difficult task (14,15). Türkvatan reported that 1 of 26 T1 tumors and 4 of 11 T2 tumors were overstaged as T3a. In our study, 17 of 158 T1a tumors, 20 of 80 T1b tumors, 6 of 20 T2a tumors, 3 of 8 T2b tumors were overstaged as T3a. As indicated by a recent retrospective analysis of 5339 patients, 5 years cancer specific survival was 94.9% in pT1a, 92.6% in pT1b, 85.4% in pT2a, 70% in pT2b and 64.7% in pT3a (16), and patients with different staged tumors may require different treatments. Accurate stage of pT1a tumors is essential because infiltration to the perinephric fat is a contraindication to NSS. NSS is most appropriate for tumors located over the upper or lower pole or in a peripheral location and with a clear demarcation to the renal vasculature and collecting system. In our study, 158 patients with pT1a tumors; 83 were submitted to radical nephrectomy, indicating that 52.54% patients were overtreated, and 43.37% of them were due to overstaging. However, although perinephric invasion characterized by perinephric stranding and enhancing nodule in perinephric fat have not a good sensitivity and accuracy, it still should be reserved in imaging evaluation, because understaged tumors receiving more conservative therapy may lead to disastrous clinical outcome.

Approximately 23% of RCC invade the renal veins and 7% invade the inferior vena cava (17). Accurate definition of the presence and level of tumor thrombus preoperatively is critical for surgical planning and patient counseling. Patients with the level of tumor thrombus located inferior to the diaphragm only require laparotomy, while the detection of supradiaphragmatic extension will require a thoracoabdominal surgical approach. Although MRI has been proved superior to other modalities in tumor thrombus detecting and predicting the tumor thrombus level (18,19), it is not easily available and not proper for patients with pacemaker or altered cardio-pulmonary function. In a prospective study, Hallscheidt found no difference in tumor thrombus staging of 23 patients who underwent MRI plus MDCT preoperatively (20). More recently, Guzzo reported that accuracy rate of MDCT in predicting the superior level of tumor thrombus is 96% (21). A low attenuation filling defect within the vein seen after injection of contrast material is the most prominent feature for venous involvement on CT scan. In our series MDCT correctly identified and localized the extent of the tumor thrombus in all patients, and the agreement between MDCT and pathological finding was excellent. However, four patients with venous wall invasion were not detected by MDCT, probably due to the local extension. Invasion of the inferior vena cava will significantly complicate surgical procedure because prosthetic reconstruction is usually required. Though negative vascular margins were achieved in all four cases, it is important to note that no imaging modality is 100% accurate and the surgeon must be prepared if more advanced disease is noted than anticipated.

Because of the low incidence of ipsilateral adrenal gland involvement (16), the current surgical trend is to spare adrenal gland during surgery. Türkvatan reported that MDCT correctly identified all six cases of adrenal involvement (15). In our study, only 3 of 13 (18.75%) suspected adrenal involvement were confirmed by pathological findings. Because direct extension of large RCC into adrenal always compresses it into a thin tiny organ and causes local inflammation, it is difficult to distinguish it from the tumors. Our radiologist tended to be conservative and loss of tissue planes and irregular margins between the tumor and neighboring organ were all considered adrenal involvement. As indicated by Novara, patients with adrenal gland invasion had much lower 5 years cancer specific survival (17.9%) than other subgroups (16), that conservative assessment of the adrenal gland is necessary preoperatively since an extensive resection applied in patients with abnormality suggested on CT scan may yield a better clinical outcome.

Lymph node involvement occurs in about 15% of patients in the absence of other metastasis (22,23). CT has in the past been insensitive to detect nodal metastasis in normal sized nodes.

A cutoff value in node size of 1 cm has been reported with a false negative rate of 10% due to reactive hyperplasia (12). Even with spiral CT, the false positive rates up to 43% have been reported (24). However, in a study by Catalano, using MDCT, 13 of 14 true positive cases for nodal metastasis were identified, reducing the false positive rate due to reactive hyperplasia to 6.3% (7). In our study, 33.33% of patients with lymph node involvement were correctly staged, with a false positive rate of 64.7%. The agreement between MDCT and pathological findings were fair (Kappa = 0.322), which is consistent to the findings of Türkvatan (15). This indicated that the MDCT is not a reliable modality in nodal involvement detection, and 1 cm size as the cutoff value is not proper. Currently, regional lymph node dissection is considered of no clinical benefit to patients with clinically negative lymph nodes (25); however, in patients with positive lymph nodes suggested preoperatively or those with progressive disease, lymph node dissection is associated with improved survival (26,27).

Organ metastasis of RCC is most frequently found in the lung, bone, brain and liver (28). Likely, the metastatic lesions tend to be hypervascular. The detection of visceral metastasis is of great importance because patients with metastatic disease still benefit from radical nephrectomy combined with systemic immunotherapy (29,30). In our study, all seven but one metastatic diseases were correctly detected by MDCT. Other study has proved excellent performance of this technique in metastatic lesions detection as well (15). However, lesion from an 83-year-old male incorrectly staged by MDCT was finally proved an adenoma from the gastrointestinal system, suggesting that in high risk population, multiple tumors from different tissues may occur. Therefore, a thorough preoperative search of tumors with different imaging modalities may be necessary.

CONCLUSIONS

In conclusion, MDCT scan can delineate RCCs with high accuracy, including tumor size, the presence and level of tumor thrombus and distant metastasis. However, a great proportion of tumors were overstaged by MDCT because of overestimation of tumor size and poor visualization of infiltration of the perinephric fat. In addition, as micrometastasis can not be identified and nodes with diameter > 1 cm may be caused by reactive hyperplasia, nodal metastatic lesion evaluation remains a difficult task.

CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Can we predict which patients will evolve to chronic kidney disease after nephrectomy for cortical renal tumors?

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ABSTRACT

Introduction: While some studies show that patients submitted to radical nephrectomy have a higher risk of developing chronic kidney disease (CKD), some studies report that carefully selected living kidney donors do not present a higher risk for CKD. Here, we aim to study predictive factors of CKD after radical nephrectomy.

Materials and Methods: Between January 2006 to January 2010, 107 patients submitted to radical nephrectomy for cortical renal tumors at our institution were enrolled in this study. Demographic data were recorded, modified Charlson-Romano Index was calculated, and creatinine clearance was estimated using abbreviated Modification of Diet in Renal Disease (MDRD) study equation. Pathological characteristics, surgical access and surgical complications were also reviewed. The end-point of the current study was new onset estimated glomerular filtration rate (eGFR) less than 60 and less than 45 mL/minute/1.73 m^2.

Results: Age, preoperative eGFR, Charlson-Romano Index and hypertension were predictive factors of renal function loss, when the end-point considered was eGFR lower than 60 mL/minute/1.73 m^2. Age and preoperative eGFR were predictive factors of renal function loss, when the end-point considered was eGFR lower than 45 mL/minute/1.73 m^2. Moreover, each year older increased 1.1 times the risk of eGFR lower than 60 and 45 mL/minute/1.73 m^2. After multivariate logistic regression, only age remained as an independent predictive factor of eGFR loss.

Conclusion: Age is an independent predictive factor of GFR loss for patients submitted to radical nephrectomy for cortical renal tumors.

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Key words:
Neoplasms; Nephrectomy; Renal Cancer; Renal Insufficiency

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INTRODUCTION

Surgical treatment of renal cortical tumors is changing in the last decade. Best management must consider preservation of renal function in order to increase global survival rate. Radical nephrectomy (RN) was considered the gold standard treatment for localized renal cortical tumor (1), however the waste of nephrons causes loss of renal function that is being implicated in the global survival rate decrease. Nephron sparing surgery has specific survival rates similar to those achieved with RN while preserves renal function. Despite the paradigm change, partial nephrectomy represents only 30-65% of all surgical procedures for renal cortical tumors in tertiary-care centers in the United States (2-4). Low volume centers present an even worse figure and RN remains the standard treatment for most cases (5).

Advances in imaging exams have led to increased detection of incidental, small (< 4cm), localized renal cortical tumors. But some tumors cannot be treated by nephron sparing surgery without compromising safety and oncological efficacy, due to a challenging central localization. Even small tumors in the central region may compromise the renal fat around the hilum and despite the low tumor volume, the possibility of a T3 should be considered and therefore RN may still be appropriate in this scenario (6). It has been reported that although experienced surgeons have similar outcomes with radical or partial nephrectomy (7,8), the partial approach can be technically challenging and associated with greater risk of complications, especially if done laparoscopically (9,10).

Huang et al. showed that patients submitted to radical nephrectomy have a higher risk of developing chronic kidney disease (CKD) than patients subjected to partial nephrectomy (11). However, carefully selected living kidney donors do not present a higher risk for CKD (12). Moreover, it has been shown that long-term mortality rate is not significantly increased in living kidney donors (13). These findings led us to looking for patients and tumors features that might influence and could predict which patients will evolve to renal function loss.

In this study, we aim to evaluate demographic data, pathological features, surgical access, and postoperative complications as predictors of CKD after radical nephrectomy for cortical renal tumors.

MATERIALS AND METHODS

Between January 2006 and January 2010, 180 patients were submitted to RN for cortical renal tumors at our institution. We included only patients treated after 2006 because nephron sparing surgery was already consolidated as the best option for small renal tumors at this time. Patients with end-stage renal disease, bilateral renal tumors or metastases at surgery, and those with less than 12 months of follow-up or incomplete data were excluded from our analysis. Only patients with images of a normal contralateral kidney before surgery were selected. Thus, 107 patients were enrolled in this retrospective study.

Clinical characteristics, including age at surgery, gender, hypertension, cerebrovascular and pulmonary disease, and liver dysfunction were recorded. Modified Charlson-Romano Index (14,15) was calculated as an objective measure of comparing comorbidity among patients. Also, preoperative serum creatinine was recorded and Glomerular Filtration Rate (GFR) was estimated using the abbreviated Modification of Diet in Renal Disease (MDRD) study equation (186 x serum creatinine in mg/dL-1.154 x age in years-0.0203 x 0.742 if female). Pathological characteristics, including size and pT stage were also reviewed. Surgical access (laparoscopic versus open) was analyzed as well as Clavien-Dindo classification of surgical complications (16) used to estimate if postoperative period could have influenced the renal function outcome.

The end-point of the current study was new onset eGFR less than 60 and less than 45 mL/minute/1.73 m^2, which defines chronic kidney disease more accurately and has been shown to be associated with a significantly higher risk of complications and comorbidity (17-19). Patients had their postoperative eGFR assessed 6 months after surgery and at the end of the follow-up period. Since the main outcome measures were the new onset of GFR lower than 60 or 45 mL/minute/1.73 m^2 after surgery, patients with pre-existing GFR values lower than these two thresholds were not included for outcome analysis.

In order to determinate p values, we used Mann-Whitney test or Student t test for continuous variables and Chi-square test for categorical variables. Because of the small frequency of postoperative complications, Fisher exact test was used for Clavien-Dindo classification. After univariate analysis, multivariate logistic regression was done to determine the real effect of each hypothesized risk factor. All statistical analysis was performed with $p < 0.05$ considered significant.

RESULTS

Mean (range) age at surgery was 58.5 (25 - 86) years and 62 (57.9%) patients were male. Mean (range) Charlson-Romano Index was 1.8 (0 - 9) and 43 (40.2%) patients had hypertension. Eighty and four (78.5%) were submitted to open radical

nephrectomy, while 23 (21.5%) to laparoscopic approach. Demographic data, surgical access (open vs. laparoscopic), surgical pathology, and Clavien-Dindo classification are summarized in Table-1. Mean (range) operative time was 108 (70 - 210) minutes. Mean (range) estimated blood loss was 180 (50 - 700) mL. Mean (range) preoperative eGFR was 72 (60 - 150) mL/minute/1.73 m^2. Mean (range) follow-up was 32 (18 - 54) months.

After 6 months and at the end of the follow-up period mean eGFR was 58.2 and 57.6 mL/minute/1.73 m^2, respectively. Thirty-two patients had eGFR lower than 60 mL/minute/1.73 m^2 six months after surgery, while 14 patients had eGFR lower than 45 mL/minute/1.73 m^2 at the same period. At the end of the follow-up, 29 patients had eGFR lower than 60 mL/minute/1.73 m^2, because three presented a small improvement in the renal function, and 16 patients had eGFR lower than 45 mL/minute/1.73 m^2.

When the end-point considered was eGFR lower than 60 mL/minute/1.73 m^2, age (p = 0.002), Charlson-Romano Index (p = 0.01) and hypertension (p = 0.04) were significant predictors of renal

Table 1 – Demographic data, surgical access, surgical pathology, and Clavien-Dindo Classification.

Mean (range) age	58.5 (25 - 86) years	
Median age	59 years	
Gender	62 (57.9%) male	45 (42.1%) female
Mean (range) Charlson Index	1.8 (0 - 9)	
Median Charlson Index	1	
Hypertension	43 (40.2%) yes	64 (59.8%) no
Surgical access	84 (78.5%) open	23 (21.5%) laparoscopic
Pathology	97 (90.6%) clear cell carcinoma	
	10 (9.4%) chromophobe carcinoma	
Pathological stage	pT1	37 (34.6%)
	pT2	25 (23.4%)
	pT3	37 (34.6%)
	pT4	8 (7.5%)
Clavien-Dindo classification	0	100 (93.5%)
	1	4 (3.7%)
	2	1 (0.9)
	3	2 (1.9%)
	4	0
	5	0

function loss at six months of follow-up. At the end of that period, these factors and preoperative eGFR (p = 0.02) were significant predictors of renal function loss (Table-2). Moreover, for each year older, the risk of eGFR lower than 60 mL/minute/1.73 m^2 increased 1.1 times, and for each point acquired in the Charlson-Romano Index, the risk of eGFR lower than 60 mL/minute/1.73 m^2 increased 1.24 times. However, after multivariate logistic regression, only age (p = 0.005) remained as an independent predict factor of eGFR loss.

DISCUSSION

This study presents original and relevant data. Multivariate logistic regression shows that age is the only predictor related to renal function loss after radical nephrectomy (OR: 1.1, 95% CI 1.04-1.15). Moreover, the risk of eGFR lower than 60 or 45 mL/minute/1.73 m2 after RN increases 10% for each year older.

The 3-year probability of absence of new onset of GRF lower than 60 mL/min per 1.73 m^2 is

Table 2 – Predictive factors of development of chronic kidney disease following radical nephrectomy (univariate analysis).

	eGFR < 60 mL/min/1.73 m^2		eGFR < 45 mL/min/1.73 m^2	
	6 months	End	6 months	End
Age	p = 0.002	p < 0.001	p = 0.003	p = 0.001
Gender	p = 0.61	p = 0.07	p = 0.51	p = 0.16
Preoperative eGFR	p = 0.23	p = 0.02	P = 0.01	P < 0.001
Charlson-Romano Index	p = 0.01	p = 0.01	p = 0.08	p = 0.09
Hypertension	p = 0.05	p = 0.04	p = 0.72	p = 0.11
Surgical access	p = 0.67	p = 0.41	p = 0.96	p = 1.00
Pathological stage	p = 0.17	p = 0.44	p = 0.65	p = 0.41
Clavien-Dindo classification	p = 1.00	p = 0.63	p = 0.21	p = 0.32
95% CI				

When the end-point considered was eGFR lower than 45 mL/minute/1.73 m^2, age (p = 0.003) and preoperative eGFR (p = 0.01) were predictors of renal function loss at six months of follow-up. Charlson-Romano Index (p = 0.08) appeared to be higher in patients with eGFR loss. All these findings persisted until the end of the follow-up period (Table-2). Again, for each year older, the risk of eGFR lower than 45 mL/minute/1.73 m^2 increased 1.1 times. After multivariate logistic regression, age remained (p = 0.001) as an independent predictor factor of eGFR loss.

80% and 35% after partial and radical nephrectomy, respectively. The corresponding values for GFR lower than 45 mL/min per 1.73 m^2 are 95% and 64% (11). However, partial nephrectomy is not always possible due to large renal tumors, central localized tumors, or in some cases, when performed mainly in small centers, because inexperienced surgeons are not used to the procedure. In these situations, it is very important for the surgeon to predict which patients are potential candidates to radical nephrectomy without compromising GFR outcome and patient survival. Even in those cases

where radical nephrectomy is the only option, it is very beneficial to know the risk of new onset of CKD to prepare patients and medical care team to this unpleasant event.

Outcome data from transplantation studies (20-22) have long supported that CKD after total nephrectomy is not a major concern in patients with two equivalent functioning kidneys and normal preoperative concentration of serum creatinine, since donors submitted to nephrectomy do not show decrease of their kidney function, kidney failure needing dialysis, or death. However, this is not observed in patients submitted to RN for cortical renal tumors. These findings encouraged us to look for which factors could influence and predict which patients would evolve to renal function loss.

Yokoyama et al. (23) retrospectively studied 416 patients submitted to nephrectomy and after univariate and multivariate analysis radical nephrectomy was an independent risk factor for new onset eGFR less than 60 mL/min per 1.73 m^2 (HR 3.19, 95% CI 1.72 - 6.75) but not for new onset eGFR less than 45 mL/min per 1.73 m^2. Age at surgery and preoperative eGFR were independent risk factors for new onset less than 45 mL/min per 1.73 m^2. Barlow et al. (24) presented similar results in another retrospectively study with 209 patients submitted to nephrectomy and with preoperative eGFR higher than 60 mL/min per 1.73 m^2. On multivariate analysis, preoperative CKD and procedure (radical versus partial) were independent predictors of new onset renal insufficiency. In this study, hypertension was an independent predictor of CKD upstage. Suer et al. (25) studied the long-term impact of hypertension and diabetes mellitus on GFR in the long term in 488 patients submitted to nephrectomy and noted that hypertension was associated with new onset of chronic renal failure only in the radical nephrectomy group (HR 1.39, 95% CI 1.02 - 1.89).

In our study, age, preoperative eGFR, and hypertension were related to renal function loss, corroborating literature data. Our study presents an original contribution that may improve the care of patients that suffer from renal tumors and are going to be submitted to RN. We found that for each year older, the risk of eGFR lower than 60 or 45 mL/minute/1.73 m^2 after RN increases 10%.

In partial nephrectomy, predicting factors of lower GFR were well studied in a large cohort of 1169 patients by Lane et al. (26). Lower preoperative GFR, solitary kidney, older age, tumor size and longer ischemic interval were all predicting factors of bad renal function outcome. However, unfortunately, this paper did not study patient's comorbidities and did not evaluate which of these factors could predict CKD in patients that are not candidate to partial nephrectomy. Santos Arrontes et al. (27) studied the survival rate after nephrectomy for clear cell carcinoma according to the Charlson comorbidity index in 192 patients submitted to nephrectomy. They reported that tumor stage and comorbidity (Charlson greater than two) were prognostic factors after one, five and ten years of follow-up. The authors concluded that the comorbidity index should be applied in daily clinical practice to assess the best therapeutic option for patients. In our study, the Charlson-Romano Index was related to renal function loss, and for each point acquired in the Charlson-Romano Index, the risk of eGFR lower than 60 mL/minute/1.73 m^2 increased 1.24 times. It appears that Charlson-Romano Index is an important factor that should be used in patients that will be submitted to nephrectomy.

Our study has some limitations. It includes a small sample, which may have affected our multivariate analysis, and as a retrospective study, patients were not randomly assigned to open or laparoscopic procedure, which means that the choice of surgical technique has been biased by the surgeon's preference. However, we may note that partial nephrectomy, when technically feasible, must be performed in older patients. In our study, as we are a tertiary reference service, we have treated complex cases and/or central localized mass, leading to a high prevalence of RN and open approach. Another limitation is that the median follow-up period of 32 months may not have been long enough to draw conclusions on long-term postoperative renal function. Maybe in a future publication this doubt can be resolved. Large studies with longer follow-up are needed to evaluate long-term renal function after radical nephrectomy.

CONCLUSIONS

Age is an independent predicting factor of GFR loss for patients submitted to radical nephrectomy for cortical renal tumors. Each year old increases 10% the risk of CKD after radical nephrectomy. Preoperative eGFR and Charlson-Romano Index also should be evaluated before nephrectomy.

ABBREVIATIONS

RN – Radical Nephrectomy
CKD – Chronic Kidney Disease
GFR - Glomerular Filtration Rate
MDRD – Modification of Diet in Renal Disease

CONFLICT OF INTEREST

None declared.

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EDITORIAL COMMENT

This is an interesting retrospective study about possible prediction factors (age, preoperative renal function, Charlson-Romano-Index and hypertension) for chronic kidney disease (CKD) in 107 patients submitted to radical nephrectomy (RN). Exclusion criteria were end stage renal disease, bilateral renal tumors, metastases at surgery, less than 12 months of follow-up and an abnormal contralateral kidney in imaging studies.

The predictive factors of kidney dysfunction after RN were age, preoperative renal function, Charlson-Romano-Index and hypertension, when the end point considered estimated was glomerular filtration rate (eGFR) lower than 60 mL/min/1.73m2. When the end point considered was eGFR lower than 45 mL/min/1.73m2, only age and preoperative eGFR remained as predictive factors. Age remained as an independent predictor factor of eGFR loss after multivariate logistic regression.

An important limitation of this paper is the short period of follow-up (18-54 months), as the authors mentioned at Discussion. Furthermore, 34.6% of the patients had small renal tumors (pT1), and missing was information on what percentage of patients was potentially eligible for partial nephrectomy (PN) but underwent RN instead. The under use of PN can easily be found in other studies, despite evidence of equivalent oncologic outcomes and an increased likelihood of developing CKD and cardiovascular comorbidity associated with RN (1-3).

Hence, RN should still be discouraged for patients with small renal tumors amenable to partial nephrectomy, including the elderly. As stated by the paper's conclusion, age is undoubtedly a predictive factor for CKD after RN. Lowrance et al. (4) evaluated age and procedure type and found no evidence indicating an increased risk of nephron-sparing surgery complications with advancing age. On the other hand, in this group of patients, active surveillance can also be used and safely avoid surgery for Stage T1a renal masses (5).

The title is very attractive, but the question still remained without a definite answer ("Can we predict which patients will evolve to chronic kidney disease after nephrectomy for cortical tumors?"). As pointed by the authors, maybe a future study with a longer follow-up and also with more strict criteria for the indication for RN could contribute even more to this issue.

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ORIGINAL ARTICLE

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Cystectomy and urinary diversion in the treatment of bladder cancer without artificial respiration

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ABSTRACT

Purpose: To assess the feasibility and performance of radical cystectomy with urinary diversion using exclusively regional anesthesia (i.e. combined spinal thoracic epidural anesthesia, CSTEA).

Materials and Methods: In 2011 radical cystectomy with extended pelvic and iliac lymphadenectomy was performed on 14 patients using urinary diversion without applying general anesthesia. Under maintained spontaneous breathing, the patients were awake and responsive during the entire procedure. Postoperatively, pain management took three days with the remaining epidural catheter before oral analgesics were administered. Mobilization and diet restoration were carried out according to the fast-track concept. Outcome measurements included operative time, blood loss, beginning of oral nutrition, beginning of mobilization, postoperative pain levels using numerical and visual analog scales (NAS/VAS), length of hospital stay.

Results: All surgical procedures were performed without any complications. The absence of general anesthesia did not result in any relevant disadvantages. The postoperative progress was normal in all patients. Particularly, cardiopulmonary complications and enteroparesis did not occur. The provided palliative care proved sufficient (NAS max. 3-4). Discharge followed 10 to 22 days after surgery. At the time of discharge, the patients described the procedure to be relatively positive.

Conclusions: Our data show that CSTEA is an effective technique for radical cystectomy, whereby spontaneous breathing and reduced interference with the cardiopulmonary system potentially lower the perioperative risks especially for high-risk patients. We recommend practice of CSTEA for radical cystectomy to further evaluate and monitor the safety, efficacy, outcomes, and complications of CSTEA.

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Feasibility Studies; Cystectomy; Urinary Bladder Neoplasms; Anesthesia, Spinal; Anesthesia, Epidural; Patient Safety

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INTRODUCTION

Radical cystectomy is the most preferred procedure in the treatment of muscle-invasive bladder cancer and involves high perioperative risks. The morbidity and mortality rates of radical cystectomy with urinary diversion are caused by patient-specific, anesthesiological and surgical factors (individual patient risk profile, selection and administration of the anesthesiological procedure, extension of the procedure, spread or metastasis of the bladder cancer, type of urinary diversion, surgical expertise, number of operations). The perioperative mortality rate of the cystectomy is between 2.9% and 7.7% (1-4). The perioperative morbidity rate is between 11% and 68% (3).

On many procedures, applying regional anesthesiological methods can lower perioperative risks. Regional anesthesia can be administered on different levels of the spinal cord and in different

areas of the spinal canal. The substances applied once or continually in these regions have varying effects, effectiveness and side effects.

Regional anesthesia is administered particularly on patients with high perioperative risks. In urology, this has been predominantly the case so far in locally restricted, endourological and low-risk procedures. Comparatively, the exclusive use of regional anesthesiological procedures during extended and high-risk operations, such as radical cystectomy, has previously been seldom described (5).

To notably minimize cardiopulmonary, patient-specific and post-operative risks, a thoracic epidural catheter is frequently used in fast-track surgery. We combined optimal analgesia with one-time spinal anesthesia for the perioperative, motoric blockade and performed radical cystectomy with lymphadenectomy with urinary diversion under CSTEA without general anesthesia.

MATERIALS AND METHODS

In 2011 radical cystectomy with extended lymphadenectomy using urinary diversion was performed on 14 patients with muscle-invasive bladder cancer.

The inclusion criterion for applying CSTEA was the presence of a muscle-invasive bladder cancer. The exclusion criterion was the non-feasibility of the catheter installation.

Individual patient features, such as age, gender, body mass index, stage of tumor, comorbidity, cardiopulmonary risk assessment as well as intraoperative and postoperative parameters, such as the type of urinary diversion, operative time, blood loss, postoperative beginning of oral nutrition, postoperative beginning of mobilization, postoperative pain levels, postoperative hospital stay are indicated on Table-1.

During bridging, patients, who were orally anticoagulated (Pt. 1, Pt. 6, Pt. 8), for instance due to chronic atrial fibrillation, received perioperatively low molecular heparin according to weight (low dose molecular weight heparin); with dose reduction immediately pre- and postoperatively (10 to 12-hour waiting period pre- and postoperatively).

Radical cystectomy with extended pelvic and iliac lymphadenectomy using urinary diversion (ileal neobladder, ileal conduit, transureterocutaneostomy) was performed in the usual open manner by means of a median lower abdominal laparotomy. A cystoprostatectomy was performed on male patients while a simultaneous hysterectomy was performed on female patients, taking the vaginal front wall. In all cases, the lymphadenectomy was extended pelvically, iliacally, presacrally up to the point of aortic bifurcation.

The applied urinary diversion was suited to the patient's age, risk factors and tumor size. Continent and incontinent urinary diversions were constructed from the terminal ileum in the form of a pouch or neobladder or a conduit with both ureters re-implanted. Simple incontinent urinary diversions were applied as transureterocutaneostomy (TUUC).

Thermoregulation was conducted with heating mats placed over and under the patient. During the entire surgical procedure, CO_2, temperature and blood pressure were continually measured as well as ECG was monitored on each patient.

To monitor breathing (O_2 and $EtCO_2$), we used the Smart CapnoLine Plus O_2 for our patients, who were not intubated and were spontaneously breathing. They could be orally and nasally applied and used for up to six hours. The nasal tube has an O_2 supply line. The Smart CapnoLine Plus O_2 can be used in pain management, emergency medicine, transport and critical care.

Premedication was administered orally with 10-20 mg chlorazepate. Perioperatively, an iso-osmolar electrolyte solution of 5-10 mL/kg BW/h was infused intravenously. Blood pressure was regulated with an arterenol perfusor (1 mg/50 mL of 0.9% NaCl).

CSTEA was administered by combining a deep thoracic epidural catheter at level T10/11 or T11/12 and a spinal anesthesia at level L3/4 or L4/5 (Figure-1).

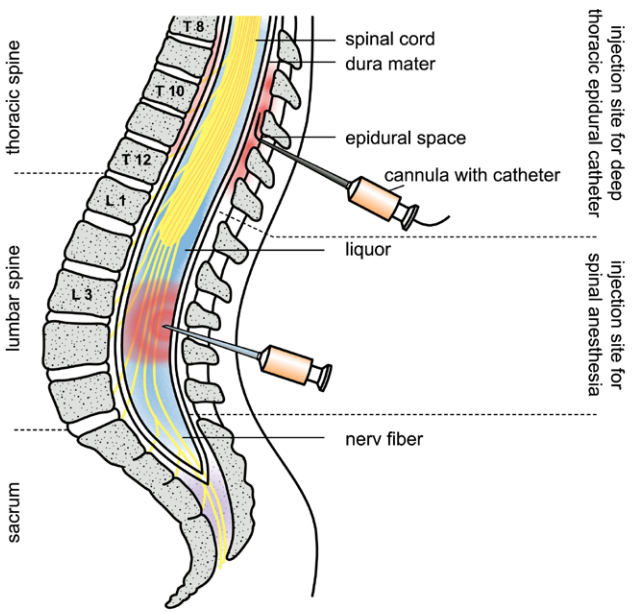
A deep thoracic epidural catheter was placed either with the patient sitting or lying in a lateral position in two stages: by placing a local anesthesia and puncturing the epidural space at level T10 to T12 between two spinous processes

Table 1 - Peri- and postoperative patient data.

Pat.	Age (yr)	Gender	BMI[1] (kg/m²)	T	N	M	G	Comorbidities	Cardio-pulmo-nary risk (ASA)	Sedation with Propofol	Urinary diversion	Operative time (min)	Blood loss (mL)	POH[2] of oral intake	POH[2] of mobilization	NAS[3] on POH 1-24 (1-10)	Hospital stayd (d)
1	64	m	24.5	3b	0	0	III	4, 7, 11	3	+	neo-bladder	110	100	6	6	0	16
2	82	f	27.7	4a	2	0	III	8	3	-	TUUC[5]	95	130	5	7	1	22
3	68	m	30.1	3a	0	0	II	7, 8, 9	3	+	conduit	290	300	6	6	3	16
4	67	m	22.3	4a	1	1	III	4, 9	3	+	conduit	210	270	5	6	0	13
5	51	m	26.2	2a	0	0	III	9	3	+	neo-bladder	330	260	6	6	4	21
6	77	m	29.5	4a	1	0	III	7, 11	3	-	TUUC[5]	100	110	6	5	0	15
7	78	m	27.4	2a	0	0	III	7, 12	3	-	conduit	105	100	6	6	0	18
8	73	f	28.1	4a	0	0	III	7, 11	3	-	conduit	110	150	6	6	2	12
9	74	m	29.5	4a	2	0	III	7, 6	4	-	TUUC[5]	105	170	6	6	0	15
10	67	f	27.4	2b	0	0	III	8, 10	3	-	conduit	125	140	6	6	0	20
11	84	f	28.1	3b	0	0	III	8, 10	3	-	TUUC[5]	155	170	6	6	1	11
12	69	f	28.9	3b	0	0	III	6	3	-	TUUC[5]	130	200	6	6	0	13
13	85	m	28.1	3b	0	0	III	8	3	+	conduit	115	170	6	6	1	16
14	91	f	30.1	3b	0	0	III	6	3	-	TUUC[5]	120	120	6	6	0	10

[1] **BMI:** Body mass index
[2] **POH:** Postoperative hours
[3] **NAS:** Numerical analog scale
4: Peripheral arterial desease
[5]**TUUC:** Transureterocutaneostomy
6 : Chronic Obstructive Pulmonary Disease
7: Hypertension
8: Diabetes
9 : Renal failure
10: Compensated congestive heart failure
11: Arrythmias / chronic atrial fibrillation
12: Coronary heart disease

Figure 1 - Method of CSTEA.



with an epidural needle (Tuohy needle, G18). The needle was inserted through the skin, the intervertebral ligaments and the ligamentum flavum in the epidural space. This was identified as the "loss-of-resistance" technique, wherein a syringe with liquid is attached to the needle. Should the needle be placed before the epidural space in the ligaments of the spinal column, fluid injection is not possible (resistance). By steadily controlling the injection resistance, the needle was carefully inserted further (usually 4-5 cm) until the resistance-free liquid could be injected (loss of resistance). This was marked by the exit of the needle point out of the ligament structure in the epidural space. After locating the epidural space, the Tuohy needle was inserted into a thin plastic catheter that could be left in the epidural space. This catheter was then tunneled under the skin. Tunneling in catheter placement supports prophylaxis against infections as well as position stability (the epidural catheter remained ca. 72 hours postoperatively, see below). Problem-free puncturing of the peridural area and placement of the catheter was tested with 0.5% bupivacaine in order to preclude the intraspinal layer. Difficult puncturing was additionally tested with adrenalin, wherein 0.5 µg/kg KG adrenalin was injected (10 µg/mL).

On the actual operative procedure and beyond, the catheter enabled an effective pain management. A pump was connected to the catheter, through which a basic quantity (basal rate) of a local anesthetic was supplied continually. Postoperatively, the pumping method also aided the patient in administering additional doses, if needed, by pressing the button (patient-controlled epidural analgesia, PCEA). As long-term medication, 0.2% (5-7 mL) of ropivacaine was administered. The rapid response of epidural anesthesia followed after 5-18 minutes and its effect lasted about two hours.

Spinal anesthesia was administered on the sitting patient by puncturing with a spinal cannula (G22 with Introducer) between the lumbar vertebrae (L 3/4 or L 4/5). This was done from the rear (median) in the layers of the spinous processes. Once the cannula penetrated the ligament structure, the actual, thin puncture needle was inserted through it, thereby puncturing the subarachnoid space. After passing through the dura, the clear liquor cerebrospinalis dripped out of the needle, indicating that the needle was correctly placed. Then a local anesthetic dosed according to the patient, was administered (approximately 3 mL of 0.5% hyperbaric bupivacaine (15 mg) and 5 µg sufentanil). The effect was almost immediate and started with a warm sensation on the legs or buttocks. The anesthetic and analgesia settled in within minutes, while movement decreased. With the hyperbaric local anesthetic, the patient's positioning influenced the extent at which the numbed area spread. The effect lasted 3 to 4 hours.

The effectiveness of epidural and spinal anesthesia depended on the dose of the administered substance. The following nerve characteristics were disabled in this order: sensors, coldness, sympathetic, vasomotor, motor skills.

All patients respired spontaneously during the entire surgical procedure. Especially in longer operative times, some patients were administered an escalating sedation by intravenously applying propofol. For this purpose, a bolus of 10 mg of propofol was administered followed by a propofol infusion (0.5% propofol) of 0.5-1 mg/kg BW/h. Postoperatively, pain therapy comprised of using the remaining epidural catheter with ropivacaine (0.1%, 1-2 mL).

The epidural catheter was removed after a 10 to 12 hour waiting period for heparin. We observed no epidural hematoma or other bleeding complications.

After removing the epidural catheter, pain therapy was resumed by orally administering a combination of novaminsulfon and oxycodone dosed according the patient's weight (4x500 mg / 2x10-20 mg). The pain level was documented using numerical and visual analog scales (NAS / VAS).

Postoperative mobilization and diet restoration were carried out according to the fast-track concept of early mobilization and early enteral nutrition. After surgery, patients were asked about their subjective assessment regarding the pre-, intra- and postoperative procedures.

RESULTS

Individual patient features as well as the intra- and postoperative data are summarized in Table-1.

When asked, the patients did not indicate perioperatively experiencing uneasiness, discomfort or pain.

All surgical procedures could be performed as preoperatively planned without complications and caused no anesthesiological or surgical features. Only muscle fasciculation was apparent on several patients upon severing the abdominal wall.

Vital signs of all patients were intraoperatively stable. On three patients with hypotension (side effect of the sympathetic blockade), temporarily administering a minimal dose of noradrenalin was necessary. Further side effects were not apparent.

The postoperatively continuous epidural analgesia showed a very good effect on all patients (NAS max. 3-4). After removing the epidural catheter after 72 hours, oral medication proved sufficient (NAS max. 3-4).

Postoperatively, all patients showed normal progress. Cardiopulmonary complications did not occur.

Mobilization and diet restoration were carried out according to the fast-track concept immediately postoperatively. No postoperative enteroparesis were observed. The patients could be discharged after 10 to 22 days.

When asked upon discharge, the patients described the surgical procedure pre-, intra-, postoperatively to be consistently positive under the circumstances.

DISCUSSION

To minimize perioperative risks, surgical techniques as well as preoperative anesthesiological management must be constantly improved. Better surgical techniques and anatomical understanding have continually been developed in the last years. Particularly, developments in high-frequency surgery and fast-track concepts have contributed to the quality of minimally invasive techniques. Firstly, from an anesthesiological perspective, it could be proven that applying a combination of epidural and general anesthesia is more effective than solely general anesthesia. Data from meta-analyses show that through a neuroaxial blockade, a decreased mortality rate of 30% can be reached while the morbidity rate is considerably reduced. This is achieved with a decreased incidence of deep venous thrombosis, pulmonary embolism, myocardial infarction, transfusions, pneumonias, infections, respiratory depression and liver failure (4,6,7). Secondly, it is generally known that dispensing with general anesthesia leads to a clear relief of the cardiopulmonary system, from which particularly patients with increased cardiopulmonary risks benefit.

On low-risk surgical procedures in the areas of the lower abdomen and lower extremities with a sensorial blockade of up to T12 (deep spinal anesthesia) or up to T10 (medium-high anesthesia), spinal anesthesia is today considered to be an established and safe method, which as a primary indication, is applied to older and/or high-risk patients (8,9). Combined spinal epidural methods (CSE methods) have the additional beneficial effect of optimal postoperative therapy over the epidurally applied local anesthetic (10). However, spinal anesthesia or CSE in the context of extensive and tumor-surgical procedures in the pelvis minor and in the lower abdomen is exceptional. Likewise, there is only few data on procedures in the area of the middle and upper abdomen, which requires a regional anesthesia of sometimes up to T4 (high spinal anesthesia).

According to a fast-track concept, a combination of spinal singular ("single shot") and continuous thoracic epidural anesthesia is suitable for this type of surgical procedure. With the spinal application of a local anesthetic, not only rapid response and reliable analgesia are attained, but also the intraoperatively desired muscle relaxation is achieved. In contrast to continuous administration, one-time administration prevents any occurrence of longer lasting motor blockade, which impedes early mobilization (8).

The anesthesia of up to T4 is administered through the deep-thoracically placed epidural catheter. A further advantage of epidural anesthesia is the optimally postoperative pain therapy using the postoperatively remaining catheter. Different studies on intra-abdominal procedures have shown the superiority of epidural anesthesia (PCEA) to intravenous and intramuscular opioid administrations (10-12). Moreover, sparing the opioid in the first 72 hours minimizes the risk of ileus.

CSTEA without general anesthesia reduces postoperative morbidity and mortality. Moreover, it combines the advantages of spinal anesthesia with a rapid starting effect and limited effectiveness on good motor blockade with highly effective as well as optimally controllable analgesia using the postoperatively remaining epidural catheter (6,13-16).

Perioperatively and postoperatively, we observed no increased complication rate regarding CSTEA and experienced no restrictions in view of pain management and early mobilization. In all cases, the administration of opioids could be dispensed with directly postoperatively.

Undesired side effects of CSTEA can include hypotension, cerebral ischemia, bradycardia, cardiac arrythmia, cardiac arrest in rare cases, respiratory insufficiency due to high spinal anesthesia, paraplegia as a result of an epidural hematoma or abscesses, arachnoidea, nausea and vomiting, motoric blockade, pruritus, post-dural puncture headache (PDPH) (17,18). Intraoperatively, we observed mild hypotension on all of our patients as the only undesired side effect.

In individual cases, patients may experience unrest, which can be caused by such patient factors as longer surgery time, hypothermia, and inadequate positioning. Should this cause discomfort or problems for the patient or the surgical team, CSTEA can be supplemented with an escalating sedation.

CONCLUSIONS

With adequate patient selection and careful application, CSTEA appears as a safe, reliable and effective option for patients undergoing radical cystectomy. Dispensing with general anesthesia resulted in no relevant perioperative disadvantages. Simultaneous, moderate sedation must be determined in individual cases.

Our data show that CSTEA is an effective technique in radical cystectomy, as particularly demonstrated by maintained spontaneous breathing and reduced interference with the cardiopulmonary system. Thus, CSTEA can be a gentle method, maybe associated with reduced perioperative risks. Larger series are necessary to prove safety, efficacy, outcomes, and complications of CSTEA, and they are under construction by our team.

ABBREVIATIONS

CSTEA: Combined spinal thoracic epidural anesthesia
CSE: combined spinal epidural
NAS: Numerical analog scale
PDPH: Post-dural puncture headache
PCEA: Patient-controlled epidural analgesia
TUUC: Transureterocutaneostomy
VAS: Visual analog scale

CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Detrusor overactivity in diabetic and non-diabetic patients: Is there a difference?

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ABSTRACT

Purpose: To compare urodynamic characteristics in patients with idiopathic detrusor overactivity (IDO) with those of an age matched cohort with diabetes mellitus (DM) and detrusor overactivity (DO). Secondly, to determine whether urodynamic features could help distinguish these two groups of patients.

Materials and Methods: Urodynamic data was collected on 58 female patients; 29 with IDO and 29 with DM and detrusor overactivity. Eight urodynamic parameters were selected for analysis: amplitude of the first overactive contraction (AOFC), the volume at the first contraction, cystometric capacity, maximal detrusor pressure, maximal flow rate, voiding pressure at maximal flow, voided volume and postvoid residual (PVR) urine volume. Finally, sensitivity analysis for distinguishing urodynamic parameters between studied groups was performed.

Results: AOFC, volume at AOFC and maximal detrusor pressure were statistically greater in diabetic patients, compared with the non-diabetic group of women (16.00 cm H_2O versus 9.00 cm H_2O, 309.00 mL versus 167.00 mL and 76.48 cm H_2O versus 55.41 cm H_2O respectively). A specificity of 72.41% and positive predictive value of 71.43% were achieved for AOFC with cutoff value of 12 cm H_2O. These parameters were further improved with cutoff value of 258 mL for volume at AOFC and were 75.86% and 73.08% respectively.

Conclusions: Certain urodynamic parameters in diabetic female patients with DO are shown to be significantly different than those in women with IDO. Further prospective study should provide additional information about the pathogenesis and progression of DO in diabetic patients as well as the validity of diabetic screening in patients with IDO.

ARTICLE INFO

Key words:
Diabetes mellitus; Urodynamics; Urothelium

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INTRODUCTION

Diabetes mellitus (DM) has been shown to alter vesicourethral function in a number of ways, from decreased detrusor contractility to bladder overactivity present in up to 61% of diabetic patients (1).

The etiology of DO in diabetic patients is not fully understood and is most likely multifactorial. Both central and peripheral mechanisms have been implicated; namely, diabetic cerebral vasculopathy and peripheral nerve stimulation as well as changes in the detrusor muscle and urothelium (2-4).

Idiopathic overactive bladder can be observed in approximately 17% of the general adult population; however, the incidence of IDO is not known (5,6). The pathophysiology of idiopathic bladder overactivity is not clear. Although it is defined as DO in the absence of a definite cause, some authors have reported on the presence of neurological signs (7). Despite the fact that not

all individuals with idiopathic overactive bladder require treatment, the condition has been shown to significantly impact on patient quality of life; often leading to isolation and depression (8,9).

Given that lower urinary tract symptoms are not disease specific and it is still unclear what can initiate IDO, the aim of the present study was to determine if there are differences in urodynamic characteristics between patients with overactive bladder secondary to diabetes mellitus and patients with overactive bladder without diabetes or any known neurologic abnormalities. Sensitivity and specificity analyses were performed to assess their ability to predict diabetic overactive bladder according to various urodynamic parameters.

MATERIALS AND METHODS

Urodynamic data of all female patients who underwent urodynamic studies in Departments of Urology and Urogynaecology of the Cork University Hospital over the period 2004 and 2008 were reviewed retrospectively. Patients with objective signs of overactive bladder during the study, defined as an involuntary rise in detrusor pressure of greater than 5 cm H_2O during filling associated with urgency or bladder fullness, were selected. Then, medical charts of all selected patients were reviewed and a database with patients' blood glucose levels and HBA1c were searched. Only patients with a known diagnosis of diabetes mellitus were included in the database of patients with DM while non-neurogenic patients with no history of diabetes and normal glucose levels were selected for the study in the idiopathic bladder overactivity group.

Exclusion criteria included patients with urodynamic evidence of bladder outlet obstruction, defined as maximum flow rate less than 12 mL/min. and detrusor pressure at maximum flow of more than 45 cm H_2O. Those with presence of concurrent neurologic disorders such as stroke, Parkinson disease, spinal cord injury, and multiple sclerosis were also excluded. Lastly, patients with medical conditions that could interfere with voiding function such as prior pelvic surgery, anterior pelvic prolapse of stage 2 or greater (Baden-Walker) or those on medication that could affect bladder function such as diuretics, calcium channel blockers and narcotics were excluded from the study. A total of 58 patients fulfilled the inclusion criteria and of these, 29 were diabetic with DO and 29 patients had IDO.

Urodynamic studies were performed by two experienced urodynamic nurses using the Solar Silver (MMS, Enschede, The Netherlands) and Dantec Menuet (Medtronic Functional Diagnostics A/S, Slovlunde, Denmark) urodynamic measurement systems. A standard protocol was employed in accordance with the guidelines established by the International Continence Society (ICS) (10). All anticholinergic medications were stopped at least 72 hours before study and all patients who underwent urodynamic evaluation had confirmed negative urinalysis findings prior the procedure. The studies were performed with patients in the seated position. Urinary bladders were filled at 50 mL/min. rate using room temperature sterile saline. A dual lumen 8F vesical catheter and 4.5 F rectal catheter were used. Eight urodynamic parameters were selected for analysis: amplitude of the first contraction (AOFC), the volume at the first contraction, cystometric capacity, maximal detrusor pressure (Pdetmax), maximal flow rate (Qmax), voiding pressure at maximal flow (PdetQmax), voided volume and postvoid residual (PVR) urine volume. Also Bladder Voiding Efficiency (BVE), an index that defines bladder voiding function, was calculated as described previously by Abrams, and then statistically analyzed (11). BVE was obtained by the formula: BVE=100% x voided volume/(voided volume+PVR).

All measurements were repeated three times by the same investigator to avoid bias.

Statistical analyses were performed using SPSS package version 11.5. The Shapiro-Wilk test was used to examine for normal distribution. Results were presented as mean values ± standard deviation when data were normally distributed, otherwise as median, 25th and 75th percentile (AOFC, volume at AOFC, PVR and BVE). Non-parametric t test and Mann-Whitney U tests were applied as appropriate.

Multiple logistic regression analysis using the forward stepwise regression with Wald test method was subsequently applied to select

a set of variables distinguishing diabetic patients with DO from IDO.

The associations between age and urodynamic parameters were examined using Pearson's correlation analyses for normally distributed data, otherwise Spearman's rank correlation coefficient was used. Sensitivity and specificity analyses for the ability to predict diabetic DO on the basis of AOFC, volume at AOFC and Pdetmax were also performed. For all statistical tests $p < 0.05$ was considered significant.

RESULTS

A total of 97 urodynamic studies were carried out on female diabetic patients referred from urology or urogynaecology departments in our university hospital over the period 2004 -2008. Of these, 41 women had DO. Strict inclusion criteria were fulfilled only in 29 patients. Three patients had type 1 DM and 26 type 2 of at least 3 years duration. Their average age was 53.84 ± 16.0 years. Average HBA1c level measured over one year preceding urodynamics was determined in 26 patients and was 6.05 ± 2.38% (5.1-12.1%). Five patients had HBA1c level checked after or more than a year before the study. In 15 diabetic women (51% of total), the major reported complaints were frequency and urge incontinence; 8 cases reported urgency without incontinence (28%); mixed urinary incontinence was the main problem in a further 4 (14%) and recurrent bladder infections in 2 more cases (7%).

Urodynamic data were also collected from 29 female patients complaining of symptoms suggestive OAB and who had no previous history of diabetes mellitus or neurological disorder and who were referred for evaluation of their lower urinary tract. The average age of this group was 50.42 ± 20.23years. The most common symptoms of women with IDO were urge incontinence in 11 cases (38%), mixed urinary incontinence in 7 (24%), urgency without incontinence in 5 (17%), voiding symptoms (hesitancy, dribbling, incomplete emptying) in 4 (14%) and stress incontinence in 2 cases (7%).

Table-1 shows characteristics of the groups studied and comparison of the analyzed urodynamic parameters between the two investigated groups. Greater amplitude of the first overactive contraction was observed in patients with DM than in females with IDO (18.31 cm H_2O versus 11.03 cm H_2O). Also, these patients had a stronger maximal detrusor contraction compared to those with IDO (76.48 cm H_2O versus 55.41 cm H_2O). The initial contraction occurred later during the filling phase in diabetic women than in those patients with IDO (333.83 mL versus 208.72 mL).

The remaining analyzed parameters were not statistically different in both groups under investigation. Also, BVE was within the normal range and showed no statistical difference in both diabetic DO and IDO group.

Multiple logistic regression analysis using the forward stepwise regression with Wald test method showed that a set of three urodynamic parameters (AOFC, volume at AOFC and Pdetmax) distinguished diabetic overactive bladder from IDO. AOFC showed to be the most independently aspect with ability to differ diabetic overactive bladder and IDO with classification accuracy of 70.7%. The combination of AOFC, volume at AOFC and Pdetmax improved accuracy to 79.3%.

To examine associations between age and parameters distinguishing diabetic DO and IDO, Pearson's correlation analyses or Spearman's rank correlation coefficient were performed. No relationship between age and parameters under investigation was found.

Since AOFC, volume at AOFC and maximal detrusor pressure between diabetic patients and women with IDO differed significantly, cutoff values were established to evaluate sensitivity, specificity, positive (PPV) and negative predictive values (NPV). A cutoff value of 12 cm H_2O or greater for AOFC produced sensitivity, specificity and positive predictive value of 68.97%, 72.41% and 71.43% respectively. Whereas analyses of using a cutoff value of 258 or greater for volume at AOFC resulted in further improvement of specificity and PPV (75.86% and 73.08% respectively). Evaluation of maximal detrusor pressure did not led to reasonable results (Table-2).

DISCUSSION

In this study we analyzed and compared bladder function in 29 female patients with dia-

Table 1 - Characteristics of the groups and comparison of urodynamic parameters and index of bladder voiding function in diabetic patients with DO and IDO.

Parameter	Diabetic patients with DO (n=29)	Patients with idiopathic DO (n=29)	P Value
Age (years)	53.84 ± 16.00	50.42 ± 20.23	0.619
HBA1C	6.05 ± 2.38	N/A	N/A
Major symptoms			
Frequency and urge incontinence	51%	38%	N/A
Urgency without incontinence	28%	17%	N/A
Mixed urinary incontinence	14%	24%	N/A
Stress incontinence	0%	7%	N/A
Voiding symptoms	0%	14%	N/A
Recurrent UTIs	7%	0%	N/A
AOFC (cm H_2O)	16.00 (11.00;22.00)	9.00 (6.5;15.00)	0.001(*)
Volume at AOFC(mL)	309.00 (208.00;496.00)	167.00 (84.00;277.00)	0.001(*)
Cystometric capacity (mL)	447.00 ± 118.95	432.66 ± 183.75	0.828
Pdetmax (cm H_2O)	76.48 ± 32.45	55.41 ± 20.95	0.010(*)
Qmax (mL/s)	22.331 ± 9.99	25.890 ± 12.36	0.304
$PdetQ_{max}$ (cm H_2O)	40.69 ± 22.00	33.07 ± 17.534	0.194
Voided Volume (mL)	414.59 ± 154.87	401.17 ± 201.30	0.703
PVR (mL)	5.00 (0.00;35.00)	2.00 (0.00;30.00)	0.483
BVE%	98.54 (86.65;100.00)	99.57 (92.87;100.00)	0.663

* Statistically significant.

Key: HBA1C = glycosylated haemoglobin level, **N/A** = not applicable; **UTIs** = urinary tract infections, **DO** = detrusor overactivity; **IDO** = idiopathic detrusor overactivity; **AOFC** = amplitude of first overactive contraction; **$Pdet_{max}$** = maximal detrusor pressure; **Q_{max} =** maximal flow rate; **$PdetQ_{max}$** = detrusor pressure at maximal flow; **PVR** = postvoid residual (urine volume); **BVE =** Bladder Voiding Efficiency.
All data are presented as mean ± standard deviation except AOFC, Volume at AOFC, PVR and BVE which are expressed as median, 25th percentile (first figure in the brackets) and 75th percentile (second figure in the brackets).

betic cystopathy and 29 female patients with DO without DM or known neurological abnormalities. All of them were referred from urology or urogynaecology departments at our university hospital for an evaluation of lower urinary tract function. In our patient population, we demonstrated that the amplitude of the first detrusor contraction was greater in diabetic patients than in women without known glycaemic and neurologic abnormalities. Similarly, the maximum detrusor pressure generated was higher in the group of patients with DM. These findings may suggest less controllable symptoms of DO in diabetic individuals. Although comparable data on the lower urinary tract symptoms between patients with IDO and diabetic DO are not available, a recent large observational study reported on an increase in urge incontinence frequency in women with DM (12). This symptom has been shown to have a profound effect on patients' quality of life (13,14).

Table 2 - Sensitivity, specificity, positive predictive value and negative predictive value for AOFC, Volume at AOFC and Pdetmax at developed cutoff values.

Parameter	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
AOFC > 12 cm H_2O	68.97	72.41	71.43	70.00
Volume at AOFC > 258 mL	65.52	75.86	73.08	68.75
Pdetmax > 63 cm H_2O	62.07	65.52	64.29	63.33

Key: PPV = positive predictive value; **NPV =** negative predictive value; **AOFC =** amplitude of first overactive contraction; **$Pdet_{max}$ =** maximal detrusor pressure; **$PdetQ_{max}$ =** detrusor pressure at maximal flow; **PVR =** postvoid residual (urine volume)

Interestingly in our study, detrusor contractions occurred later during filling in the DM group than in patients with IDO. However, PVR in both patients with diabetes and DO and IDO was not increased thus functional capacity in diabetic patients was not reduced and bladder contractions were occurring at different intervals in both investigated groups.

These findings are different from other reports that noticed increased PVR and decreased functional capacity in diabetic DO individuals (2,15); but similar to those suggesting that conditions affecting nervous system may induce stronger overactive contractions at higher volume (16).

The greater amplitude of the first detrusor contraction, volume at first contraction, and maximum detrusor pressure observed in patients with diabetes can be explained by diuresis-induced bladder wall tissue remodeling and neuropathy. Several studies in the past have demonstrated that the high rate of bladder filling during cystometry may result in an increase in intravesical pressure and threshold volume (17-19). In addition, such non-physiological filling rates may mechanically damage the afferent limb of the micturition reflex resulting in the later generation of action potentials and, consequently, an urge to void at volumes which are larger then normal. Although the mechanisms involved in triggering bladder tissue hypertrophy and hyperplasia in patients with diabetes are not very clear, it seems that a high filling rate is a primary factor in the stimulation of hyperplasia of bladder smooth muscle, urothelium and connective tissue (20,21). An increase in bladder weight is also related to alterations in bladder volume as well as the rate of stretch of the bladder wall, both caused by polyuria, a consequence in itself of diabetes (22). Increases in fluid output likely contribute to faster and greater increases in bladder weight in diabetic patients with DO than in patients with idiopathic detrusor overactivity.

Both peripheral autonomic neuropathy and central nervous system dysfunction due to cerebral vasculopathy are implied in the aetiology of DO in diabetic patients (2,23). However, this kind of diabetic bladder dysfunction can also be present in the absence of CNS lesions (2). In our study, patients with history of stroke were excluded, thus peripheral pathology as the cause of development of DO need to be considered. In addition, alterations in bladder innervation, the bladder smooth muscle cells and urothelium have been proposed to be involved in early stages of diabetic bladder dysfunction (4). M2 receptors up-regulation with partial autonomic denervation leading to the decreased cholinergic transmission are involved in the aetiology of DO with altered contractility in early stages of diabetic bladder dysfunction (24).

Although the chronology of bladder dysfunction in DM and its correlation with diabetic control is not fully known, one group led by Rundles investigated the initial manifestation of the diabetic neurogenic bladder (25). In their series, 83% of diabetic patients with neuropathy had an abnormal cystometrogram and enlarged bladder indicating neurogenic bladder. However, most of them had no residual urine. This differs from advanced diabetic neurogenic bladder with paralysis. These findings were consistent with those in recent reports garnered from laboratory animals (21).

Up to 61% of patients with diabetic bladder dysfunction have DO (1). Currently diagnostic evaluation of patients with IDO does not include determination of diabetes (26). Although routine assessment prior to urodynamics includes questions about DM there is no additional check for diabetes except for urine testing for glycosuria. However, urine testing for glycosuria as screening for DM is not recommended particularly in patients with type 2 diabetes, because of the low sensitivity of the test (27-29). Glucose tolerance test and fasting glucose measures have been the standard tests for screening and diagnosing of diabetes mellitus. Recently hemoglobin A1c testing for the diagnosis of type 2 diabetes has been recommended (30).

The utility of fasting glucose and HBA1c measurements have not yet been evaluated in patients with IDO.

In our study we used a cutoff value of 12cm H_2O for the amplitude of the first overactive contraction in diabetic patients with DO. Specificity was 72.41%, however positive predictive value was 71.43%. These parameters were further improved when analyzed for volume at AOFC. Using a cutoff value of 258 mL specificity and PPV were 75.86% and 73.08% respectively. Therefore, we suggest diabetic screening in the IDO patient with greater amplitude and bladder volume at the first overactive contraction.

Our study was a retrospective, two-unit analysis of patients and as such is subjected to biases and limitations that surround these study types. Data obtained from medical charts may not have revealed undiagnosed neurologic condition which could affect all women, however to our knowledge none had been diagnosed at this point. In addition, study participants may not be a representative cohort from the greater community; thus limiting the ability to generalize findings. Finally, amplitude of the first overactive contraction is not an ideal parameter quantifying detrusor overactivity as it may be affected by various factors during bladder filling. Although all urodynamic studies were performed under the same conditions and in accordance with the strict guidelines established by the ICS to maintain their objectivity, accuracy and reliability it is possible that some discrete factors could affect AOFC. However, combining AOFC, volume at AOFC and Pdetmax improved accuracy for identifying diabetic female with DO and minimized potential bias. Further prospective study in a larger cohort of patients would be useful to stratify certain subgroups based on type and duration of diabetes, symptom levels and glycaemic control.

CONCLUSIONS

Certain urodynamic parameters are important for the detection of diabetes-related DO. It seems that stronger overactive contractions in the presence of larger threshold volume at which they occur characterize the DO in diabetic female patients and suggest different pathogenesis then that involved in IDO. Also diabetic screening of women with IDO and greater amplitude of the first overactive contraction may have a role in identifying patients who do not have a true IDO.

Further prospective studies will provide additional information about pathogenesis and progression of DO in diabetic patients as well as validity of diabetic screening in patients with IDO who have high amplitude and volume at first overactive contractions. Comparison of urodynamic parameters in diabetic patients with and without urodynamically demonstrable DO as well as in patients with neurogenic detrusor overactivity may provide important information on chronology of bladder dysfunction in DM and mechanisms involved in the development of diabetic cystopathy.

ABBREVIATIONS

AOFC = amplitude of first overactive contraction
BE = bladder voiding efficiency
CNS = the central nervous system
DM = diabetes mellitus
DO = detrusor overactivity
HBA1c = glycosylated haemoglobin level
IDO = idiopathic detrusor overactivity
ICS = The International Continence Society
NPV = negative predictive value
$Pdet_{max}$ = maximal detrusor pressure
$PdetQ_{max}$ = detrusor pressure at maximal flow
PPV = positive predictive value
PVR = postvoid residual (urine volume)
Q_{max} = maximal flow rate

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CONFLICT OF INTEREST

None declared.

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EDITORIAL COMMENT

This is a nice, small study that deserves some considerations. The authors described urodynamic over activity particularities related to diabetes mellitus. In my opinion, more important than isolated numbers themselves, are the implications of such increasing data relating diabetes mellitus and urinary tract disorders.

We cannot forget the potential impact that systemic illness and its repercussions can lead to the urinary tract, and how its treatment could change things, over time. Although small, the numbers showed by the authors bring some questions in mind.

1. Diabetic patients in the study have had this diagnosis for at least, 3 years, but we do not know for how long, exactly, they have this condition. Probably, the fact that all others variables analyzed did not show statistical differences when compared to the control group, can be related to the fact that these patients don´t have very lasting disease timing.
Clinical and experimental data confirm that detrusor over activity, both neurogenic and myogenic can be present in diabetic neuropathic bladders. Moreover: these findings are normally seem in earlier stages of the disease, whereas detrusor under activity appears to be linked to later stages of DM. (Does diabetes mellitus-induced bladder remodeling affect lower urinary tract function? Kirschner-Hermanns R, Daneshgari F, Vahabi B, Birder L, Oelke M, Chacko S.Neurourol Urodyn. 2012; 31(3): 359-64. ICI-RS 2011). So, if the sample was bigger, and the lasting time of the disease analyzed, could the results associated to the other variables be different? This is a nice question to be answered, in the future. The same doubt can be extended to the glycemic control. Do the patients that have betters long lasting glycemic controls show less symptoms and urodynamic changes over time? If so, could we act as prophylactic agents of chronic urinary tract disturbances in such group?
2. The authors also raise the question about diabetic screening of patients with IDO and some urodynamic findings. Thinking on the clinical nature of the diagnosis of hyperactive bladder, I agree with such screening but I rather do it on all patients with this clinical picture, instead of doing it only in patients submitted to such study with critical findings. It looks like the practical application of an increasing body of knowledge on the complex etiology of urinary tract disorders.

I expect that more studies like this can help us to understand the real relationship of chronic illness like diabetes and urinary tract disorders in order to open new perspectives of, perhaps, prophylactic treatments in the future.

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ORIGINAL ARTICLE

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A comparative analisys of pelvic floor muscle strength in women with stress and urge urinary incontinence

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ABSTRACT

Aims: To assess pelvic floor muscle (PFM) strength in women with stress urinary incontinence (SUI) and urge urinary incontinence (UUI).

Materials and Methods: 51 women were prospectively divided into two groups, according to the symptoms as SUI (G1 = 22) or UUI (G2 = 29). Demographic data, such as number of pads/ 24 hours, number of micturations/ 24 hours and nocturia, delay time of urgent void (i.e., the time period for which an urgent void could be voluntarily postponed), number of parity and vaginal deliveries were obtained using a clinical questionnaire. Objective urine loss was evaluated by 60-min. Pad Test, subjective urine stream interruption test (UST) and visual survey of perineal contraction. Objective evaluations of PFM were performed in all patients (vaginal manometry).

Results: Median of age, mean number of pads / 24 hours, nocturia and warning time were significantly higher in UUI comparing to SUI group. During UST, 45.45% in G1 and 3.44%, in G2, were able to interrupt the urine stream ($p < 0.001$). The 60-min. Pad Test was significantly higher in G2 compared to G1 women (2.7 ± 2.4 vs 1.5 ± 1.9 respectively, $p = 0.049$). Objective evaluation of PFM strength was significantly higher in the SUI than in the UUI patients. No statistical difference was observed regarding other studied parameters.

Conclusion: Pelvic floor muscle weakness was significantly higher in women with UUI when compared to SUI.

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Pelvic Floor; Women; Urinary Incontinence; Stress; Urinary Incontinence; Urge

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INTRODUCTION

Stress urinary incontinence (SUI) is considered when involuntary leakage of urine on effort or exertion occurs (1,2). Urge urinary incontinence (UUI) is defined as urinary incontinence (UI) accompanied by urgency (1). This work will be restricted to SUI and UUI.

Urinary incontinence may involve important psychosocial implications; there is a significant greater proportion of patients (60%) with urge incontinence with previous history of depression than those with SUI (14%) (3).

Pelvic floor muscle (PFM) function evaluation may play an important role in the diagnosis and treatment of female urinary incontinence. Amaro et al. reported significant decreased in PFM strength on incontinent women when compared with continent ones (4), showing that the anatomical and functional dete-

riorations of these striated muscles may cause urinary and fecal incontinence. Vaginal delivery causes several degrees of PFM and connective tissue damage (5). The recovery of these muscles could be therapeutic (6). Some authors observed a positive correlation between increase in PFM strength and improvement in SUI and quality of life (7-10).

Pelvic floor muscle evaluation can be performed using objective and subjective parameters. However, there is no consensus about the best clinical assessment of this muscle (11).

Kegel (12) was the first to describe the perineometer using an endovaginal probe to evaluate the pelvic floor muscle contraction.

Morked (13) et al. demonstrated that continent women had significant higher maximal vaginal squeeze pressure and muscle thickness increment compared with incontinent women.

Visual inspection and digital tests are easy and reliable methods by which insight can be gained into the multi-muscular activity and coordination of the PFM and lower abdominal muscles in continent and incontinent women (14). Lynch and Aronoff (15), in a small sample size, observed better agreement using the tampon scale than the digital scale. Several approaches for evaluating PFM have been described, but without documented validation.

Measurement of PFM function and strength is important in analyzing which is the best training protocol to use, and may be an important tool to provide biofeedback and motivation throughout the training period.

The aim of this study was to assess pelvic floor muscle function and its correlation with SUI and UUI.

MATERIALS AND METHODS

Fifty one women were prospectively distributed into two groups. Group G1 (n = 22) included women with SUI and Group G2 (n = 29), with UUI. Urodynamic studies were not realized for any patient; they were selected using a non validated questionnaire which classified the responders as UUI patients that were not able to postpone the urgency time period in more than 15 minutes. Patients were classified as SUI when ordinary movements such as coughing, walking etc, caused urine leakage. This study was approved by the Bioethics Commission of the Paraná State University.

Demographic data, such as daily fluid intake, number of pads and micturations for 24 hours (24-hour voiding diary), delay time of urgent void (i.e., the time period for which an urgent void could be voluntarily postponed), visual analog scale (VAS) (3) for assessing the level of degree of wetness and discomfort sensation, number of parity and vaginal deliveries were obtained using a clinical questionnaire (3).

Subjective assessment consisted of urinary stream interruption test (UST) and visual analysis of perineal muscle contractions. The patients were evaluated in supine position with a pillow under their head, straight knees and legs abducted. The PFM contractions were evaluated as present or absent depending on visualization.

Objective urine loss was evaluated by the 60-min. pad test (4,16).

Objective evaluation of perineal muscle strength was made using a portable perineometer (Peritron 9300+) connected to a balloon catheter, size 11x 2.6 cm, inserted into the vagina. The balloon was located 1 cm from the outside of vaginal conduit, positioning the middle of the balloon 3.5 cm inside the introitus vagina (4,6). Measurement of maximum and mean squeeze pressure, and holding period in seconds were assessed in supine position. Only contractions with simultaneous visible inward movement of the perineum were accepted as correct. All evaluations were assessed by a single physiotherapist.

Statistical Analysis

Analysis of the association between incontinent groups (SUI and UUI) and PFM contraction was performed by the Goodman's test with multinomial distribution (17).

Perineum muscle force comparison was made by Student T test for independent samples (17). Differences were considered significant for p value < 0.05.

RESULTS

The average age of UUI patients was significantly higher than the SUI ones (54 yrs. vs. 45 yrs. respectively, $p < 0.05$).

The daily fluid intake was significantly lower in UUI group than in SUI (Table-1).

UUI patients had to use a significantly higher number of 24 hours pads when compared to SUI group (Table-1).

Regarding the average number of micturitions in 24 hours, there was no statistical difference between groups (Table-1). However, nocturia was significantly higher in UUI patients (Table-1).

The average delay time of urgent void was significantly lower in UUI than in SUI group (Table-1).

In VAS, the dry perception was significantly lower in UUI patients compared to SUI ones (38% vs. 68% respectively, $p = 0.04$). The discomfort sensation was significantly higher in the UUI group than SUI (76% vs. 50% respectively, $p = 0.05$).

There was no statistical difference between groups considering body mass index, number of parities and vaginal deliveries (Table-1).

The number of patients who were able to interrupt the urinary stream was significantly higher in SUI group (Table-2).

There was no statistical difference between both groups in the visual analysis of perineal muscle contractions (Table-3).

PFM strength was significantly higher in the SUI group (Table-4).

DISCUSSION

We observed that women were significantly older in the UUI. Other authors have observed more prevalent overactive bladder during the aging process, that could be considered an important factor in urgency genesis (18,19).

In our series, the fluid intake was significantly lower in UUI group, this demonstrates that these women may have attempted to decrease the fluid ingestion to avoid urinary leak due to urgency as self-treatment. Other authors (20) reported that avoiding excessive fluid intake can contribute to reduce the UUI symptoms in women taking anticholinergic medications; however, additional individualized instructions along with other be-

Table 1 - Population characteristics of patients with stress urinary incontinence (SUI) (n = 22) and urgency urinary incontinence (UUI) (n = 29).

Variable	Group		Statistical Results (p- value)
	SUI (G1)	UUI (G2)	
Body Mass Index	27.3 ± 3.7	28.6 ± 5.5	p = 0.373
Daily Fluid Intake (l)	1.8 ± 0.7	1.3 ± 0.5	p = 0.041
Number of Pads / 24 Hours	3.0 ± 1.5	5.0 ± 2.5	p = 0.004
Micturations / 24 Hours	6.1 ± 1.2	6.7 ± 1.8	p = 0.236
Nocturia	0.9 ± 0.9	1.7 ± 0.9	p = 0.010
Delay time of urgent void (min)	20.1 ± 12.2	2.1 ± 2.2	p < 0.001
Number of parity	5.9 ± 2.9	5.5 ± 2.6	p = 0.691
Vaginal deliveries	4.4 ± 2.8	3.9 ± 2.0	p = 0.551
60-min. Pad Test (g)	1.5 ± 1.9	2.7 ± 2.4	p = 0.049

Table 2 - Assessment of pelvic floor performance with stream interruption test expressed in proportional value.

Group	Proportion of patients with capacity to interrupt the urinary stream		Total
	YES	NO	
G1	45.45% (10)	54.55%(12)	100% (22)
G2	3.44% (1)	96.56% (28)	100% (29)
Statistical Results (p-value)	p < 0.001	p < 0.001	

Table 3 - Visual analysis of perineal muscle contractions.

Group	Contraction		Total (n)
	Absent (n/%)	Present (n/%)	
1 (SUI)	5 (22.7%)	17 (77.3%)	22
2 (UUI)	8 (27.6%)	21 (72.4%)	29

$X^2 = 0.16$ ($p = 0.69$)

Table 4 - Perineometer evaluation of perineal muscle strength.

Group	Perineometer			Total
	Maximum Peak ($cm\ H_2O$)	Mean Peak ($cm\ H_2O$)	Duration (S)	
1 (SUI)	26.50 ± 3.00	16.56 ± 1.19	9.54 ± 0.18	22
2 (UUI)	21.70 ± 0.79	13.72 ± 0.56	8.43 ± 0.42	29
Statistical results	p < 0.001	p < 0.001	p < 0.001	

havioral therapies did little to further outcome improvement. It shows that little is known about the effect of fluid management in women with UUI. Despite this, some authors (21) have proposed to reduce bladder irritants such as acid food, alcohol, and caffeine which may decrease the number of UI episodes in patients with overactive bladder.

As for number of pads/24 hours, nocturia, delay time of urgent void, VAS to wetness sensation and discomfort, we observed worse results in UUI group, demonstrating that urgency may affect people's daily routine. Stewart et al. (22) observed that UUI is bothersome and is associated with decrease of quality of life. UUI patients may also change their behavior such as prophylactic urination and fluid restriction. Incontinence episodes have also shown to be perceived by women as a barrier to perineal exercises (23).

The urine stream interruption test (UST) is considered an objective method to quantify pelvic floor muscle strength (4). Using UST to assess contraction ability and pelvic floor muscle strength, we

observed that only 1% of UUI patients and 45% of SUI women were able to interrupt the urine stream. This suggested that women with urge incontinence may have a lower perception and strength of pelvic floor muscle. Amaro et al. (4), studying the effects of UST in incontinent patients in a control trial, concluded that incontinent women also have a lower perception of PFM, demonstrating that the strength of perineal muscle during exercises can also be used as rehabilitation treatment of SUI and UUI women.

The visual analysis of perineal muscle contractions showed no statistical difference between groups, what may demonstrate the ineffectiveness of this modality of PFM evaluation, whereas the perineometer test presented a significant deficit of muscular strength in the UUI group compared to the SUI one. Despite this, Deveuse et al. (14) observed that visual inspection and digital test are easy and reliable methods to evaluate PFM strength.

Women with weakness in perineal muscles were unable to effectively contract their PFM to inhibit detrusor contractions. This fact may inactivate the mechanism that provides negative feedback worsening the urgency symptoms (24). In our study, the pelvic floor muscle weakness was significantly higher in women with UUI when compared to SUI. This demonstrated that the status of the levator ani muscle can be important to functional and objective evaluation of pelvic floor muscle to assess UUI and SUI patients.

CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Prognostic value of urethral mobility and valsalva leak point pressure for female transobturator sling procedure

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ABSTRACT

Purpose: To analyze the influence of urethral mobility and Valsalva leak point pressure on postoperative outcomes of transobturator sling (TOT) for female stress urinary incontinence.

Materials and Methods: A prospective cohort was conducted including 66 patients submitted to TOT from March 2006 to May 2009. Urethral hypermobility was defined as mobility ≥ 30º on Q-tip test, and Valsalva leak point pressure (VLPP) was classified as greater than 60 cmH_2O or 60 and less on preoperative urodynamics. These parameters were compared through well defined postoperative objective and subjective success criteria. Intensity of urinary leakage and quality of life was analysed by ICIQ-SF. Statistical analysis was accomplished and the results rendered significant if $p < 0.05$.

Results: Mean follow up was 10 months (3 to 28). Mean age was 55 years (33 to 80), 70% were white and 30% African descendent, mean body mass index was 27 (21 to 38), average vaginal and abdominal deliveries were 2.8 and 0.5 respectively. A quarter had prior stress incontinence surgery. Patients with urethral hypermobility had higher objective success rates (98% versus 81.25%, $p = 0.04$). The subjective success rate was also greater in the hypermobility group (84% versus 62.5%), but statistical significance was not reached ($p = 0.07$). VLPP had no influence on either objective or subjective postoperative success rates ($p = 0.17$ and 0.34, respectively). In the subgroup analysis, those with low mobility and high VLPP had worse objective success rates in comparison to the group with hypermobility and low VLPP ($p = 0.04$) and also in relation to the remaining of the studied population. Other possible prognostic factors (previous surgery, mixed incontinence, gestational status) had no influence on success rates.

Conclusions: High urethral mobility, regardless of the sphincteric status indicated by VLPP, is a favorable prognostic factor for tension-free transobturator tape procedure. No relationship was demonstrated between postoperative success rates and VLPP.

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Key words:
Urinary Incontinence; Suburethral Slings; Outcome Assessment (Health Care)

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INTRODUCTION

Stress urinary incontinence (SUI) is a significant health problem worldwide, affecting up to 30% of adult women (1). Despite extensive studies in this field, the exact pathogenesis is still imperfectly comprehended. Nonetheless, recent evidence-based theories introduced more realistic insights about the mechanisms responsible for continence and, more importantly, about how to restore it in incontinent women.

The most accepted theory, the "hammock theory", states the urethra is closed during straining as a result of compression against a ham-

mock-like, supporting layer of connective tissue, and not by assuming truly intra-abdominal position, as previously thought (2). Base on this hypothesis, mid-urethral sling procedures were introduced, providing reinforcement of a defective suburethral support rather than correcting hypermobility, leading bladder neck procedures to be virtually abandoned (3). While this is true, it's still debated the prognostic significance of urethral mobility and Valsalva leak point pressure (VLPP), which is a marker of intrinsic sphincter function (4-7). So we decided to study the influence of these two determinants on postoperative outcomes of the transobturator sling procedure. This study aims to analyze independent influence of urethral mobility and Valsalva leak point pressure on postoperative outcomes of transobturator sling (TOT) for female stress urinary incontinence in the same group of patients.

MATERIALS AND METHODS

The study protocol was approved by the Ethics Committee of the Hospital. All the patients understood and signed the informed consent.

From March 2006 to May 2009, 73 consecutive patients with urodynamically proved stress or mixed urinary incontinence submitted to tension-free transobturator tape procedure (TOT) were included into a prospective cohort. The operations followed Delorme's description, using a polypropylene mesh, under spinal anesthesia (3).

Exclusion criteria included neurogenic bladder, concomitant urogenital prolapsed greater than POP-Q (Pelvic Organ Prolapse - Quantification) stage 1, pregnancy and malignancy. Patients who had received previous surgical treatment, either a sling or a colposuspension operation, were not excluded.

Preoperatively, data including age, body mass index (BMI), previous surgeries and parity were collected. Urethral mobility was measured using Q-tip test in the supine position and VLPP (at maximal cystometric capacity) through multichannel urodynamics following International Continence Society (ICS) standardisation (8). Hypermobility was defined as mobility ≥ t^o and VLPP was classified as high when > 60 cmH_2O and low when 60 or less, according to previously established criteria (9-11).

For Q-tip tests, a sterile lubricated cotton swab was placed into the bladder through the meatus and withdrawn until resistance indicated correct positioning at urethrovesical junction. The angle between the cotton swab at rest and after maximal Valsalva maneuver in degrees was defined as the urethral mobility (measurements were taken using a goniometer placed against the patient's perineum).

The sling material used was monofilament polypropylene mesh (Intracorp™, Venkuri, Brazil) through outside-in technique.

On postoperative visits, 3 and 6 months, and after that every 6 months, patients responded questions about their continence status and were classified as total continence, stress incontinence, urgency incontinence or both (mixed incontinence). They also were questioned about the need of using pads and satisfaction with the procedure according to a visual analogic scale from 0 to 10. A full bladder standing Valsalva and cough maneuver completed the evaluation. If the patient did not leak she was asked to repeat the maneuver bending the knees. A post test void of, at least, 200 mL was necessary for the test to be considered valid.

Intensity of urinary leakage and quality of life was assessed before and after surgery using the International Consultation on Incontinence Questionnaire - Short Form (ICIQ-SF), as validated in the Portuguese language for Brazilian population (12).

Analysis of outcomes

Postoperative objective success was defined as absence of any urinary loss during full bladder standing Valsalva/cough maneuver and no need of pads, while subjective success was achieved when patients considered themselves much better or cured (Question: How do you feel today about your bladder problem comparing to before the surgery? Answers: cured, much better, better, unchanged, worse and much worse), the level of satisfaction was ≥ 8 (visual analogic scale from 0 to 10) and there was no report of

stress incontinence after surgery. Objective and subjective success rates were compared between the groups (high versus low mobility, low VLPP versus high VLPP). To analyze independent outcome influence of the two variables (mobility and VLPP), we also divided the patients into four subgroups: subgroup I, low mobility and high VLPP; subgroup II, low mobility and low VLPP; subgroup III, high mobility and high VLPP; subgroup IV, high mobility and low VLPP.

All terminology followed that proposed by the ICS (13). Statistical analysis was performed by a professional, applying the Student t-test or Mann-Whitney test for continuous variables, according to the distribution (parametric or nonparametric). The Chi-square test, Fisher's exact test or its extension were used for categorical variables. The results rendered significant if $p < 0.05$.

RESULTS

Among the 73 patients submitted to the TOT procedure, seven were excluded due to incomplete preoperative data (VLPP or urethral mobility) and 66 patients were included for analysis. Mean follow-up was 10 months (3 to 28). Mean age was 55 years (33 to 80), 70% were white and 30% African descendent, mean body mass index was 27 (21 to 38), average vaginal and abdominal deliveries were 2.8 and 0.5 respectively. A quarter had prior stress incontinence surgery. Overall, 94% and 79% of our patients were considered as a success according to objective and subjective success criteria, respectively. There were two cases of mesh extrusion to the vaginal wall and one case of erosion to the bladder neck. None of them presented local severe infection and were submitted to the correcting surgery electively. They were all healthy and continent on completion of follow-up.

Baseline characteristics (age, BMI, gestational status, preoperative ICIQ-SF, type of incontinence and previous incontinence surgery) had similar distribution when different groups were compared. These possible prognostic factors were individually analysed and had no influence on postoperative outcomes. Mean preoperative ICIQ-SF was 15.5 and 16.7 in patients with low and high mobility ($p = 0.2$ - Mann-Whitney's test), respectively.

Urethral hypermobility was an important determinant of objective success ($p = 0.04$), as just one patient with mobility $\geq 30^{o}$ failed therapy (Table-1). Subjective success rate was also higher in this group, but the difference did not reach statistical significance ($p = 0.07$). Conversely, VLPP had no role as prognostic factor, either when analysed as categorical variable (Table-2) or when numeric VLPP values were compared. In the objective success analysis, mean VLPP was 77 cmH_20 and 95 cmH_20 in successful and unsuccessful cases, respectively ($p = 0.21$ - Mann-Whitney's test). In the subjective success analysis, patients who failed therapy had mean VLPP similar to those who succeed (79.3 cmH_20 versus 73.6 cmH_20, respectively) ($p = 0.31$ - Mann-Whitney's test).

There was no statistical difference between mean postoperative ICIQ-SF in the presence of high and low mobility (mean 3.7 and 2.8, respectively) ($p = 0.16$).

In the subgroup analysis, patients with concomitant low mobility and high VLPP (subgroup I) had worse objective success rates when

Table 1 - Influence of urethral mobility on postoperative success rates.

Urethral mobility	Objective success - n(%)		p*	Subjective success - n(%)		p*
	Yes	No		Yes	No	
Mobility $< 30^{o}$	13 (81)	3 (18.7)		10 (62.5)	6 (37.5)	
Mobility $\geq 30^{o}$	49 (98)	1 (2)	0.041	42 (84)	8 (16)	0.07
Total	**62 (94)**	**4 (6)**		**52 (78.8)**	**14 (21.2)**	

* Fisher's exact test.

compared to subgroup VI, those with hypermobility and low VLPP (Table-3). The former also presented lower rates of objective (p = 0.01) and subjective (p = 0.04) success in relation to the remaining of the studied population, subgroups II, III and IV (Table-4).

DISCUSSION

This paper reiterates the effectiveness of TOT for the treatment of SUI and has similar results to previously published data (14,15). Notwithstanding, as the number of surgeries per-

Table 2 - Role of Valsalva leak point pressure on postoperative success rates.

Valsalva leak point pressure	Objective success – n(%)		p*	Subjective success – n(%)		p*
	Yes	No		Yes	No	
≤ 60 cmH_20	23 (100)	0 (0)		17 (73.9)	6 (26.1)	
> 60 cmH_20	39 (90.7)	4 (9.3)	0.17	35 (81.4)	8 (18.6)	0.34
Total	**62 (93.9)**	**4 (6.1)**		**52 (78.8)**	**14 (21.2)**	

* Fisher's exact test.

Table 3 - Comparison between specific subgroups.

Subgroups	Objective success – n(%)		p*	Subjective success – n(%)		p*
	Yes	No		Yes	No	
Mobility < 30º and VLPP > 60 cmH_20	8 (72.7)	3 (27.3)		6 (54.5)	5 (45.5)	
Mobility ≥ 30º and VLPP ≤ 60 cmH_20	18 (100)	0 (0)	0.04	13 (72.2)	5 (27.8)	0.28
Total	**26 (89.7)**	**3 (10.3)**		**19 (65.5)**	**10 (34.5)**	

* Fisher's exact test.

Table 4 - Subgroups compared to the remaining of the studied population.

Subgroups	Objective success – n (%)		p*	Subjective success – n (%)		p*
	Yes	No		Yes	No	
Mobility < 30º and VLPP > 60 cmH_20	8 (72.7)	3 (27.3)		6 (54.5)	5 (45.5)	
			0.01			0.04
Other patients	54 (98.2)	1 (1.8)		46 (83.6)	9 (16.4)	
Mobility ≥ 30º and VLPP ≤ 60 cmH_20	18 (100)	0 (0)		13 (72.2)	5 (27.8)	
			0.27			0.31
Other patients	44 (91.7)	4 (8.3)		39 (81.2)	9 (18.8)	

* Fisher's exact test.

formed progressively increases in the contemporary scenario of minimally invasive procedures indicated more indulgently, surgeons frequently faces the difficult situation of a patient, with high expectations, with persisting incontinence after surgery. The need to refine selection criteria for TOT motivated us to conduct a study to shed some light on what are the determinants of postoperative outcomes, considering that they are imperfectly understood for the TOT procedure (introduced in 2001 by Delorme (3)) and that controversy exists about its efficacy in patients with sphincter deficiency. (7,16-19).

There are few prospective series addressing prognostic factors for TOT and results are conflicting (16). Minaglia (6) and Karateke (17) found that low urethral mobility was associated with higher rates of postoperative incontinence, while Paick (20) states the cure rate was not significantly lower in the group without a mobile urethra (< 30º). None of them concomitantly analysed the role of VLPP, although the last showed that mean VLPP was similar in patients with and without hypermobility. Two other studies concluded that VLPP was a prognostic determinant, but urethral mobility was not discussed (18,19).

To more adequately analyze the interaction between urethral mobility and sphincter intrinsic dysfunction (indicated by VLPP ≤ 60 cmH_20), we compared subgroups and demonstrated that high urethral mobility predicts cure, even when VLPP indicates a theoretically more dysfunctional sphincter. Low mobility was a predictor of failure even in women with good intrinsic sphincter function (high VLPP), although cure rates remain acceptable. This type of analysis seeks to circumvent selection bias, as analyzing urethral mobility or VLPP singly. A contemporary Turkish study had a similar conclusion (21).

Current evidence suggests not only that urethral hypermobility is equally common in women with lower urinary tract symptoms and SUI, but also that intrinsic sphincteric deficiency and urethral hypermobility may coexist and do not define discrete classes of patients with SUI (10). Additionally, previous reports demonstrate that correction of hypermobility is not required to obtain continence either for retropubic or transobturator sling procedure (14,22).

For all these reasons, urethral hypermobility should be no more considered an etiology of incontinence as leak of urine always implies some degree of sphincteric insufficiency. This expression was elaborated about half-century ago when suspension procedures were the rule, according to the belief that incontinence resulted from excessive downward movement of the urethra, leaving the abdominal cavity and leading abdominal pressure to be transmitted to the bladder and not to the urethra (23,24). The term itself is incorrect because "hypermobility" implies a mobility that exceeds normal values producing disease and, in reality, theses values are unknown (despite classic textbook teaching) and all evidence suggests high mobility does not cause incontinence. Accordingly, patients with SUI should be characterized by VLPP and urethral mobility, but not classified by them (4,10,16). Nonetheless, a general agreement in this issue has not been reached (11).

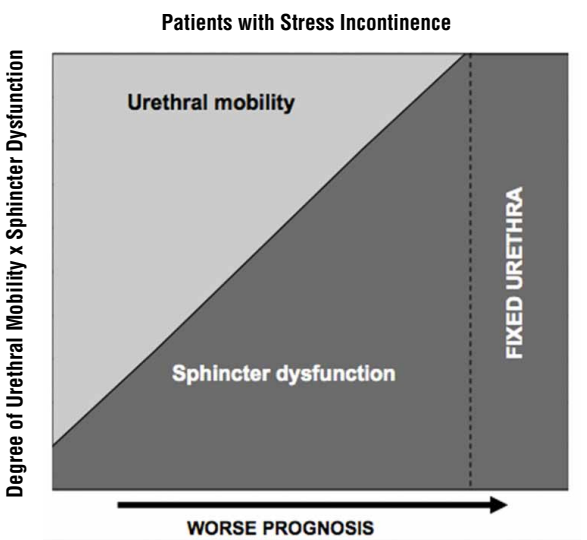
Theoretically, a successful sling procedure restores continence not by increasing resting urethral pressure but by providing a support the holds the mid-urethra in place while the proximal urethra descends under stress, allowing better pressure transmission and, more importantly, a kinking of urethra during straining (16,25). When urethra doesn't move well, this kinking does not occur. That's the advocated mechanism for urethral mobility as a prognostic factor. We propose a graphic to illustrate how sphincter dysfunction and urethral mobility interact to determine prognosis following sling procedure (Figure-1). Similar illustration has been presented in lectures and plenary sessions, but note that in this graphic SUI is not possible without some degree of sphincter deficiency.

This study presents several limitations including the low number of subjects, short follow-up time and lack of multivariable analysis, but it was clearly possible to demonstrate and differentiate prognostic influence of urethral mobility from VLPP.

Finally, this paper also addresses subjective improvement and, interestingly, some of the differences favoring urethral mobility as a prognostic factor were not significant when subjective success rates were compared. Although this may seem discordant initially, subjective success allows a wider

and deeper evaluation of response to treatment as it takes into account patient's satisfaction and opinions. The lower subjective success rates may be explained by diverse patient's expectations that would not be accomplished by the sling procedure. Therefore, subjective success depends on how well patients are informed about the goals of surgical treatment. More cases will probably be needed to render differences, according to subjective success criteria, significant.

Figure 1 - Prognostic interaction between urethral mobility and sphincter dysfunction in women with SUI after sling procedure. Note that some degree of sphincter dysfunction is required for incontinence to occur.



CONCLUSIONS

Urethral mobility was an important prognostic factor for TOT surgery. Low urethral mobility predicts higher failure rates, but it does not preclude surgery as most of these patients are cured following the procedure. No association was found between postoperative outcomes and preoperative VLPP.

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CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Stereological and Biochemical Analysis of the Urethral Edges in Patients Submitted to End-to-End Anastomosis for Bulbar Urethral Stricture

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ABSTRACT

Purpose: To study the morphologic alterations in the proximal and distal urethral edges from patients submitted to end-to-end bulbar urethroplasty.

Materials and Methods: We analyzed 12 patients submitted to anastomotic urethroplasty to treat bulbar strictures less than 2.0 cm in length. After excision of the fibrotic segment to a 28Fr urethral caliber, we obtained biopsies from the spongious tissue of the free edges (proximal: PROX and distal: DIST). Controls included normal bulbar urethras obtained from autopsies of 10 age matched individuals. The samples were histologically processed for smooth muscle cells (SMC), elastic system fibers and collagen. Stereological analysis was performed to determine the volumetric density (Vv) of each element. Also, a biochemical analysis was performed to quantify the total collagen content.

Results: Vv of SMC was reduced in PROX (31.48 ± 7.01 $p < 0.05$) and similar in DIST when compared to controls (55.65 ± 9.60%) with no statistical difference. Elastic fibers were increased in PROX (25.70 ± 3.21%; $p < 0.05$) and were similar to controls in DIST (15.87 ± 4.26%). Total collagen concentration in PROX (46.39 ± 8.20 µg/mg), and DIST (47.96 ± 9.42 µg/mg) did not differ from controls (48.85 ± 6.91 µg/mg). Type III collagen was similarly present in all samples.

Conclusions: After excision of the stenotic segment to a caliber of 28Fr, the exposed and macroscopically normal urethral edges may present altered amounts of elastic fibers and SMC, but are free from fibrotic tissue. When excising the peri-stenotic tissue, the surgeon should be more careful in the proximal end, which is the most altered.

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INTRODUCTION

Male urethral stricture is characterized by a fibrous process, which leads to the development of a low compliance tissue, associated with reduction of the luminal diameter (1). Previous studies documented a negative correlation between urethral diameter and both the AUA urinary symptoms score and maximum flow rate (2).

These alterations can severely worse the voiding pattern and consequently compromise patient's quality of life (3). The extracellular matrix (ECM), notably its fibrous components such as collagen and elastic fibers, participates

actively in the tissue organization and in urethral physiopathology (4,5).

Structural changes observed in urethral stenosis were first described by Singh (1), who through light microscopy, observed that the collagen was increased and less organized. They also noted a replacement of muscle cells by dense collagen.

In urethral stenosis treatment, it is important to take into account its localization, extension and degree of fibrosis of the corpus spongiosum (6). The endoscopic treatment of stenosis through the incision of the fibrotic tissue, with its posterior reepithelization, can be an option when the stenotic segment is short and has a limited degree of fibrosis (7).

Surgical treatment should preferentially be done by an anastomotic urethroplasty with complete resection of the fibrous tissue. The anastomosis should be done with macroscopically wealthy edges. This technique is limited to stenotic segments not greater than 2.0 cm. In more extended strictures, substitution of the fibrous tissue by grafts is recommended (8). A previous study characterized the bulbar urethra stenosis and its fibrous tissue (9); however, there is little data on the surgically exposed edges. Indeed, it is noteworthy that investigations on this disease have concentrated on the fibrotic tissue (5,9).

A recent study has evaluated the surgically exposed urethral ends, but only qualitative histological techniques were used (10). This fact, together with other technical limitations, may render these results less reliable. Thus, knowledge about the exposed urethral ends, whose quality may affect the surgical success of the anastomotic urethroplasty, is still unsettled.

Therefore, the aim of the present study was to perform a stereological and biochemical analysis of the urethral edges in patients undergoing end-to-end anastomotic bulbar urethroplasty for stricture disease.

MATERIALS AND METHODS

The present work was approved by the Bio-Ethics Committee of our institution. This study was carried out in accordance with the ethical standards of the responsible institutional committee on human experimentation. From May 2006 to August 2010, we analyzed 12 patients aged 13 to 43 years (mean = 32) who had been submitted to end-to-end bulbar urethroplasty. The stenotic segment was shorter than 2.0 cm in all patients and was estimated by urethrocystogram and confirmed during surgery.

The etiology of the stenosis was idiopathic in 5 patients, inflammatory in 5 and traumatic in 2. All patients were submitted to uroflowmetry study and presented a maximum flow rate under 15 mL/s (mean 5.3 mL/s). No patient was diverted prior the surgery. The proximal (PROX) and distal (DIST) extremities of the stenosis were excised by the usual technique (11) and by the same surgeon. The macroscopically fibrotic tissue was completely removed until a 28Fr dilator could pass through the luminal openings of both urethral edges. If the urethral edges thus exposed were macroscopically healthy, based on the criteria of Mundy (12), but the luminal openings were not large enough for a 28Fr dilator, approximately 2-mm circular slices were consecutively sectioned from the free ends until the desired diameter was obtained. Afterwards, a biopsy was obtained from these edges.

The control group comprised macroscopically normal bulbar urethras obtained during autopsy of 10 individuals (mean age 29 years) who died of causes not related to the urinary system or to pelvic trauma.

Histology and Stereology

Tissue specimens were fixed in 10% buffered formalin and routinely processed for paraffin embedding. Sections of 5-µm were obtained and stained with: (a) Masson Thricome´s to label smooth muscle cells, (b) Weigert's resorcin-fuchsin with previous oxidation to stain elastic system fibers, and (c) Picrosirius red under polarized light to detect differences in overall collagen organization.

Also, we performed immunohistochemical analysis to specifically detect collagen type-III. The specificity of the Weigert's method was confirmed by immunolabeling with an anti-elastin antibody (monoclonal, E 4013, Sigma, Saint

Louis, MO, USA), and that of the smooth muscle staining by immunolabeling with an anti-smooth muscle alpha actin (Zymed Laboratories, 08-0106 predilute antibody).

Morphological data was quantified using stereological methods. For each Individual and for each histological staining technique, 5 sections of each edge (PROX, DIST, Control) were obtained, and for each section, 5 fields were analyzed. All images were photographed with a digital camera directly coupled to the microscope at a final magnification of X200 to SMC and X400 for Elastic Fibers. The volumetric density (Vv) of histological structures was then evaluated by superimposing the 100 point grid test system on the digital images, using the Image J program (NIH Image ®), (13), Figure-1A.

Biochemical Analysis

Immediately after excision during surgery or autopsy, urethral tissue samples were fixed in cold acetone for 24 hours at 4º C. The samples were then finely minced and submitted to two changes of 24 hours each in 40 mL of chloroform/methanol (2:1, v/v) at room temperature. The solvent was then decanted, and after incubation at 60º C for 30 minutes, a preparation of dry and defatted urethral tissue was obtained and weighed.

The concentration of total collagen in the urethral tissue was determined by a colorimetric hydroxyproline assay. Thus, 5 to 14 mg of dry, defatted urethral tissue were hydrolyzed in 6N HCl for 18 hours at 118º C as previously described (14).

The assay was then carried out in the neutralized hydrolyzates using a chloramin T method (15). Results were expressed as micrograms of hydroxyproline per milligram of dry, defatted tissue.

Statistics

Statistical procedures followed Sokal and Rohlf (16). Variations among the three groups for each quantitative parameter were analyzed by one-way ANOVA. When significant differences were detected, pairwise planned comparisons using the Bonferroni method were done between the control group and either the PROX or the DIST group. All numerical results are given as means ± standard deviations (SD), and statistical significance was considered when $p < 0.05$.

RESULTS

The quantitative analysis showed a decrease in the volumetric density (Vv) of smooth muscle cells in PROX (43.40%) edges, when compared to controls ($p < 0.05$), with no significant difference in the DIST edge (Figures 1B, 1C and 1D and Table).

The elastic system fibers quantification showed a 44.40% increase in the Vv in the PROX edges, when compared to controls ($p < 0.05$), while in the DIST edges the difference was not significant (Figure-2 and Table).

The Picrosirius Red stain showed a prevalence of greenish color in PROX edges whereas in the DIST edges there was a prevalence of a red/yellowish color, similar to controls. The imunohistochemical analysis revealed the presence of similar amounts of type III collagen in all samples (Figure-3).

Total collagen concentration in controls (48.85 ± 6.91 µg/mg), and in samples from the PROX (46.39 ± 8.20 µg/mg) and DIST (47.96 ± 9.42 µg/mg) edges did not differ significantly. Thus, the exposed edges of the two urethral ends after excision of the stenotic segment had similar collagen content and were free from fibrotic tissue.

DISCUSSION

In the best of our knowledge, this is the first study to perform a stereological and biochemical analysis on the urethral edges (proximal and distal) that will be anastomosed after resection of the stenosis in an end-to-end bulbar urethroplasty.

In the present work, we have found a decrease in the Vv of smooth muscle cells and an increase in the Vv of elastic fibers, both of which were more intense in the proximal urethral edge. Probably this happens since the proximal urethra is submitted to a much higher hydrostatic pressure, which can cause an increase in fibroblast activity (17). On the other hand, the urethra distally to

Figure 1 - Smooth muscle cells (SMC) distribution in urethral samples. A) Photomicrography of a control urethra showing the morphometric analysis. Quantification of smooth muscular cells using the software Image J Test grid. Masson's trichrome X200. B) Smooth muscle cells (SMC) distribution in control group. Smooth muscle cells (SMC) distribution in proximal edge of stenotic group and D) Smooth muscle cells (SMC) distribution in distal edge of stenotic group. Note marked decrease of SMC(red) in the proximal edges and less marked decrease in the distal edge, when compared to controls (differences statistically significant). Masson's trichrome, X200.

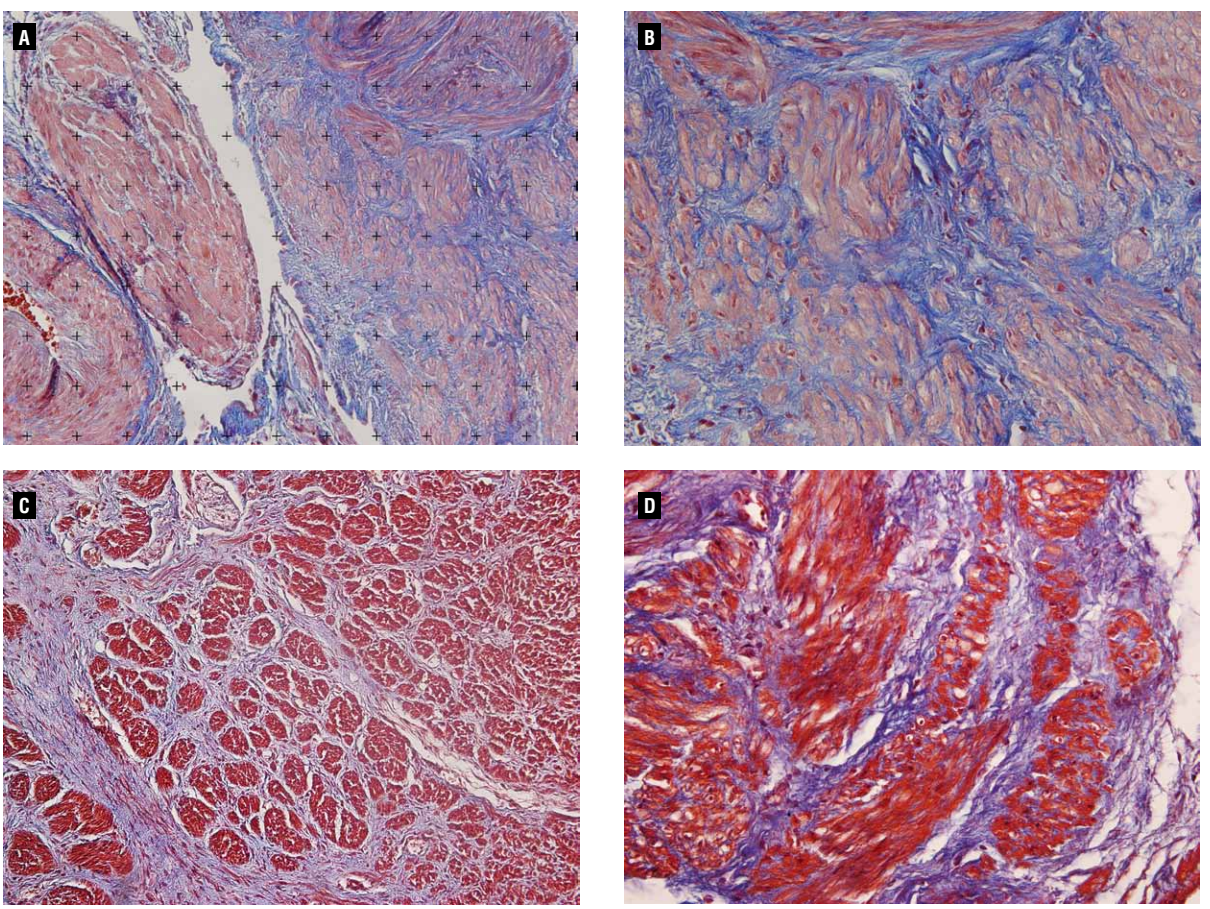


Table 1 - Stereological analysis of smooth muscle and elastic system fibers of urethral edges.

	Mean (%)	SD (±)
SM Controls	55.64	9.60
SM Prox	31.48	7.01
SM Dist	50.97	10.27
EF Controls	17.79	2.82
EF Prox	25.70	3.21
EF Dist	15.87	4.26

SM = smooth muscle; PROX = proximal urethral edges in stenotic group; DIST = distal urethral edges in stenotic group; EF = elastic fibers. $P < 0.01$ when comparing SM PROX with SM Controls. $P < 0.01$ when comparing EF PROX with EF controls.

Figure 2 - Elastic system fibers distribution in urethral samples from Controls (A), Proximal edge of stenotic group (B) and Distal edge of stenotic group (C). Note marked increase of elastic fibers in the proximal edge and similar content in the distal edge, when compared to controls. Weigert's Resorcin-Fuchsin, X400.

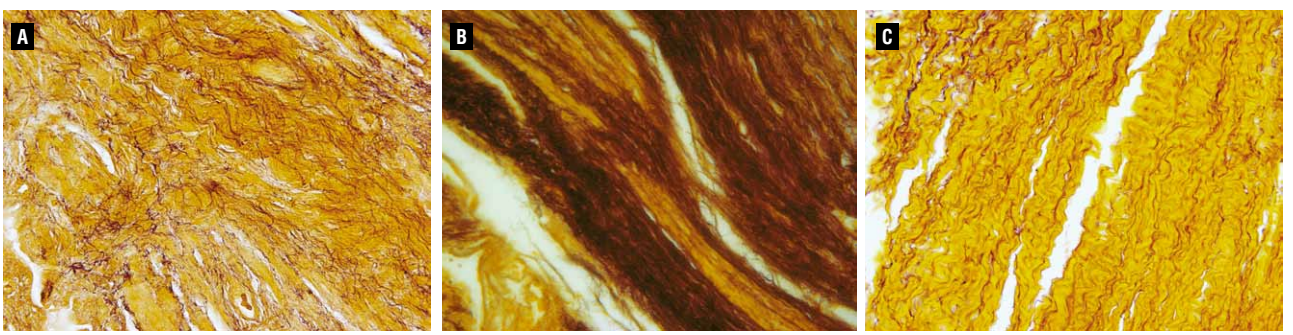
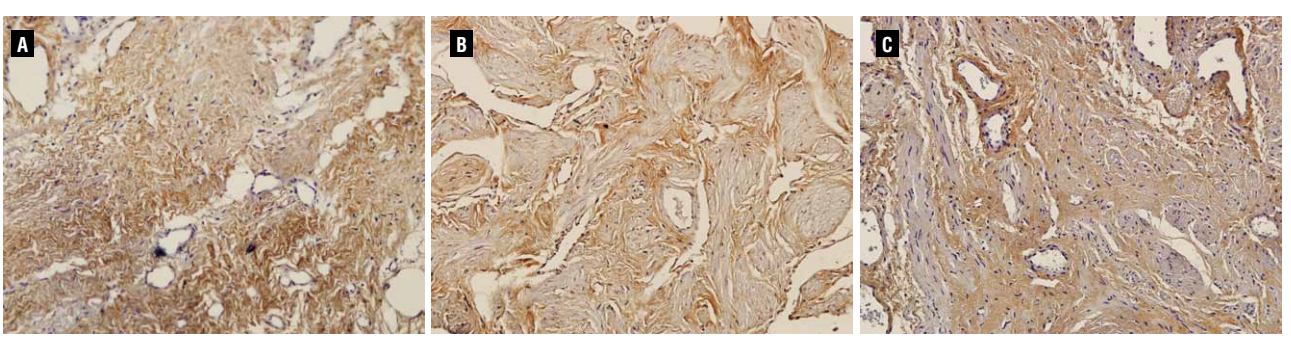


Figure 3 - Imunohistochemical staining for Type III collagen from Control group (A), Proximal edge of stenotic group (B) and Distal edge of stenotic group (C), showing a similar distribution in all groups (Immunolabelling, X200).



the stenosis is protected from high pressure and it could explain the less pronounced alterations.

It has been previously demonstrated that the lesions of nerves that carries the nitric oxide synthase (nNOS), in the cavernous body, leads to a fibrotic process associated to smooth muscle degeneration (18). A previous study of Cavalcanti et al. (19) documented an association of urethral stricture with alteration in nNOS imunorreactivity, even in small amounts of spongiofibrosis.

Based on these observations we could speculate that the smooth muscle reduction should be the primary event in urethral stenosis and fibrotic tissue formation. This event is intense in PROX margin, probably correlated to a hydrodistension process, which could also explain the structural differences between the urethral ends after excision of the stenotic area.

In the urethra distal to the stenosis, the tissue would be protected from higher pressure, and this could explain the less pronounced alterations at the distal edge, as commented previously. It is important to point out that none of the patients had cistostomy tubes (not diverted) prior surgery, and therefore, the patients had to void under high pressure due to the obstructive process.

Bastos (20) previously described the elastic system's fibers distribution in male fetal urethra and demonstrated that the concentration of elastic fibers in the spongy urethra increases significantly with age and this high concentration of elastic fibers may partially explain its high extensibility. Also, the elastic fibers concentration has a progressive increase during the fetal period gestation in a linear fashion, and continues to increase to the adulthood, which implies functional adaptation of the fetal male urethra.

In the present study, we observed an increase in elastic fibers volumetric density in

the proximal margin when compared to control group. Thus, increased synthesis of elastic fibers may be associated with excessive distension of an organ, as it also occurs during bladder outlet obstruction (21,22). In our analysis, the area once before occupied by smooth muscle cells, has been replaced by elastic fibers, suggesting, once more, the hydrodistension process suffered by the proximal margin.

When using the fibrotic resection to a 28Fr luminal caliber, the biochemical analysis demonstrated that all fibrotic tissue was satisfactorily excised, as collagen concentrations were similar to control group. These results show that the surgical technique described in the present study guaranteed complete resection of the fibrotic segment.

It has been variously shown that a shift towards greenish color in the Picrosirius polarization method is associated with less organized and/or degraded collagen (23). This color change may also occur in earlier phases of the remodeling and repair of connective tissues, when the synthesis of collagen type III is enhanced (24). In fact, a green shift in the staining pattern produced by the Picrosirius-polarization method is more related to the organization or packing state of collagen fibrils in the tissue (24), and therefore it is not necessarily an indicative of the presence of collagen type III.

To test between these possibilities in our samples, we immunostained urethral sections with an anti-type III collagen antibody, which showed that the intensity and distribution of the labeling was similar in all three groups. Additionally, the biochemical assay indicated equal amounts of total collagen in the urethral edges.

Thus, and based on these findings, the results of the Picrosirius-polarization method suggest that collagen matrix at the proximal urethral edge is disrupted or degraded, rather than fibrotic, which is consistent with a higher hydrostatic pressure proximal to the stenotic segment. It should be mentioned that these results are in stark contrast with a recent report that used this same surgical procedure (10). In that study by Da Silva (10), urethral samples were stained with the Picrosirius-polarization method, and based solely on the generated color patterns, the results were expressed as a ratio of collagen types I and III.

Such a result implies that colors were quantified, although no information was provided on how this was done. Further, in their description of the results, the authors of that study limited themselves to stating that the ratio was altered at the proximal urethral edges. This again is odd because, insofar as putative collagen types, or ratios thereof, were quantitated, one could tell whether their amounts were increased or decreased.

Although we did not directly assess inflammatory cells at the urethral edges, the fact the collagen type III remained unchanged in samples from stricture patients suggests that there is no major inflammatory reaction in the tissue. This is also at variance with what was found by Da Silva (10), and it might be explained by differences in the surgical technique.

For example, after excision of the stenotic segment, the criteria normally used to consider the exposed urethral edges as suitable for anastomosis are their macroscopic aspect (10,12). However, in our evaluation of tissue status, a healthy edge had in addition a normal luminal opening, as determined by the passage of a 28Fr dilator.

This approach may set the position of excision farther from the stenotic area, so that the exposed edges would be less altered. A healthier urethral end is likely to increase the success rate of the surgical anastomosis (25). However, because we do not have follow-up data for our patients, we cannot ascertain whether our particular technique improved the long-term outcome of the anastomosis.

Da Silva et al. (10) claimed to have followed their patients, yet no data on this regard was reported.

The study of tissue components in anastomotic margins should improve our understanding of the physiopathology process in urethral stricture and can be applied directly to the development of new therapeutic options.

In conclusion, our results have shown that, after excision of the stenotic segment to a luminal caliber of 28Fr, the exposed and macroscopically proximal edge may present altered

amounts of elastic fibers and SMC, as well as structural modifications in collagen, but are free from fibrotic and overtly inflammatory tissue.

These alterations are consistent with a higher hydrostatic pressure in the proximal urethra. All histological quantitative and qualitative findings of the present research indicate that when excising the peri-stenotic tissue, the surgeon should be more careful in the proximal end.

ACKNOWLEDGEMENTS

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ABBREVIATIONS

SMC = smooth muscle cells
PROX = proximal urethral edge
DIST = distal urethral edge
Vv = volumetric density

CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Finasteride for recurrent priapism in children and adolescents: A report on 5 cases

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ABSTRACT

Purpose: Recurrent priapism is prevalent in children. Different medications have been used to avoid new episodes, however, there is no consensus regarding the best option. The use of finasteride to treat priapism in adults has already been tested. The aim of the present study was to test the hypothesis that a low dose of finasteride would be effective in preventing recurrent priapism in children.

Materials and Methods: Since 2007, five children and adolescents with recurrent episodes of priapism have been treated with finasteride in our department, and the medical records of these patients were reviewed for this study. In four cases, the dose used was 1 mg a day, while the remaining patient used 1 mg twice a day.

Results: Prior to initiating finasteride treatment, one patient reported having had 6 episodes of acute priapism, while the remaining patients had more than 10 episodes. One of the patients reported having stuttering priapism almost daily. With a mean follow-up of 20 months, four patients had no episodes and only one patient complained of sporadic and shorter duration episodes.

Conclusions: These initial results suggest that a low daily dose of finasteride appears to represent an effective and safe form of treatment for recurrent priapism in children and adolescents with SCD. However, in order to confirm these initial findings, studies with a large population and a control group are essential.

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Priapism; Child; Anemia, Sickle Cell; Finasteride

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INTRODUCTION

Priapism is defined as an involuntary erection unassociated with sexual arousal that may be painful and persistent. It is considered a medical emergency that may result in erectile dysfunction if not treated promptly. Priapism in children is generally associated with sickle cell disease (SCD) (1-3). The incidence of priapism in children with SCD is estimated to be between 35% and 89% (1,3). Approximately 27% of patients with sickle cell disease between 5 and 20 years of age have at least one episode of priapism and the probability of having at least one episode of priapism by age 20 was reported to be 89% (4). It is generally presented clinically as a painful, self-limiting event of short duration; however, recurrent episodes of priapism may result in physical and emotional damage in both children and adults (5).

Recurrent priapism is common in children (2,5). Different medications have been used to prevent the occurrence of new episodes; however, there is no consensus on the best option (6). Hydroxyurea is an alternative for the prevention of attacks of severe vaso-occlusive disease, but its benefits for the treatment of priapism are not yet fully established (7,8).

The guidelines of the American urological Association (AUA) cite the use of GnRh ana-

logues to prevent new episodes of priapism in adults; however, this treatment, aside from being expensive, may lead to a decrease in libido and should be avoided in children (9).

Anti-androgens have been used to treat priapism in adults with satisfactory results (2,5,6,10). The dose of 100 mg/day of cyproterone acetate is associated with a reduction in nocturnal priapism attacks and a 50% reduction in serum LH, FSH and testosterone levels. On the other hand, patients experience a loss of libido (5). Treatment with bicalutamide (50 mg) was reported in three young men with recurrent priapism (10). No new episodes occurred while the patients were on the medication, but one patient developed breast swelling and gynecomastia. No change in libido was reported.

Finasteride has already been evaluated by Rachid-Filho et al. for the prophylactic treatment of priapism in adolescents and adults (6). In that study, 35 patients were treated with decreasing doses of 5 mg, 3 mg and 1 mg of finasteride daily for 4 months. The age of the patients ranged from 15 to 53 years, and they all had sickle cell disease and a history of recurrent priapism. After treatment, 46% of the patients reported a cessation of priapism episodes. The authors failed to describe how many patients were under 18 years of age and did not report the outcome for this specific group. The aim of the present study is to test the hypothesis that a low dose of finasteride would be effective in preventing recurrent priapism in children.

MATERIAL AND METHODS

Since 2007 all children and adolescents with recurrent episodes of priapism have been treated with finasteride in our department. The medical records of these patients were reviewed for this study. In four cases, the dose used was 1 mg a day, while the remaining patient received 1 mg twice a day.

All patients were contacted and interviewed together with their parents. Data were obtained on the number of episodes of priapism prior to and following the beginning of medication, the maximum and minimum duration of the episodes, whether or not the attacks were painful, whether any surgical procedure had been needed, and erectile function before and after the use of finasteride. Priapism was defined as the presence of a persistent, spontaneous, painful erection. None of the patients had ever had sexual intercourse before treatment. To evaluate erectile function, we asked the mothers of children and adolescents who had not started sexual life on the presence of nocturnal or morning erections. Adolescents who had initiated sexual life during follow-up, reported the presence of erections during sexual activity. Two were prepubescent and 3 were going through puberty.

There was no signing of informed consent, since the data were obtained through chart review and follow-up was obtained by telephone. The decision to use finasteride was made due to the high frequency of episodes of priapism. The study was submitted to the ethics committee of our institution during the course of treatment.

RESULTS

Five patients with recurrent priapism, all of whom had SCD, were evaluated. Prior to initiating finasteride treatment, one patient reported having had 6 episodes of acute priapism, while the remaining patients had had more than 10 episodes. One of the patients reported having non painful sporadic episodes of shorter duration (stuttering priapism) almost daily. The duration of the episodes ranged from 30 minutes to 17 hours. During the emergency, one patient could only effectively treat his episode through venous hydration. In three patients, both venous hydration and drainage of the corpora cavernosa were necessary. One patient underwent distal spongious cavernosa fistula after failure of a more conservative treatment.

Finasteride was used for a mean of 7.5 $\pm$ 3 months (range 6-14 months). The mean age of the patients at beginning of treatment with finasteride was 12.0 $\pm$ 3.08 years (range 8-16 years). During treatment, two patients had 2 episodes of stuttering priapism and one had eight. These patients were among those who reported more than 10 episodes previously. However, all episodes

were of short duration and involuted spontaneously. After treatment, four patients had no episodes at all, and one patient reported stuttering priapism. In an attempt to treat the episodes, this last patient used finasteride intermittently without his doctor's consent (self-medication). The mean duration of follow-up was 20 months (range 9-31 months). No side effects were reported. All patients reported normal, full erections (Table-1).

DISCUSSION

Priapism is a medical emergency that mainly affects patients with SCD. It may result in permanent damage to the patient (11). It is believed that the phenomenon of vaso-occlusion, very common in patients with SCD, may hamper blood circulation to the penis (12). Since there is no standard treatment for the prevention of further attacks, it is very common for children with priapism to have been submitted to several different forms of management (2,6,13).

Priapism often occurs as an episode of short duration. More conservative forms of management have been attempted as a means of treating the attacks. It is very important that patients with SCD who experience episodes of priapism be managed first with hydration, pain and anxiety relief, and alkalinization of the blood; this approach does not resolve the priapism episode, that can be treated with drainage of the corpora cavernosum and possibly the injection of adrenergic agents into the corpora cavernosum. More invasive approaches such as surgery to decompress the corpora cavernosa are restricted to the refractory acute attacks.(11,14). The recurrence of the acute episodes is common. The use of vasodilator agents such as pentoxifylline (12), as well as several anti-androgenic drugs such as flutamide, bicalutamide (10) and cyproterone acetate (5,10,12) have been used to prevent further attacks.

Table 1 - Details of the treatment.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Age at first episode	14	4	13	9	13
Age at the beginning of treatment	16	8	13	10	13
Age at final follow-up	18	9	14	11	14
Treatment duration (months)	14	6	12	6	6
Dosage of Finasteride	Twice a day	Daily	Daily	Daily	Daily
Previous surgical treatment	Drainage of the corpora cavernosa	No	Drainage of the corpora cavernosa	Drainage of the corpora cavernosa and distal spongious cavernosa fistula	No
Episodes before treatment	36	frequently (nearly 2 times per week)	6	10	Frequently (almost daily)
Episodes during treatment	8	3	3	0	0
Episodes after treatment	0	Occasional	0	0	0
Follow-up (months)	31	24	12	9	21

Some authors recommend managing recurrent priapism in selected cases of adults with the use of self-administered intracavernosal injections of alpha-adrenergic agonists such as metaraminol, etilefrine and epinephrine (15-17); however, this is not practical in the case of children.

Okpala et al. (13) reported a good clinical response with the alpha-adrenergic agonist, etilefrine (50-100 mg/d), in 13/18 adults with recurrent priapism (72%), 17 of whom had SCD. Despite these positive findings, however, side effects such as headache, tachycardia and hypertension are of concern. To our knowledge, there is no record of this drug ever being tested on children.

Finasteride is currently considered an alternative prophylactic treatment for recurrent priapism in adults (6,12,14). This drug acts by inhibiting the type 2 isoform of the 5 alpha-reductase enzyme, which is responsible for the conversion of testosterone into dihydrotestosterone predominantely in hair follicles and the prostate (6). However, the exact mechanism through which this drug reduces the frequency of priapism attacks resulting from vaso-occlusive events is unclear. There is no significant androgenic activity in prepubertal children that would justify the use of this drug (18); however, it has been speculated that its effect may be mainly due to its ability to inhibit calcium efflux from smooth muscle cells (4). It is possible that finasteride may reduce the rate of nocturnal spontaneous erections. Finasteride has little effect on the level of serum testosterone and it has no other steroidal, androgenic, estrogenic or progestinic effects (19). Finasteride acts by inhibiting the action of 5-alpha-reductase type 2 and thus inhibiting the conversion of testosterone into DHT. This enzyme occurs most abundantly in hair follicles and prostate tissue. Therefore, the use of a low dose of finasteride might have little effect in the pubertal androgen development and epiphyseal closure. Of concern is the role of DHT during penis development at puberty. We did not find any complaints of stunted growth of the penis in our patients. However, we recommend that this treatment be performed in low doses and for a short period of time.

The dose of finasteride prescribed for priapism in adults ranges from 3 to 10 mg/day. At these doses, side effects such as a reduction in libido, problems with ejaculation and gynecomastia have been described (6,12). However, these side effects were not found with the use of a 1 mg dose. Rachid-Filho et al. (6) treated 35 adults with recurrent priapism using finasteride, starting at a dose of 5 mg and reducing this dose every 40 days, first to 3 mg and then to 1 mg. After the fourth month of evaluation, these investigators observed that 16 patients (46%) reported no recurrence, while another 16 patients (46%) had 1-15 recurrent episodes. At the beginning of the treatment with finasteride, the mean number of episodes of priapism per patient had been 22.7 compared to 2.1 after 4 months of treatment. These results show that finasteride successfully decreased and controlled the number of recurrent episodes of priapism in that sample.

In view of the physical and emotional sequelae resulting from fibrosis and erectile dysfunction that may occur as a consequence of recurrent priapism in this age group, attempts to avoid new episodes have been carried out. Antiandrogens are generally used in this population for the purpose of treating endocrine disorders and normalizing a state of hyperandrogenism. In these situations, the use of low-dose antiandrogens corrects the state of hyperandrogenism without causing any relevant side effects. No publications were found on the use of antiandrogens in male children without preexisting hormonal disorders. Therefore, we still do not know the long term safety risks and finasteride should be used in selected cases only.

CONCLUSIONS

To the best of our knowledge, this is the first study to evaluate the use of finasteride in children. No side effects were found at the doses of 1-2 mg a day in children and adolescents after a maximum of 14 months of use. These results suggest that a 1-mg/day dose of finasteride is effective in preventing the recurrence of priapism in children and adolescents with SCD. Despite the frequency of attacks in all five patients prior to treatment, only one had short attacks of stuttering priapism during or after finasteride treatment.

CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Identification of mechanisms involved in the relaxation of rabbit cavernous smooth muscle by a new nitric oxide donor ruthenium compound

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ABSTRACT

Purpose: The aim of this study was to evaluate the relaxation in vitro of cavernous smooth muscle induced by a new NO donor of the complex nitrosil-ruthenium, named trans-[Ru(NH3)$_4$(caffeine)(NO)]C$_{13}$ (Rut-Caf) and sodium nitroprusside (SNP).

Materials and Methods: The tissues, immersed in isolated bath systems, were pre-contracted with phenilephrine (PE) (1 µM) and then concentration-response curves (10^{-12} - 10^{-4} M) were obtained. To clarify the mechanism of action involved, it was added to the baths ODQ (10 µM, 30 µM), oxyhemoglobin (10 µM), L-cysteine (100 µM), hydroxicobalamine (100 µM), glibenclamide, iberotoxin and apamine. Tissue samples were frozen in liquid nitrogen to measure the amount of cGMP and cAMP produced.

Results: The substances provoked significant relaxation of the cavernous smooth muscle. Both Rut-Caf and SNP determined dose-dependent relaxation with similar potency (pEC_{50}) and maximum effect (E_{max}). The substances showed activity through activation of the soluble guanylyl cyclase (sGC), because the relaxations were inhibited by ODQ. Oxyhemoglobin significantly diminished the relaxation effect of the substances. L-cysteine failed to modify the relaxations caused by the agents. Hydroxicobalamine significantly diminished the relaxation effect of Rut-Caf. Glibenclamide significantly increased the efficacy of Rut-Caf (pEC_{50} 4.09 x 7.09). There were no alterations of potency or maximum effect of the substances with the addition of the other ion channel blockers. Rut-Caf induced production of significant amounts of cGMP and cAMP during the relaxation process.

Conclusions: In conclusion, Rut-Caf causes relaxation of smooth muscle of corpus cavernosum by means of activation of sGC with intracellular production of cGMP and cAMP; and also by release of NO in the intracellular environment. Rut-Caf releases the NO free radical and it does not act directly on the potassium ion channels.

ARTICLE INFO

Key words:
Nitric oxide; Nitrosyl-ruthenium complex; Endothelium, Vascular; Potassium Channel

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INTRODUCTION

Human corpus cavernosum contains vascular smooth muscle, kept under tonic contraction induced by adrenergic excitation to maintain penile flaccidity (1). Nitric oxide (NO), the endothelium-derived relaxation factor discovered by Palmer and Moncada, is the main inhibitory neu-

rotransmitter that mediates penile erection in all animals (2). NO activates soluble guanylate cyclase (sGC), which induces production of cyclic guanosine monophosphate (cGMP) from guanosine triphosphate (GTP). The cGMP acts in intracellular effectors, like protein kinase G (PKG), which cause diminishment of intracellular calcium and disassociation of actin and myosin fibers, ultimately leading to relaxation of the smooth muscle (2).

NO donors are substances that release NO, either in vivo or in vitro. One of these donors, sodium nitroprusside (SNP), is a powerful vasodilator utilized in patients with hypertensive crisis. Nevertheless, it is extremely labile, induces tolerance and releases cyanide, which is toxic to the endothelium (3).

NO is a potent vasodilator synthesized by neurons, endothelial cells, leucocytes and platelets among others. It is released by the autonomous nerve terminals, and by the vascular and sinusoidal endothelium when stimulated by acetylcholine (4).

Synthesis of NO is catalyzed by NOS, which converts l-arginine and oxygen to l-citrulline and NO. NOS exists as three isoforms in mammals: nNOS and eNOS are preferentially expressed in neurons and endothelial cells, respectively, and iNOS in virtually all cell types. All three NOS isoforms have been identified in the corpus cavernosum, with nNOS and eNOS being considered responsible for initiating and sustaining erection, respectively (5). Down-regulation of nNOS expression has been found in the corpus cavernosum of aging rats (6), a model in which corpus cavernous smooth muscle relaxation is impaired (7).

Endothelial dysfunction is present in a large group of patients with erectile dysfunction (ED) and co-morbidities like hypertension and diabetes. This syndrome is characterized by an endogenous NO production deficiency (8). About 56% of patients in this group show resistance to current ED treatment with phosphodiesterase 5 (PDE-5) inhibitors (9). Research for new drugs that increase bioavailability of endogenous NO is a permanent challenge.

Recently, new NO donor compounds with higher stability and less toxicity have been subject of research. One group of such substances, S-nitroso-glutathione (GSNO) and S-nitroso-N-acetylcysteine-etylester (SNACET), was utilized in studies with strips of human corpus cavernosum mounted in isolated bath systems, proving to be potentially useful for tissue relaxation (10).

SNP has already been utilized intracavernously in vivo, in both human and animals, to induce penile erection. In dogs and monkeys, SNP induced dose-related erection, without causing hypotension (11). In patients with ED, SNP provoked erection of shorter duration than did papaverine, without side effects - like hypotension, injection site pain, or priapism (11,12).

Ruthenium compounds, which are NO donors, have been tested in vitro and showed a similar relaxation to SNP on rat aorta smooth muscle (13). These compounds theoretically have the potential to be better than SNP because they do not release cyanide nor they react with the superoxide anion, forming peroxynitrite. However, further experimental studies are necessary to confirm this theory. Furthermore, they are stable at physiologic pH and soluble in water (13).

The aim of this study was to evaluated the relaxation in vitro of cavernous smooth muscle induced by a new NO donor of the complex nitrosyl-ruthenium, named trans-[Ru(NH3)4(caffeine)(NO)]C13 (Rut-Caf) and sodium nitroprusside (SNP).

MATERIALS AND METHODS

The study was approved by the local Committee of Ethics on Animal Use in Research. Adult male New Zealand rabbits, weight 2 - 3 kg, were used. After anesthesia, the penis was removed entirely, and placed in Krebs-Henseleit solution. Cavernous tissue was dissected free of connective tissue and albuginea, providing two corpus cavernosum strips (1 cm) from each penis.

These strips were mounted in isolated baths (10 mL) containing Krebs-Henseleit solution. This solution contains Na, K, Cl, Ca, MgSO4, HCO3, PO4, glucose, albumin, and tromethamine (THAM) and was used to maintain tissues during experiments (14). The strips were aired with a mixture of O_2 (95%) and CO_2 (5%), pH 7.4, 37°C. Tissues were placed vertically, with tension of 1g. One extremity was connected to an isometric power

transducer, while the other end was attached to a mobile unit that allowed tension adjustment.

The tissues rested for one hour. Tension was calibrated and the solution renewed every 15 minutes. Alterations on tension were registered on a polygraph (Gemini 7070, Ugo-Basile, Italy). Eight different experimental protocols were performed, as outlined next.

Experiment 1: After pre-contraction with phenilephrine (1 µM), rising concentrations (10^{-12} - 10^{-3} M) of SNP (as a control) and Rut-Caf were administered to the baths and relaxation concentration-response curves were obtained.

Experiment 2: To evaluate the NO liberation profile for Rut-Caf, oxyhemoglobin (10 µM) was added to the baths, 30 minutes before pre-contraction with phenilephrine (1 µM).

Experiment 3: To evaluate possible anion nitroxyl (NO-) liberation by Rut-Caf during the relaxation process, L-cysteine (100 µM), a specific NO- remover was added to the baths, 30 minutes before pre-contraction with phenilephrine (1 µM).

Experiment 4: To evaluate the contribution of metabolically activated potassium channels (K_{ATP}) on the relaxation process, one K_{ATP} blocker - glibenclamide (1 µM) - was added to the baths, 30 minutes before pre-contraction with phenilephrine (1 µM).

Experiment 5: To evaluate the contribution of high, medium and low conductivity calcium-activated potassium channels (K_{CA}) on the relaxation, two K_{CA} blockers - iberotoxin (1 µM) and apamine (0.1 µM) - were added to the baths, 30 minutes before pre-contraction with phenilephrine (1 µM).

Experiment 6: To evaluate the way NO is involved in the relaxation, hydroxocobalamin (0.1 mM), a NO remover, was added to the baths, 30 minutes before pre-contraction with phenilephrine (1 µM).

Experiment 7: To determine the activation of sGC by Rut-Caf, the agent 1H-[1,2,4] oxadiazole[4,3-α]quinoxalin-1-one (ODQ) - a sGC blocker - was added to the baths, 30 minutes before pre-contraction with phenilephrine (1 µM).

In all the experiments, relaxation concentration-response curves were obtained according to rising concentrations (10^{-12} - 10^{-3} M) of Rut-Caf.

Experiment 8: The RbCC strips were frozen in liquid nitrogen for dosage of cGMP and cAMP. The method of non-acetylation was utilized and samples were separated for dosage of proteins according to the Bradford method (1976) (15). Trichloroacetic acid (TCA) was added to the tissue macerate, resulting in a final TCA concentration of 10%. After centrifugation, the supernatant was washed with water saturated diethylic ether. This process was repeated six times. After the washings, the samples were dried in a nitrogen atmosphere at 60ºC, and then suspended again in the assay buffer of the immunoenzymatic kit for dosage of cGMP and cAMP.

The relaxant effect of the substances was measured from the maximal contraction plateau induced by phenilephrine, and expressed as percentages of contraction diminishment. The maximal effect (E_{max}) was considered as the maximal amplitude response induced by the relaxant agents on the concentration-response curves. The drug concentrations that induced 50% maximal relaxation (pEC_{50}) were determined after logarithmic transformation of concentration-response normal curves, and expressed as the negative logarithmic of values for each tissue (pEC_0). Percentages of contraction diminishment superior to 50% were considered significant.

The data were expressed as averages ± standard deviation. Statistical analysis was performed with ANOVA, followed by the Tukey-Kramer test. Values of $p < 0.05$ were considered significant.

RESULTS

The SNP Emax value was 100%, and its pEC50 was 6.9 ± 0.2. For Rut-Caf, Emax value was 72.6 ± 6.6%, and pEC_{50} was 6.8 ± 0.2. There was no significant difference in potency between the two substances ($p = 0.851$).

The relaxation curve induced by Rut-Caf was dislocated below with oxyhemoglobin, while the maximal response for Rut-Caf, in this setting, was 49.8 ± 6.5%.

Incubation of tissues with L-cysteine did not provoke any relaxation alterations. The values of pEC50 for Rut-Caf and Rut-Caf + L-cysteine were respectively 6.8 ± 0.2 and 6.9 ± 0.2 ($p =$

0.915). The E_{max} value for Rut-Caf was 77% and for Rut-Caf + L-cysteine was 80% (Figure-1).

Glibenclamide modified potency and efficacy of Rut-Caf, as reflected by pEC_{50} values for Rut-Caf of 5.2 ± 0.2 and Rut-Caf + glibenclamide of 7.3 ± 0.2 (p = 0.016). E_{max} value increased from 68 ± 8.9% to 92 ± 7% (p = 0.060).

Tissue incubation with ibero-apamin did not interfere with Rut-Caf relaxations. The pEC50 for Rut-Caf was 4.7 ± 0.2, compared to 4.8 ± 0.3 for Rut-Caf + ibero-apamin (p = 0.779) (Figure-2).

Hydroxocobalamin almost abolished the relaxant effects of Rut-Caf. The pEC50 for Rut-Caf was 4.9 ± 0.1, while pEC50 for Rut-Caf + hydroxocobalamin was 5.5 ± 0.2 (p = 0.713). As to E_{max}, values were respectively 85.3 ± 9.4% and 21.3 ± 2.2% (p < 0.001).

The relaxation induced by Rut-Caf was significantly inhibited by ODQ. This is evidenced by the Emax values for Rut-Caf - 58 ± 9.2% – and Rut-Caf + ODQ - 34.6 ± 3.7% (p = 0.038) (Figure-3).

Both SNP and Rut-Caf produced cGMP in the cavernous tissue in amounts significantly higher than the basal control value of 8.26 pmoL/mg. Rut-Caf generated 143.52 pmoL/mg (p < 0.01) and SNP 43.50 pmoL/mg (p < 0.05) (Figure-4).

Finally, Rut-Caf (100 µM) produced cAMP in the corpus cavernosum in quantity similar to that of forskolin (10 µM) and significantly superior to the control amount (p < 0.001) (Figure-5).

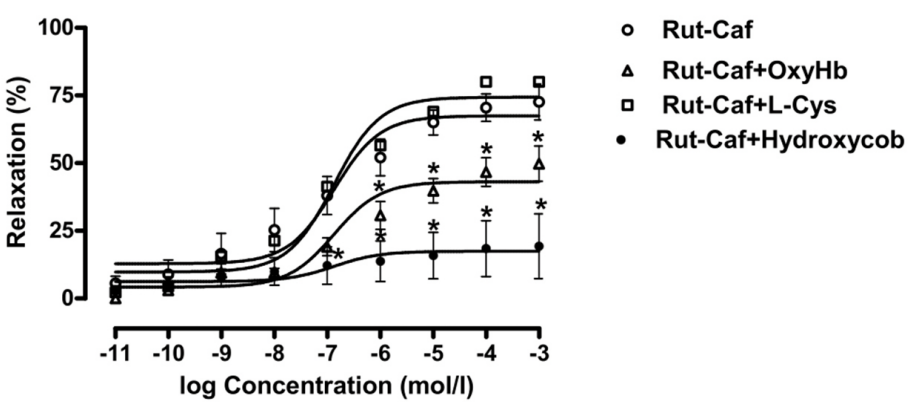
DISCUSSION

This study evaluated a new ruthenium compound and its capability to promote relaxation of cavernous smooth muscle.

The substance Rut-Caf provoked significant relaxation of cavernous smooth muscle (Emax = 80%) with potency similar to SNP; however, with lower maximum effect. The most probable hypothesis is that SNP acts on relaxation of vascular smooth muscle activating the sGC and as a hyperpolarizing agent through direct activation of potassium ion channels and Rut-Caf acts mainly on activation of sGC (16).

The soluble isoform sGC plays a pivotal role in erectile function because it provides the link between NO and cGMP, which represent the

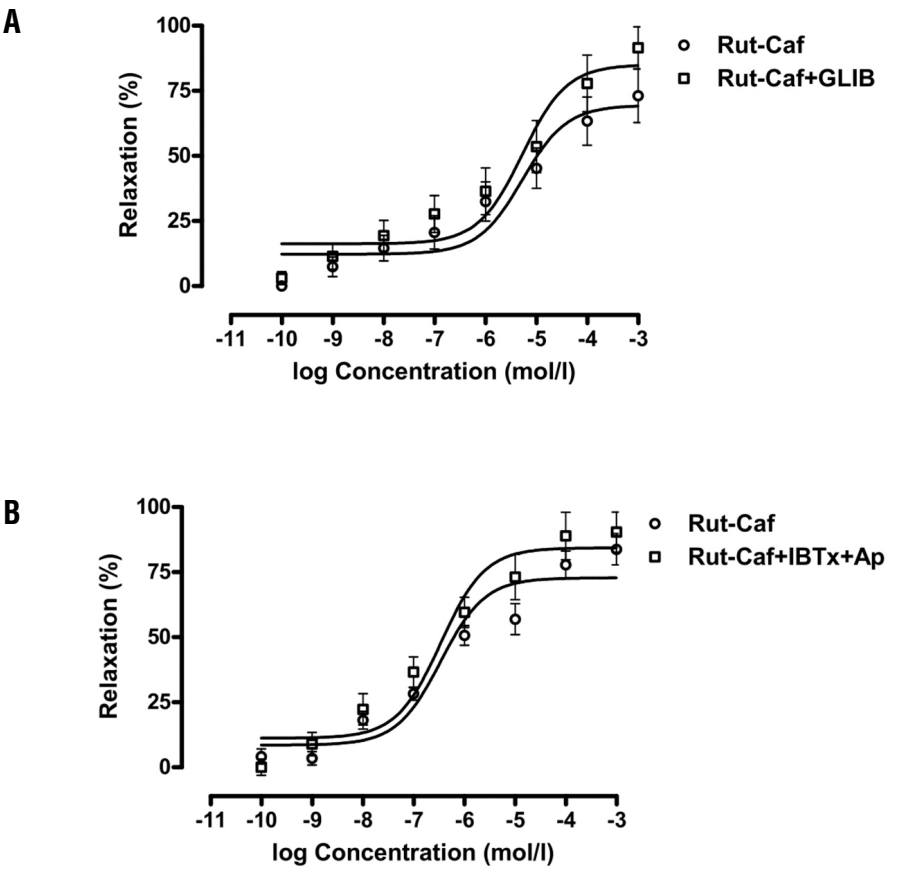
Figure 1 - Effect of Rut-Caf upon rabbit corpus cavernosum strips with and without pretreatment with 10 µM/L oxyhemoglobin, 1 mmoL/L L-cysteine or 0.1 mmoL/L hydroxocobalamin. Concentration/response curves were plotted for graded concentrations (10-12 to 10-3 M) of Rut-Caf. Results were expressed as average ± standard error based on seven experiments. The statistical significance was verified with ANOVA followed by Buonferroni's test.



*p<0.05 vs. Rut-Caf without oxyhemoblin pretreatment.

*p<0.05 vs. Rut-Caf without hydroxocobalamin pretreatment.

Figure 2 - A. Relaxation induced by Rut-Caf in rabbit corpus cavernosum strips pre-contracted with 1 µM phenylephrine with and without glibenclamide pretreatment. Results were expressed as average ± standard error based on seven experiments. B. Relaxation induced by Rut-Caf in rabbit corpus cavernosum strips pre-contracted with 1 µM phenylephrine with and without iberiotoxin/apamin pretreatment. Results were expressed as average ± standard error based on seven experiments.



extracellular and intracellular signaling molecules, respectively, in physiologic erection (17).

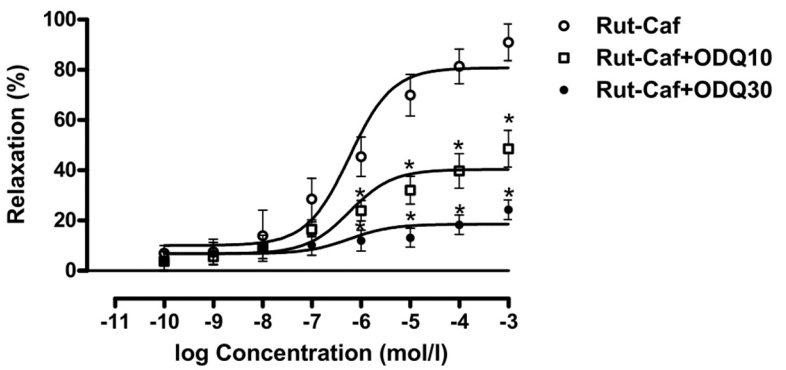
Bonaventura et al., studying substances that also belong to the nitrosyl-ruthenium complex, in rabbit aorta rings, demonstrated a maximal relaxation effect of 102% with pEC50 of 6.61 ± 0.09. These data confirm, as showed in this study, that ruthenium compounds are powerful vasodilators (13).

In vivo, NO arranges with hemoglobin creating a stable iron-nitrosyl complex that does not release NO. Bonaventura et al., evaluating the effects of ruthenium compounds in rabbit aorta rings in the presence of oxyhemoglobin, observed a decreased potency without significantly modifing its maximum effect, due to intracellular release of NO.

In this in vitro study, the addition of oxyhemoglobin to the organ baths reduced the maximum effect of Rut-Caf, but could not abolish it. Therefore, the relaxant effect of this substance is also probably due to the intracellular release of NO (13).

McDonald and Murad demonstrated that NO acts on the relaxation of vascular smooth muscle through activation of soluble guanylate cyclase, increasing the synthesis and bioavailability of intracellular cGMP (18). In this study, tissue incubation with a specific inhibitor of soluble guanylate cyclase (ODQ; 10 µM) abolished the re-

Figure 3 - Concentration/response curves showing relaxation induced by Rut-Caf in rabbit corpus cavernosum strips with and without pre-treatment with 10 µM ODQ or 30 µM ODQ. Results were expressed as average ± standard error based on seven experiments.



*p < 0.05 vs. Rut-Caf with 10 µM ODQ pretreatment

*p < 0.01 vs. Rut-Caf with 30 µM ODQ pretreatment

Figure 4 - cGMP dosage of rabbit corpus cavernosum strips exposed to saline solution (control), 100 µM Rut-Caf or 3 µM SNP (positive control).

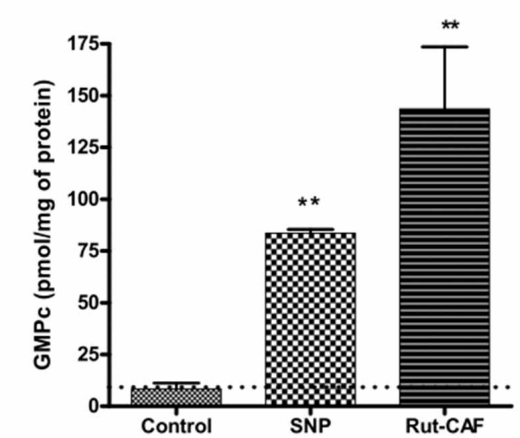
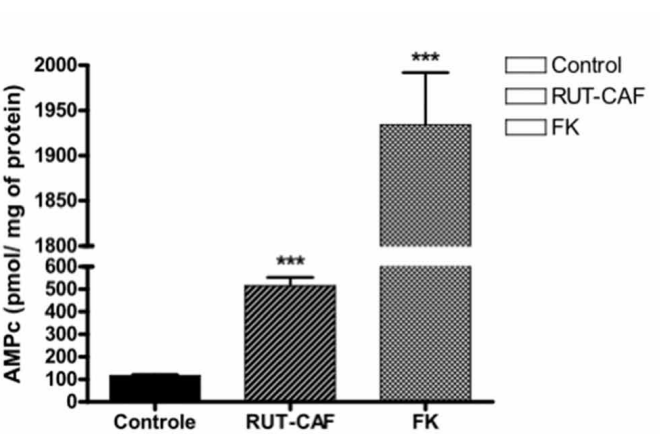


Figure 5 - cAMP dosage of rabbit corpus cavernosum strips exposed to saline solution (control), 100 µM Rut-Caf or 10 µM forscolin (positive control).



laxation response provoked by Rut-Caf. This demonstrates the action of the substance studied on the NO-cGMP cell signaling system.

According to the studies with compounds of the group nitrosyl-ruthenium, it was observed that these substances act by means of donation of free NO and nitroxyl anion (NO-) (13). Utilizing a specific nitroxyl anion remover, L-cysteine, the potential of relaxation remained unchanged. However, the addition of the NO free radical remover, hydroxocobalamin, to the baths significantly decreased the relaxation induced by Rut-Caf. In contrast with Bonaventura et al. findings, Rut-Caf does not release the nitroxyl anion during relaxation. In consequence, probably its relaxation effect is related to release of the NO free radical.

Smooth muscle has neither a T-tubule system nor a well-developed sarcoplasmatic reticulum. Therefore extracellular calcium plays an

important role, and calcium must enter the cytoplasm through the plasma membrane during an action potential.

A direct hyperpolarizing action of the Rut-Caf was not observed. The addition of calcium activated potassium ion channels of high, medium and low conductivity inhibitors did not modify the relaxation effect of Rut-Caf. When glibenclamide, which is an ATP dependent potassium channel blocker, was added to the baths, an increase on the efficacy of Rut-Caf was observed (pEC50 4.04 x 7.69, $p < 0.05$).

The results obtained in this study were similar to the findings of Lee and Kang in human corpus cavernosum (19). These researchers, studying the effect of relaxation of a NO donor - SIN-1, could observe an increase in the probability of opening of calcium activated potassium channels in an indirect way, through activation of soluble guanylate cyclase and activation of the cGMP protein kinase. However, it was not demonstrated direct action of SIN-1 on the ion channels studied.

The best explanation for the efficacy of Rut-Caf would be the action of glibenclamide as a reducing agent. Bates et al. showed that the bioactivation of NO in biological medium needs the presence of a reducing agent. In consequence, glibenclamide acting as a reducing agent increase the efficacy of Rut-Caf (3).

This hypothesis was supported by the spectroscopic profile. The product formed (UV-Vis) is compatible with the formation of the species Rut-Caf-H_2O. Initially, there is a reduction of NO0 by glibenclamide with posterior liberation of the metal coordination sphere. In conclusion, this suggests that amplification in the presence of glibenclamide occurs due to reduction of NO+ present in the metal coordination sphere, increasing the concentration of NO^0 in the reactive environment.

Although it is known that ion channels effectively act on the control of the basal tone of the cavernous smooth muscle (1), they do not participate in the relaxation process induced by Rut-Caf.

The production of intracellular cGMP from activation of sGC by NO in different tissues was initially demonstrated by Arnold et al. (20). The production of cGMP in corpus cavernosum of animals and humans by NO donors, during the relaxation process, was demonstrated in studies (10,21).

Similar to these studies, it was demonstrated that the production of cGMP induced by Rut-Caf in the relaxation of cavernous smooth muscle was significantly higher than that induced by saline solution and like that produced by SNP.

It was also observed production of cAMP induced by Rut-Caf during the relaxation process. Uckert et al. demonstrated the interaction of cGMP-cAMP cell signaling systems in the relaxation of cavernous smooth muscle (22). These authors demonstrated the presence of cAMP-specific phosphodiesterases, PDE-3 and PDE-5 in the cavernous endothelium. They also noted that the cAMP specific kinase PKA inhibited the relaxation induced by PDE-5 inhibitors, specific to cGMP, characterizing the integration of the cGMP-cAMP cell signaling systems on the cavernous smooth muscle relaxation. Although the mechanisms for that interaction are not clear, the authors have proposed that the increase in cAMP would be secondary to the drop in the level of cGMP, which would diminish the activity of PDE-3, increasing the level of cAMP. So, the regulation of cAMP-specific phosphodiesterases by cGMP could explain the increase of cAMP observed in this study, as a direct effect of Rut-Caf in the relaxation of cavernous smooth muscle.

Lindaman et al. studied the gallbladder smooth muscle in vitro. They noted that caffeine acting as an unspecific phosphodiesterases inhibitor 1 to 5, increased the production of cGMP and cAMP in the relaxation process (23). Similarly, the presence of caffeine in the structure of Rut-Caf could contribute to the increase of cAMP produced by that substance.

It should be emphasized that the results of this study were obtained in in vitro experimental model and future studies in vivo and clinical studies are necessary in order to confirm them.

CONCLUSIONS

In conclusion, Rut-Caf causes relaxation of smooth muscle of corpus cavernosum by means of activation of sGC with intracellular production of cGMP and cAMP; and also by release of NO

in the intra-cellular environment. Rut-Caf releases the NO free radical and it does not act directly on the potassium ion channels.

CONFLICT OF INTEREST

None declared.

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Umbilical KeyPort bilateral laparoscopic orchiectomy in patient with complete androgen insensitivity syndrome

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ABSTRACT

Main Findings: A 22-year-old woman with complete androgen insensitivity syndrome (CAIS) presenting with primary amenorrhea and normal female external genitalia was referred for laparoscopic gonadectomy. She had been diagnosed several years earlier but was reluctant to undergo surgery.
Case Hypothesis: Diagnosis of this X-linked recessive inherited syndrome characterizes by disturbance of virilization in males with an AR mutation, XY karyotipe, female genitalia and severely undescended testis with risk of malignization. The optimal time to orchidectomy is not settled; neither the real risk of malignancy in these patients. Early surgery impacts development of a complete female phenotype, with enlargement of the breasts. Based on modern diagnostic imaging using DCE-MRI and surgical technology with single port laparoscopic access we hypothesize that the optimum time for gonadectomy is not at the time of diagnosis, but once feminization has completed.
Promising Future Implications: An umbilical laparoendoscopic single-site access for bilateral gonadectomy appears to be the first choice approach as leaves no visible incision and diminishes the psychological impact of surgery in a patient with CAIS absolutely reassured as female. KeyPort, a single port access with duo-rotate instruments developed by Richard Wolf facilitates this surgery and allows excellent cosmetic results.

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Androgen-Insensitivity Syndrome; Magnetic Resonance Imaging; Neoplasms, Germ Cell and Embryonal; Sertoli Cell Tumor; Leydig Cell Tumor; Testis

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INTRODUCTION

Most of the patients with disorders of sexual differentiation are diagnosed on the basis of cytogenetic and biochemical tests. Patients with complete androgen insensitivity syndrome (AIS) are raised as females because they have female external genitalia. At puberty, following LH response, normal androgen amounts produced by the testis are aromatized to estrogens, thus leading to breast development (1). Untreated patients are often tall phenotypic females with well-formed breasts, scarce pubic and axillary hair and shallow vagina that consult due to primary amenorrhea. Bilateral cryptorchidic testis, usually intra-abdominal, and absent wolffian and müllerian duct derivatives complete the clinical picture of this type of female pseudohermaphroditism also known as testicular feminization. Incomplete or partial AIS is possible in phenotypic males or in patients with ambiguous genitalia.

The pathogenetical basis of testicular feminization is androgen insensitivity, not androgen absence. Testosterone and DHT levels are normal or elevated, but unable to stimulate development of wolffian duct system and male external genitalia, due to mutations in AR gene that occur at Xq11-q12 (2). Most often absence of menarche,

sometimes consulted at the time of infertility work-up, initiates investigation. Female siblings of a patient diagnosed of AIS follow genetic counseling of this X-linked recessive disorder that often leads to an earlier diagnosis. This entity may also be discovered by the finding and biopsy of cryptorchidic testicles in infants or children with bilateral inguinal hernias. Very rarely this syndrome presents as a pelvic mass since germ cell malignancy before puberty is exceptional. On the other hand, it is accepted that 30% of untreated patients develop germ cell malignancy by the age of 50 years (3). Early laparoscopic gonadectomy with subsequent estrogen replacement is the current management of these patients.

However, the optimal timing of gonadectomy is not completely defined. When diagnosis is made before puberty there is not firm consensus upon the moment at which testis should be removed. Some authors strongly encourage the tendency to perform early orchiectomy right after diagnosis of testicular feminization and use estrogen replacement therapy straightforward (4,5). In children operated estrogen replacement with ethinyloestradiol is needed around the age of 11 on to rise normal female puberal development. Despite absence of uterus, combined estrogen-progesterone treatment in a cyclic fashion may diminish risk of breast cancer and cardiovascular disease associated with un-opposed estrogens (5).

Women with CAIS are increasingly likely to defer or decline orchiectomy (6). The most common reasons for bad acceptance of surgery include inconvenience, psychological impact, concern about the risks of surgery and reluctance to take hormone replacement therapy (6). It is understandable then that some specialists consider the optimum time for gonadectomy is not at the time of diagnosis, but once feminization has completed. After puberty, estrogen replacement in selected cases can be delivered through transdermal patches. Genetic and endocrine counseling need psychological reinforcement.

CASE HYPOTHESIS AND RATIONAL

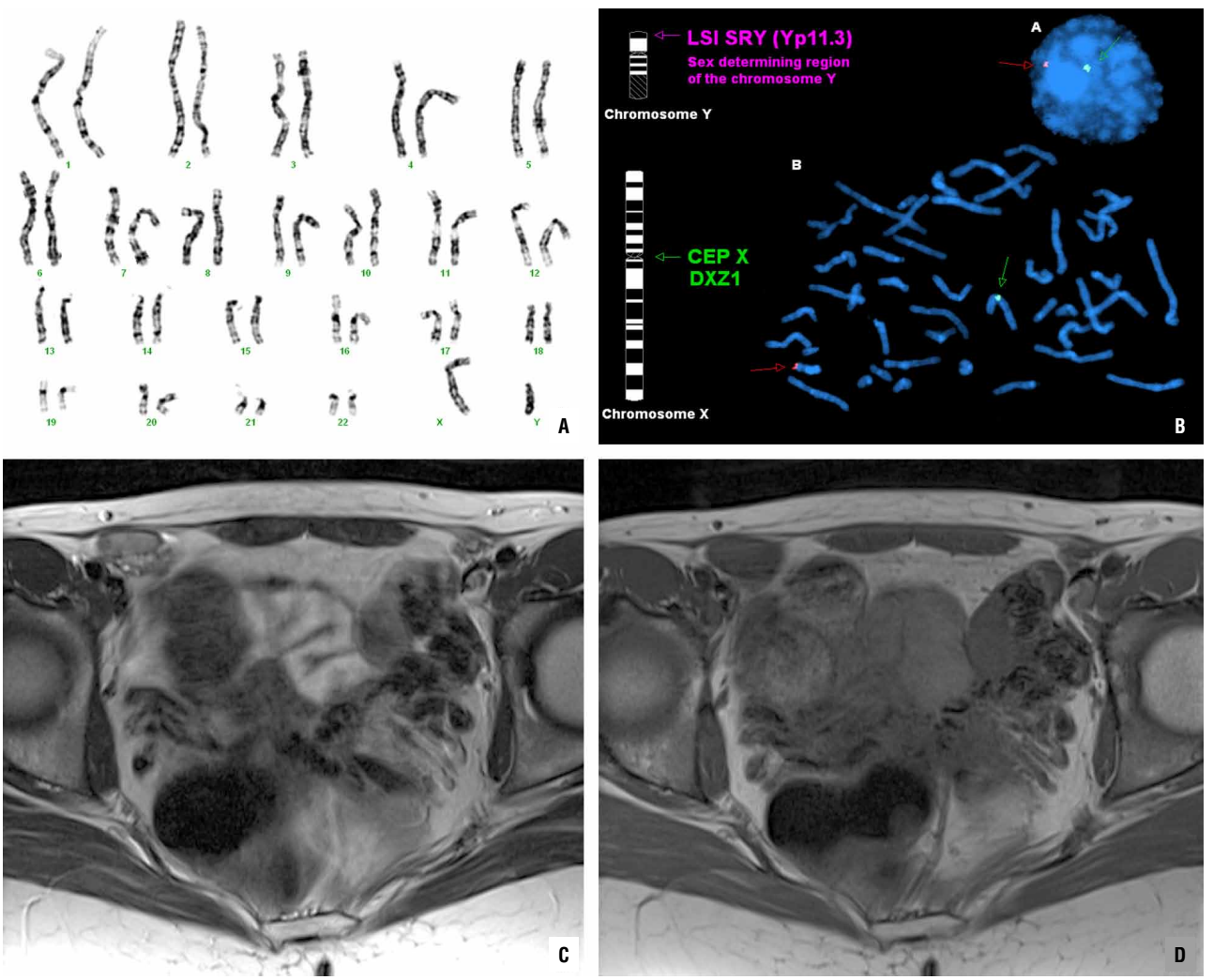
A single 22 years-old phenotipically female Arabic had been diagnosed of complete AIS 4 years earlier when she consulted due to primary amenorrhea but was reluctant to undergo surgery and refused it. She was referred to our institution for psychological counseling and to evaluate the risks and benefits of receiving bilateral orchiectomy through the umbilicus with the new KeyPortTM system (Richard Wolf, Knittingen, Germany).

Hormonal evaluation performed showed normal testosterone (6.4 ng/ml), LH (14.2 mIU/ml) and FSH (6.6 mIU/ml) levels and elevated β-HCG (2.8 mg/ml). Chromosomal study revealed a normal male 46-XY karyotype. FISH was performed using Vysis Probe (Izasa) that revealed the presence of the SRY gene on the short arm of the Y chromosome (Yp11.3) and also the presence of AR gene on the X chromosome (Xq11-12) (Figure-1). The patient was submitted to automatic sequencing of AR gene to show the causative mutation. This genetic study has also been proposed to her mother and sisters, and is now pending.

The external genitalia appeared entirely female and pelvic ecography highlighted absence of internal genital organs and dead-end normal vagina. No sexual glands or uterus was visible on computed tomography (CT) or magnetic resonance imaging (MRI). MRI revealed pelvic (right inguinal and left paravesical) gonads of altered signal intensity (Figure-1). Apparent diffusion coefficient (ADC) map was reconstructed from diffusion-weighted images and evidenced a nodular pattern with higher ADC values in both testes suggestive of high cellular density.

The patient accepted laparoendoscopic examination of the pelvic cavity and umbilical bilateral orchiectomy assuming diminished psychological impact caused by a scar hidden inside the umbilicus. Under general anesthesia a 25 mm long incision was performed without need of augmenting the skin or aponeurotic incision to introduce the KeyPort and create the pneumoperitoneum. A long 30º 5.3 mm lens and two duorotate curved instruments, scissors and grasping forceps, combined with Eragon system (Richard Wolf) were used (Figure-2), together with Forcetriad (Covidien) 5 mm hemostatic system and transparent jelly application for lubrication. Both

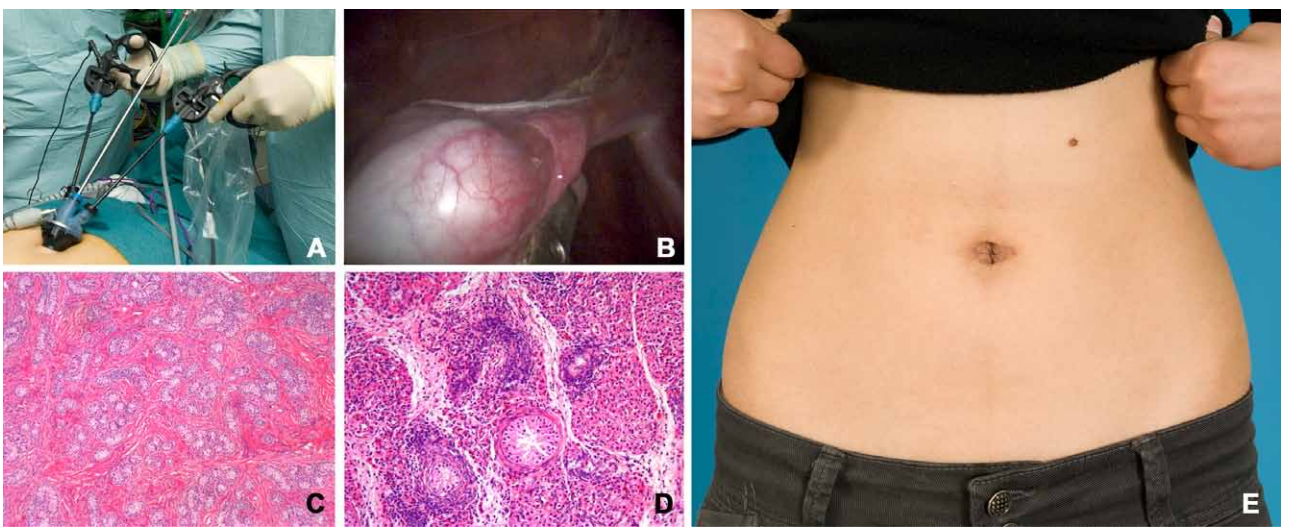
Figure 1 - Preoperative work-up: Chromosomal study with 46-XY karyotype (A); FISH using Vysis Probe (Izasa) revealed SRY (Yp11.3) and AR (Xq11-12) genes (B); Pelvic gonads on MRI of altered signal on T2 TSE (C) and homogeneous contour on T1 SE (D).



gonads were easily retracted and dissected and no inguinal hernia was evidenced. Hem-o-lok placement in the testis cord facilitated orchiectomy. The specimens were packed and extracted through the umbilicus without leaving a drain. Duration of surgery was 45 min. and bleeding was 40 cc. Postoperative recover was uneventful and the patient left hospital after 24 hours without the need of analgesics. Two weeks later the wound appeared absolutely concealed within the umbilicus (Figure-2) and the patient was very happy both with her immediate recovery to normal day-life activities and with the excellent cosmetic aspect of the incision.

Cryptorchidic testis revealed a hard consistency produced by firm, tan nodules measuring approximately 1 cm surrounded by dark brown testicular parenchyma. The nodules comprised small tubules populated by Sertoli cells, demarcated from testicular tissue with marked Leydig cell hyperplasia and a spindle-cell proliferation very similar to ovarian stroma (Figure-2). These nodules were of hamartomatous nature and histologically defined as Sertoli adenomas.

Figure 2 - Operative and postoperative results: Umbilical KeyPort with 30o 5.3 mm lens and duo-rotate instruments (A); laparoendoscopic view of the gonad (B); hamartomatous nodule composed of small tubules filled with Sertoli cells (C); Leydig cell hyperplasia and also a spindle-cell proliferation very similar to ovarian stroma (D); absence of visible incision two weeks after surgery (E).



Intratubular germ cells that stained positively to placental alkaline phosphatase were present inside some seminiferous tubules, thus revealing intratubular germ cell neoplasia. Three months after surgery the patient initiated estrogen replacement using transdermal patches and both genetical and molecular analysis have been proposed to all the female siblings in the family.

DISCUSSION AND FUTURE PERSPECTIVES

Transumbilical single-port surgery has greatly evolved in the last few years in the urological literature (7). Although it is still a minority, with recent advent of new instrumentation, transumbilical laparoendoscopic single-site surgery has become more popular and the cosmetic benefit is well accepted both by physicians and patients (8).

We have proved that a great variety of laparoendoscopic surgeries can be performed through the umbilicus with the new KeyPort system (9,10). We believe this single port is specially indicated for reconstructive surgery because under these circumstances patient recovery and excellent cosmetic results are very important. Neobladder construction with orthotopic anastomosis to the urethra after cystoprostatectomy is possibly the best example of how this new system can be used to achieve an excellent postoperative recovery (11). It is not surprising then that patient acceptance of the umbilical approach is higher than that of conventional laparoscopy and much better than open surgery to face a reconstructive procedure, because cosmetics are greatly improved. In this case no additional 3.5 mm accessory trocar was needed. However, needlescopic material can be very helpful in complicated surgeries such as partial nephrectomy, adrenalectomy or pyeloplasty.

It is well accepted that laparoscopic access is primarily indicated in intersex patients and also in the management of impalpable gonads in the normal male population (4,12). In fact, laparoscopy has been used for diagnostic biopsy of intersex patients since 1986 (13). Apart from the case here presented laparoendoscopic single site surgery to perform orchiectomy has been described in a 20-year patient to treat AIS in the Japanese literature (14). The patient we treated exemplifies that this surgery is better accepted than other conventional options to manage these complex patients that often need psychological and sociological reassure.

Of course there is a risk of developing germ cell tumors in the testis of patients with

AIS. However accurate estimate risk of adult malignancy is not really available. With the limitations of combining historic case series, it has been recently estimated that 14% (0-22%) of untreated adults with complete AIS (6) could develop germ cell malignancy. That means the risks often quoted may have been exaggerated.

Modern diagnostic imaging using MRI and particularly DCE-MRI allows a better definition of the structure of the gonads that may justify a delayed biopsy or excision as far as there is no suspicion of malignancy (15). Delayed orchiectomy allows a better development of female characters at puberty, thus resulting in a better acceptance of the patient's condition. We therefore believe that laparoscopic access through umbilical single port is the best option to plan bilateral gonadectomy in these patients because no visible incision is left and this fact minimizes the psychological impact of surgery. The most appropriate time to perform it is after the patient has been reassured as a female with infertility alone. KeyPort system, the single port access with duo-rotate instruments developed by Richard Wolf facilitates this surgery, allows excellent cosmetic results and can be used safely after sufficient practice both in dry lab and experimental animals (9).

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CONFLICT OF INTEREST

None declared.

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Sigmoid colon ureteral fistula presenting with urosepsis

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This 72-year-old white male presented in the emergency room with symptoms of urosepsis. He had been sick for about ten days, reporting increasing malaise, temperature elevation, left flank pain, and "foul-smelling" urine. He had three prior episodes of left lower quadrant pain associated with diarrhea; which his physician had diagnosed as diverticulitis and treated with antibiotics and dietary restrictions.

At admission, vital signs of the cachectic patient were recorded as BP 160/78, pulse rate 92, respiration 22, and temperature of 38.4 Celsius. Physical exam demonstrated the lungs clear to auscultation and percussion; tenderness to percussion in the left back, and rebound tenderness in the left lower abdominal quadrant. Laboratory data were: RBC 3.8, Hb 9.4, HCT 36, WBC 24,000, BUN 28, Creatinine 2.6; K 4.2, and Na & Cl within normal limits. Urinanalysis and cytology demonstrated a murky appearance, specific gravity of 1.024, cellular debris, WBC 120/hpf, RBC 80/hpf, gram negative bacteria, and vegetable fibers. An admission chest radiograph was negative.

A three-phase contrast-enhanced CT (with intravenous contrast medium reduced to 60 mL, because of elevated creatinine) was performed with both coronal and sagittal reconstructions. An axial slice showed a hugely dilated left ureter with an air fluid level (Figure-1). A coronal reconstruction (the area of interest enlarged to156%) demonstrates gas in the fistula to the thickwalled segment of the sigmoid colon as well as at the level of the UPJ (Figure-2). Diverticula are shown in the third portion of the sigmoid colon. An aneurysm of the infra-renal segment of the aorta was noted. Another coronal reconstruction (206% enlargement of area of interest) at a slightly more posterior level shows the entire left ureter dilated by gas (Figure-3). A sagittal reconstruction shows the dilated gas-filled left ureter (Figure-4).

Fistula from the GI tract to the ureter is uncommon. Uretero-ileal fistulae as consequence of Crohn's disease occur with higher frequency then uretero-colonic fistulae (1,2). Uretero-colonic fistulae may be secondary to inflammatory disease of the large bowel, such as diverticulitis, obstructing ureteral calculi, or neoplasm of the colon that contiguously involves the ureter (3,4). A non-functioning kidney and ureter are often the consequence. CT is the modality of choice in working up urosepsis or renal colic, while antegrade or retrograde ureterogram is the most sensitive in the detection and characterization of a fistulous tract (5). In a like pathologic process, fistulization to the fallopian tubes can occur from diverticular abscesses. However, the incidence of colo-vesical fistulae caused by diverticulitis is higher (6).

Figure 1 - Axial slice showed a hugely dilated left ureter with an air fluid level.

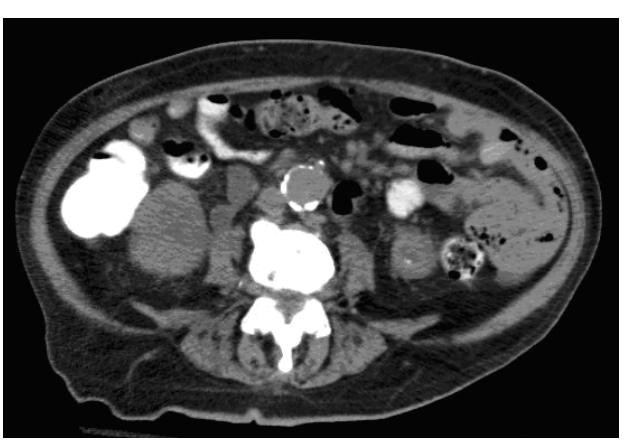


Figure 2 - Axial scan of pelvic CT, (a) noncontrast and (b) contrast enhanced, bone window. A curvilinear hyperdense material (black arrow) was demonstrated at left anterolateral wall of urinary bladder.

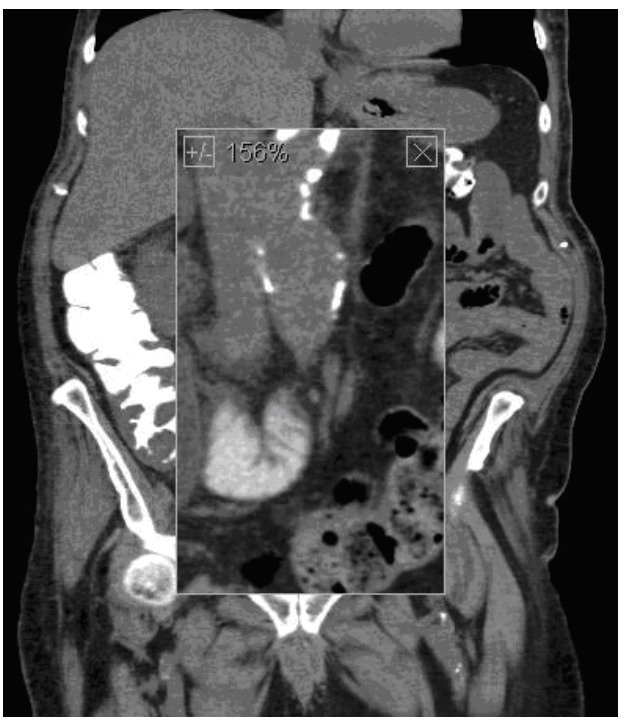


Figure 3 - Coronal reconstruction (206 % enlargement of area of interest) at a slightly more posterior level shows the entire left ureter dilated by gas.

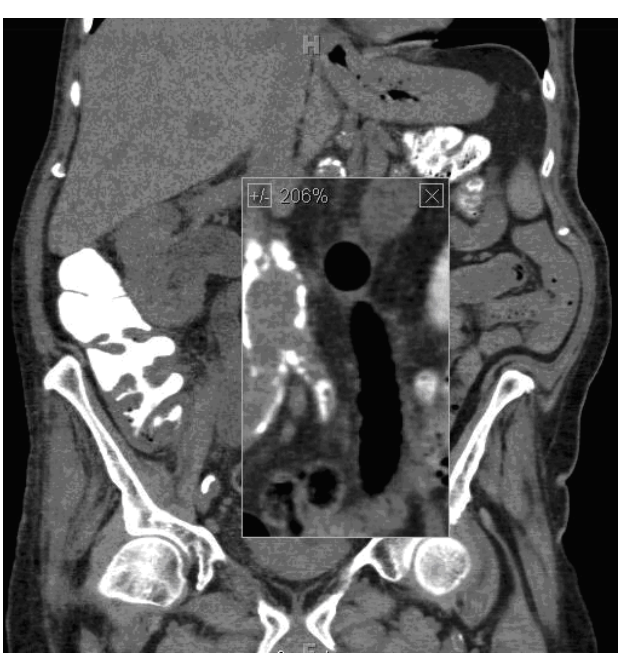
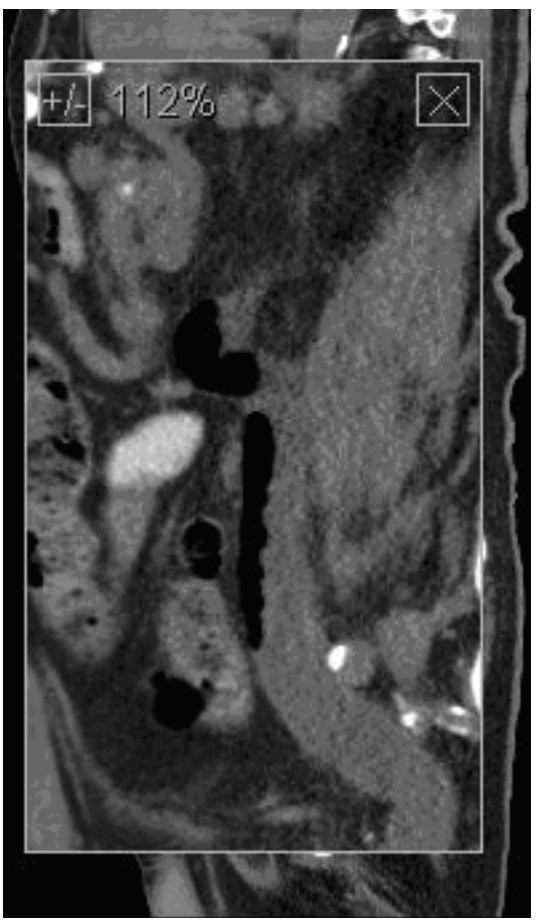


Figure 4 - A sagittal reconstruction shows the dilated gas--filled left ureter.



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Erosion of inferior vena caval filter noted during robotic assisted laparoscopic partial nephrectomy

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ABSTRACT

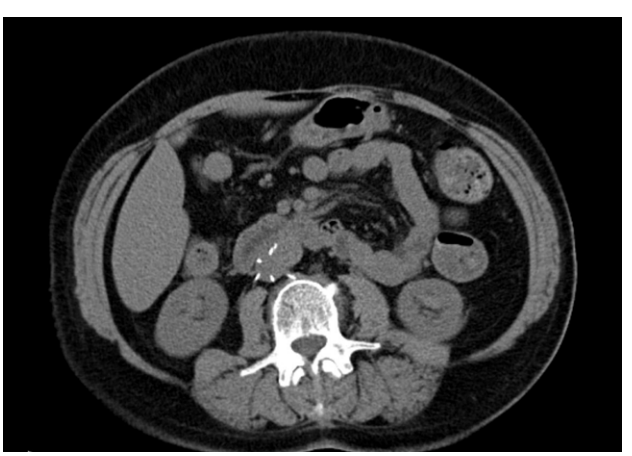
Inferior Vena Cava (IVC) filters are mechanical devices implanted to provide prophylaxis against pulmonary emboli in patients for whom standard anticoagulation is either inadequate or contraindicated. A 67-year-old female with a 10-year-old indwelling IVC filter underwent robotic assisted laparoscopic partial nephrectomy for a right upper pole renal mass. Renal hilum dissection was complicated by adhesions secondary to eroded IVC filter struts. IVC filter erosion is a well-described phoenomena in both the radiologic and surgical literature. As many as 25% of filters are noted to be radiographically eroded; however, the incidence of clinically significant erosion is much less. Given the placement of endovascularly delivered IVC filters in close proximity to many urologic operative fields, it is important for urologists to be aware of the potential of eroded devices when pursuing para-caval dissections.

BACKGROUND

A 67-year-old female with a history of recurrent deep vein thrombosis on warfarin presented with an incidentally identified 1 cm enhancing upper pole right renal mass and stress type urinary incontinence. She had previously undergone laparoscopic cholecystectomy, appendectomy, and placement of a Simon Nitinol IVC filter. Options, including surgical intervention and surveillance, were discussed, and she elected to pursue a combined robotic assisted laparoscopic partial nephrectomy with simultaneous transbobturator urethral sling placement. Pre-operative imaging noted her previously placed IVC filter near the region of the renal veins (Figure-1).

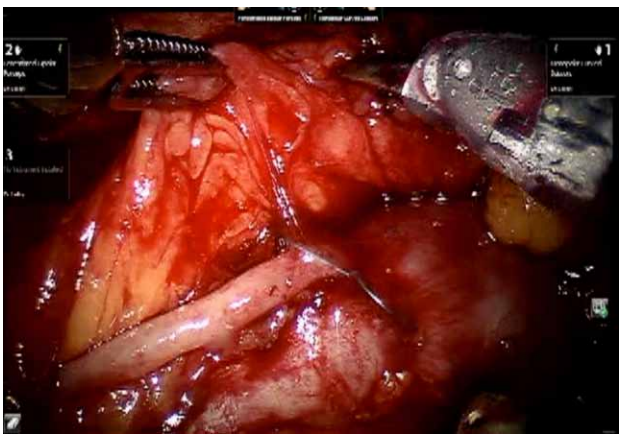
During the renal hilar dissection, dense adhesions surrounding protruding prongs from her previously placed IVC filter were noted in the region of the gonadal vein and renal vein (Figure-2). Adhesion around the prongs made mobilization of the duodenum more difficult than usual. Careful dissection was used to free up the protruding prongs (Figure-2). Once the prongs were isolated, the renal artery and vein were able

Figure 1 - CT scan image of IVC filter.



This CT image notes an IVC filter in the region of the gonadal vein and renal veins. Protruding prongs can be noted posteriorly.

Figure 2 - Intra-operative image of the IVC prongs.



2 metal prongs are noted protruding through the IVC near the gonadal vein and renal vein. These prongs were carefully dissected free of their attachments in order to minimize interfere with duodenum or renal vein dissection.

to be circumferentially dissected in anticipation of renal hilum clamping prior to mass excision. The patient then underwent standard partial nephrectomy. Following partial nephrectomy, there was no further manipulation of the filter prongs. The final pathology was consistent with hemangioma. The patient tolerated the procedure well and was discharged after an uneventful postoperative course.

DISCUSSION

The Inferior Vena Caval (IVC) filter is a mechanical prophylactic option for patients at high risk for thromboembolic complications where medical anticoagulation is contraindicated or inadequate to control risk (1). Since Greenfield's seminal paper in 1973, they have been a part of the surgical armamentarium to prevent perioperative complications (1). Currently placed endovascularly through femoral or jugular venous access, these filters offer a low peri-procedural complication rate. However, the long-term implications of an indwelling foreign body within the vena cava are less well characterized and can present operatively challenging pathology (2). We present the case of an eroded inferior vena caval strut discovered incidentally during robotically assisted laparoscopic partial nephrectomy.

Erosion of inferior vena caval filters is not rare with as many as one in four (25%) demonstrating radiographic evidence of erosion. Clinically, these erosions are typically asymptomatic, with rates of symptomaticity reported in less than 1% of known filters (2). Given the anatomic location of these filters adjacent to the aorta, spine, renal hilum, portal vein, and duodenum, there is the potential for serious complication from protruding struts. Pain, ureteral injury, aortic perforation, intracardiac migration, and duodenal perforation have all been reported as late complications of indwelling IVC filters (2).

There is often disparity between CT scan appearance of IVC prong protrusion and actual appearance intra-operatively. Examination of the fat planes surrounding the vena cava may hold more valuable information. Visualization of the fat planes around the IVC can be predictive of prong protrusion that may cause adhesion or bleeding during surgery. A perfect example of this is in our Figure-1. There is evidence of protrusion of an IVC prong posteriorly on CT scan but the fat planes are in-tact. One pronge near the renal vein anterior-lateral has no surrounding fat plane, hence possibly predictive that there might have been some bleeding causing adhesions.

Newer filters are sometimes equipped with a mechanism allowing for later retrieval in situations where thrombotic risk is temporary, as in the perioperative period or after trauma. Retrievable IVC filters can offer the advantage of a non-permanent prophylaxis and are retrievable in approximately 90% of attempted retrievals (3). This contrasts sharply with actual reported rates of retrieval of 2-41%; this disparity appears to persist even under thorough monitoring protocols and appears multifactorial, related to overall medical condition, ongoing indication for filter, patient preference and patient volition (4). Also, while rare, clinically significant erosions can be devastating and require multiple high-morbidity procedures to correct. Currently controversy exists as to the risks, benefits and indications for IVC filters; level one evidence will be needed to further inform this discussion (5).

To our knowledge, no guidelines exist for management of incidentally discovered eroded IVC struts. Management options for incidentally identified eroded struts include clipping protruding struts flush with the IVC wall in order to dull sharp edges and prevent viscous perforation, buffering with external surgical devices, and no action.

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VIDEO SECTION

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Urethral duplication II-A Y type with rectal urethra: ASTRA approach and tunica vaginalis flap for first stage repair

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ABSTRACT

Introduction: Urethral duplication is a rare congenital anomaly affecting mainly boys. Generally, the duplication develops on the sagittal plane; the accessory urethra may run dorsally or ventrally to the orthotopic one. We present a patient with urethral duplication in which the orthotopic urethra was patent in the penile segment but atresic in the bulbar and prostatic segment. The patient had urinary flow from the rectum and the ectopic urethra could be well identified by anal examination.
Materials and Methods: Age at surgery was 13 months. The procedure consisted of an ASTRA (anterior sagittal trans-ano-rectal) approach for dividing the urethra and rectum and was successful to move the urethra up to the perineal area. The rectum was reconstructed and the patient placed into a lithotomy position. A urethral catheter inserted in the penile urethra oriented us were the atresic urethra in bulbar area started. The scrotum was opened in the middle and the distance between the two urethral stumps proximal and distal defined the extension of no urethral tissue that consisted of 5 cm. We opened the right scrotal space and a tunica vaginalis flap was obtained and attached to the bulbar tissue for a two-stage urethroplasty strategy.
Results: Patient had a nice healing and the tunica vaginalis was nicely incorporated to the adjacent tissue, having the two urethral stumps well delineated.
Conclusions: ASTRA approach in combination with a two-stage urethroplasty with tunica vaginalis dorsal flap proved to be an excellent combination for a rare case of urethral Y duplication having the main urethra into the rectum.

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EDITORIAL COMMENT

Macedo et al. present a video that nicely demonstrates the first stage of a planned two-stage procedure. Urethral duplication are rare congenital anomalies. The urethral configurations and patient symptomatology can vary greatly. Patients can be completely asymptomatic. They can have two parallel urinary streams or, as in this case, can present with rectal urinary incontinence (1). The anterior sagital trans-ano-rectal approach (ASTRA) was described by Di Benedetto and Di Benedetto for use in clitoro-vaginoplasty (2).

Y-type duplications with a perineal or rectal urethral meatus are often accompanied by other congenital anomalies such as renal agenesis, cryptorchidism, anorectal malformations or sacral agenesis. They can also be the most challenging to repair, often requiring multiple surgeries (3). In general, the dorsally-positioned urethra is the accessory urethra. Single-stage repairs using buccal mucosa grafts as a free-tube have been reported (4).

We look forward to the authors' submission of the second-stage surgery for this child with an uncommon and challenging anomaly.

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