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Crystal/cell interaction and nephrolithiasis.

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Summary

Crystals of calcium oxalate (CaOx), the major constituents of most urinary stones, are injurious to cells, create oxidative stress and evoke an inflammatory response. Renal injury results in cell damage. The damaged and dead cells are released into the urine and are capable of promoting crystal nucleation at much lower supersaturations. Damaged cell membranes also provide sites for crystal attachment and eventual retention within the kidneys. Renal epithelial damage may assist in movement of crystals from the intratubular to interstitial location and perhaps in the formation of apatitic Randall's plaques. Inflammatory response may be responsible for Randall's plaques ulceration to the renal papillary surface.

KEY WORDS: *Urinary calculi; Crystals; Calcium Oxalate; Cells; Inflammation.*

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INTRODUCTION

Human urinary stones are polycrystalline aggregates of crystals and an organic matrix (1). While calcium phosphate (CaP) is the most common crystalline constituent of the stones, calcium oxalate (CaOx) crystals are the main component of up to 80% of the stones worldwide. The stones are anchored to the papillary surfaces in the renal calyces and pelvis. In idiopathic CaOx stone formers, crystals are restricted to the renal medulla and papilla. However in nephrolithiasis associated with primary hyperoxaluria, crystal deposits are seen in all parts of the kidneys including the cortex, and in all segments of the nephron including the proximal tubules. Necropsy studies of human kidneys have revealed calcification (CaP) in renal interstitium, mostly around the renal tubules. It has been suggested that the presence of such interstitial CaP crystals can predispose the renal papillae to form kidney stones. Randall suggested that interstitial sub-epithelial crystal deposits arising from pathologic conditions of the renal papilla erode through to the papillary surface and form a precalculus lesion or stone nidus, which, under suitable conditions will support any type of stone, whether it be oxalate, phosphate or urate (2). Randall himself described two types of precalculus lesions or plaques, subepithelial Type I lesion on the pelvic aspect of the papilla and intratubular Type II lesion in the ducts of Bellini. Randall's plaque generally refers to the Type I precalculus lesion containing subepithelial calcium phosphate deposits.

Recent studies, by *Stoller et al.* (3) and *Evan et al.* (4), of kidneys of a variety of stone patients, including idiopathic, intestinal bypass surgery for obesity, primary hyperoxaluria, brushite, cystine and distal tubular acidosis, have provided detailed descriptions of plaque and histo-pathological changes in kidneys of the stone formers. They have also shed more light on the role of RP in stone formation. Randall's plaques consist of poorly crystalline biological apatite and are suggested to originate in the renal interstitium close to the collagen fibers and the basement membrane of the loops of Henle. From there, the deposits grow outward encasing the ducts of Bellini reaching the papillary epithelium.

URINARY SUPERSATURATION

The formation of kidney stones or nephrolithiasis is the result of crystal formation in the kidneys. Crystallization is modulated by a number of urinary inhibitors and promoters which determine whether a crystal will nucleate and grow into a stone or be excreted as a crystalluria particle. The driving force behind crystal formation is urinary supersaturation with respect to the stone forming salts, which means that crystals form when the concentrations of participating ions are higher than the thermodynamic solubility for that salt. The most important determinants of supersaturation for CaOx are urinary volume and total daily excretion of calcium and oxalate. But human urine is a complex solution containing not only calcium (Ca) and oxalate (Ox) but also other ions

and macromolecules that can interact with Ca and/or Ox and modulate crystallization. Urinary CaOx supersaturation depends not only on the concentration of Ca and Ox, but also the presence of ions such as citrate and magnesium as a result, hypercalciuria, hyperoxaluria and hypocitraturia are major risk factors for calcific stone formation. Additionally, urinary pH is critically important because of its role in salt solubility. For example CaP solubility decreases with increasing pH (above 6), while that of uric acid increases. Solubility of cystine also increases with increasing pH. Supersaturation and crystallization in the urine also depend upon the presence of macromolecules such as many proteins and lipids (5), which can bind or form complexes with Ca and/or Ox.
Even though crystals do not form without supersaturation, this alone cannot explain stone formation, because people who have never formed stones can also pass highly supersaturated urine (6). Crystal formation within the urinary tract, particularly of calcium phosphate (CaP) and CaOx is widespread. Humans excrete millions of urinary crystals daily, indicating at least transient development of supersaturation. However, few develop kidney stones, probably, because either the crystals do not form in the kidneys or the crystals that form do not stay there. It has been suggested that the transit time of 5-10 minutes across the kidney is insufficient for crystals to nucleate and grow large enough to be trapped (7). We have hypothesized that transient or long-term renal injury/cellular dysfunction is critical for the formation of kidney stones. In the following brief commentary I will provide supporting evidence from human, animal models and tissue culture studies. Details are available in earlier reviews (5, 8-15).

Clinical studies

Renal biopsies from patients with primary hyperoxaluria regularly demonstrate CaOx crystals within tubular epithelial cells as well as interstitium. Crystal deposition is associated with cell proliferation, the formation of multinucleated giant cells, as well as vascular and interstitial inflammation. Similar observations have been made in other cases of increased urinary excretion of oxalate secondary to enteric hyperoxaluria, such as Crohn's disease and after an intestinal bypass. Higher than normal levels of renal enzymes, gamma-glutamyl transpeptidase (GGTP), angiotensin 1 converting enzyme (ACE), β-galactosidase (GAL), and N-acetyl-β-glucoseaminidase (NAG) were found in the urine of idiopathic CaOx stone formers (16). Since elevation of these enzymes in the urine is considered an indication of renal proximal tubular injury, it was concluded that stone patients had damaged renal tubules.
Results of recent studies also describe CaOx kidney stone patients to be under oxidative stress. Urine from stone patients had increased NAG and significantly higher β-glutathione S-transferase (β-GST), malondialdehyde (MDA) and thiobarbituric acid-reactive substances (TBARS), indicating that CaOx kidney stone-associated renal injury is most likely caused by the production of reactive oxygen species. Urinary 8-hydroxydeoxyguanosine (8-OHdG), a marker of oxidative damage of DNA, was increased in stone patients and was positively correlated with tubular damage as assessed by urinary excretion of NAG (17). All major markers of chronic inflammation including proinflammatory cytokines, adhesion molecules, microalbumin, myeloperoxidase, 8-OHdG, 3-nitrotyrosine and monocyte chemoattractant protein (MCP-1) were detectable in patients with renal stones (18).

Animal models of nephrolithiasis

Experimental CaOx crystal deposition in the kidneys or nephrolithiasis can be induced by the administration of hyperoxaluria-causing agents such as sodium oxalate, ammonium oxalate (AOx), ethylene glycol (EG), or hydroxy-L-proline (HLP) (14). Kidneys of nephrolithic rats showed deposition of CaOx crystals in renal calyces and at papillary tips. Many were located subepithelially, often anchored to the basement membrane. Ultra-structural examination of the kidneys revealed that the epithelial cells lining renal tubules that contained crystals were damaged. Cells of the proximal tubular epithelium, where the earliest noticeable changes were detected, appeared more sensitive. This injury resulted in death and detachment of many epithelial cells, thus resulting in exposure of the basal lamina. Most crystals were intraluminal and invariably associated with cellular degradation products. Intracellular as well as interstitial crystals were also seen. Crystals appeared first in the tubular lumen. Thereafter they moved into inter and intracellular locations and eventually into the interstitium. The move into interstitium was associated with inflammation, attracting many inflammatory cells including leukocytes, monocytes and macrophages. Eventually the crystals disappeared. CaOx crystals that blocked the terminal collecting ducts on the papillary surface appeared to lose the surface epithelium, become exposed to the pelvic urine and grow as large papillary deposits. The loss of papillary surface epithelium appeared to be a result of loosening of tight junctions.
CaOx crystal deposition in the kidneys also increased the expression of Tamm-Horsfall protein (THP), OPN, inter-alpha-inhibitor (ITI), prothrombin (PT), and heparin sulfate (HS), as determined by immunocytochemical localization using specific antibodies. There was no increase in either the production or excretion of THP, only increased retention of crystals that were surrounded by THP. Other studies have, however, shown either a decrease or an increase in THP expression and production by nephrolithic rats. Production and urinary excretion of OPN, PT, various ITI-related proteins and HS was substantially increased as determined by detection of their specific mRNAs. The up-regulated macromolecules play significant roles in inflammatory process. HS regulates extracellular matrix production. Bikunin, a constituent of ITI, is a proteinase inhibitor. Acute inflammatory conditions are known to up- or down-regulate transcription of inter-α-inhibitor (ITI) genes. Bikunin is associated with inflammation and stabilization of the extracellular matrix. THP is seen in the renal interstitium in several forms of tubulointerstitial diseases. Interestingly, inactivating the THP gene in mouse embry-

onic stem cells results in spontaneous formation of calcium oxalate crystals in adult kidneys. The administration of THP is shown to produce interstitial inflammation and scarring. It can activate alternate pathways, interact with neutrofils and bind to certain cytokines. Prothrombin is the precursor of thrombin and fragments 1 and 2. Thrombin is involved in platelet aggregation and blood coagulation and plays a major role in the recruitment and activation of infiltrating immune cells.

Osteopontin is a not only a modulator of crystallization but also a monocyte chemoattractant, specifically for the renal interstitium and upregulation of osteopontin precedes interstitial monocyte infiltration. Osteopontin knockout studies demonstrated a reduced influx of macrophages into obstructed kidneys of knockout mice compared to wild type mice at day 4 and 7 but not at day 14. It was concluded that osteopontin mediated early interstitial macrophage influx. Ethylene glycol administration to OPN knockout mice resulted in intratubular deposition of CaOx while there was no deposition in the wild type mice given the same treatment.

Evidence has also been presented for the activation of renin-angiotensin system (RAS) during the development of tubulointerstitial lesions of CaOx crystals. CaOx crystal deposition in rat kidneys activated the RAS, increased renin expression in the kidneys and serum and regulated OPN production. Reduction of angiotensin production, by inhibiting the angiotensin converting enzyme as well as blocking the angiotensin receptor, reduced crystal deposition and ameliorated the associated inflammatory responses.

Mild renal injury was detectable as increased urinary enzymes in hyperoxaluric rats without CaOx crystal deposition in the kidneys, indicating the nephrotoxic nature of the Ox ions, while morphologically discernible damage was associated with crystal deposition. This attests to the detrimental effect of epithelial exposure to the dual toxins, Ox and CaOx crystals.

Results from one of our studies showed a gradual increase in urinary levels of alkaline phosphatase (AP), gamma-glutamyl transpeptidase (GGTP), and N-acetyl-β-glucoseaminidase (NAG), enzymes often reflective of proximal tubular injury. Urinary excretion of NAG was most significantly increased and correlated highly with urinary excretion of Ox. Another study showed that hyperoxaluric rats had increased urinary excretion of LDH and lipid peroxides indicating the involvement of free radicals in oxalate and CaOx associated renal toxicity. Renal oxidative damage of hyperoxaluric rats was shown to be caused by changes in mitochondrial glutathione and energy homeostasis.

Support for the involvement of oxidative stress in CaOx nephrolithiasis was also provided by treating the hyperoxaluric rats with antioxidants. Administration of Vitamin E to the hyperoxaluric rats resulted in the amelioration of their renal tubular injury and reduction in CaOx crystal deposition (19, 20). Taurine treatment of the hyperoxaluric rats improved antioxidant status and resulted in reduction in CaOx crystal deposition in the kidneys (21). Reduction in renal epithelial injury and CaOx crystal deposition was seen in hyperoxaluric rats treated with atorvastatin (22).

Tissue culture studies

Tissue culture studies in which renal epithelial cells were exposed to Ox and/or CaOx have provided new insights into the pathogenesis of nephrolithiasis, both in animal models as well as in the clinical setting. Cell response is time and concentration dependent and cell specific. Both Ox and CaOx crystals are injurious to renal epithelial cells in culture. LLC-PK1 cells, which represent the proximal tubular cells, are more susceptible to injury then MDCK cells, which represent epithelial cells of the more distal sections of the renal tubules. Lower Ox levels induce expression of immediate early genes, stimulate DNA synthesis and promote cellular proliferation, while higher Ox levels induce cell damage and death. Epithelial injury promotes attachment of CaOx crystals. Attachment is mediated by Ox-induced exposure of phosphatidylserine (PS) on cell surfaces. Enrichment of cell membranes with PS also resulted in increased attachment of CaOx crystals. It has been shown that crystals bind rapidly to the surface of epithelial cells and are internalized. A variety of anionic cell surface molecules, which mediate crystal attachment, can be exposed during cell proliferation or on exposure to Ox. Several studies have, however, indicated that injury and exposure of PS may not be essential for the attachment of CaOx crystals to epithelial cells. Specific urinary substances such as citrate, glycosaminoglycans, OPN, and bikunin inhibit the process. In addition, crystals adhere to proliferating and subconfluent, but not to confluent, cultures of MDCK cells.

The response of renal epithelial cells to COM crystals is characterized by increased expression of specific genes that encode the following. 1, transcriptional activator such as early growth response-1, *c-myc*, Nur-77, *c-jun*; 2, a regulator of the extracellular matrix composition, the fast-acting plasminogen activator inhibitor-1; and 3, growth factors that could stimulate fibroblast proliferation in a paracrine manner, such as platelet-derived growth factor-A chain, connective tissue growth factor. The exposure to oxalate and CaOx crystals also up-regulates the production of both the OPN, bikunin, two known modulators of crystallization. Recent studies have shown OPN to be a monocyte chemoattractant specifically for the renal interstitium and that up-regulation of OPN precedes the interstitial monocyte infiltration. Interestingly renal cell exposure to high oxalate as well as calcium oxalate and calcium phosphate crystals leads to production of both OPN and monocyte chemoattractant protein-1 (MCP-1).

Results of studies of renal epithelial cell exposure to oxalate and calcium phosphate and CaOx crystals also confirmed the involvement of free radicals in production of various crystallization modulators, inflammatory macromolecules as well as toxicity. Renal cells exposed to CaOx crystals secrete superoxide in real time as measured by an electrochemical superoxide biosensor (23). The presence of antioxidants produced a significant reduction in cell injury and improved the antioxidant status of the cells when exposed to oxalate or CaOx or CaP crystals. In addition there was a marked reduction in the production of macromolecules such as OPN and MCP-1. Both mitochondria and NADPH oxidase appear to be involved.

Concluding remarks

Recent data indicate that kidneys of idiopathic stone formers are under oxidative stress, and show signs of damage. Their kidneys also show signs of chronic low grade inflammation. Tissue culture studies indicate that interactions between the crystals and renal cells produce reactive oxygen species (ROS), which appear to mediate many of the cellular responses. There is increased production of the chemokine, MCP-1 as well as OPN, latter being both a monocyte chemoattractant as well as mineralization modulator. CaOx crystal deposition in rat kidneys leads to oxidative stress, is associated with the activation of renin-angiotensin system, and increases the production of macromolecules such as OPN that modulate crystal formation and their retention within the kidneys. Renal injury results in cell death and sloughing of cells and membranous degradation products in the urine. These membranes have been demonstrated to promote crystal nucleation at much lower supersaturations. Floating dead cell may also assist in crystal aggregation and slowing their movement through the renal tubules. Damaged cell membranes may also be sites for crystal attachment and eventual retention within the kidneys. Renal damage may also assist in movement of crystals from the intratubular to interstitial location and perhaps in the formation of apatitic Randall's plaque. Products of interaction between renal tubular cells and Ox or CaOx crystals under hyperoxaluric conditions might play an important role in the attraction and accumulation of infiltrating inflammatory cells. The interstitial infiltrate around crystals consists mainly of lymphocytes and macrophages. They may be responsible for the renal tissue damage through the production of proteolytic enzymes, cytokines, and chemokines. Such tissue damage may assist crystalline Randall's plaques to ulcerate to the renal papillary surface. Exposed plaques when continuously bathed to the slow moving urine of the renal pelvis and urinary calcium and oxalate promote the formation of kidneys stones attached to the renal papillae.

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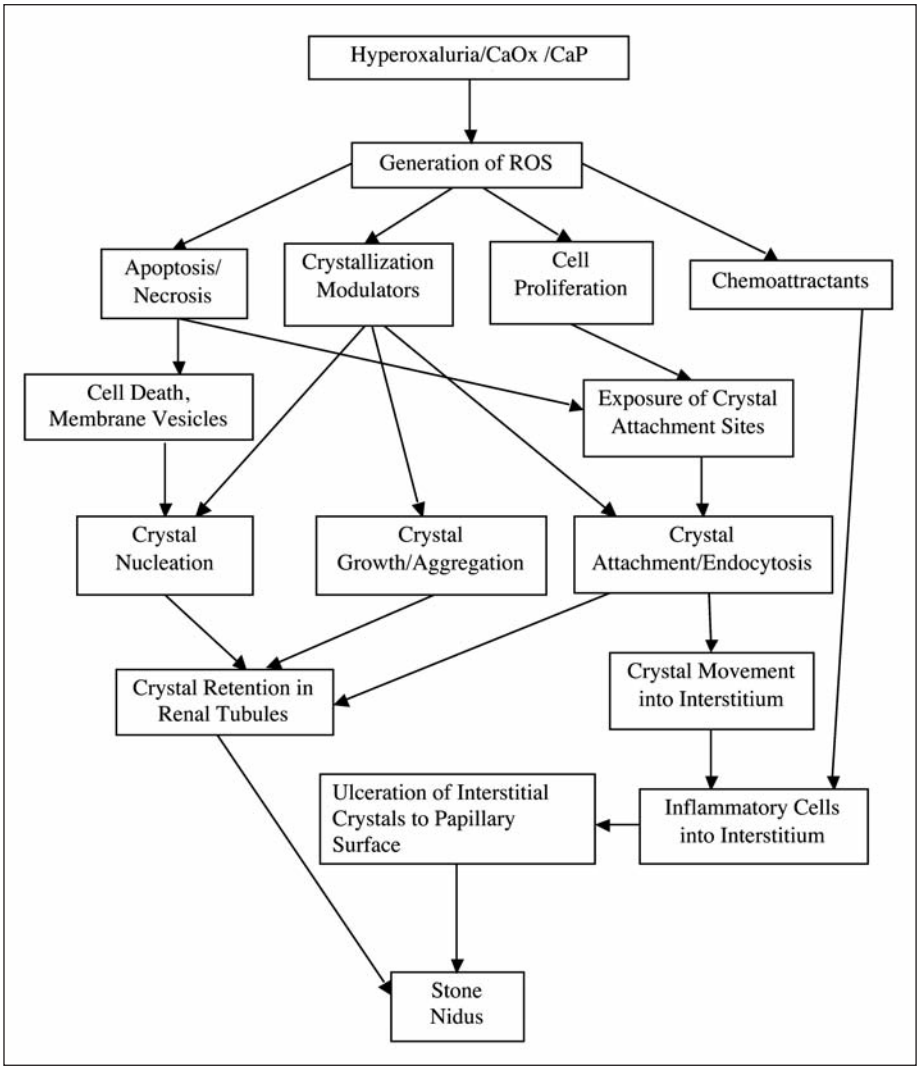

Figure 1. *Diagrammatic representation of events following exposure of renal epithelial cells to high oxalate and or calcium oxalate (CaOx) / calcium Phosphate (CaP) crystals. Reactive oxygen species (ROS) are produced which trigger signaling pathways leading to specific cellular responses. Cells produce crystallization modulators and chemoattractants. Many of the exposed cells undergo apoptosis or necrosis which is followed by cell proliferation. Membranous cellular degradation products promote heterogeneous nucleation of crystals at lower supersaturation and also assist in crystal aggregation and their retention within the tubules by becoming a part of the growing aggregates thereby increasing their mass. Apoptosis as well as cell proliferation expose crystal attachment sites which are also involved in crystal retention. Attached crystals are endocytosed by the cells at the luminal side and exocytosed at the basolateral side resulting in crystal movement into the renal interstitium. Inflammatory cells are recruited at sites of crystal deposits. These cells release proteases which help ulceration of the interstitial crystals to the papillary surface. These exposed crystals are now continuously bathed in the pelvic urine with its supply of calcium, oxalate and other ions, eventually leading to the formation of a stone attached to the papillary surface. (Modified from Khan, Urol. Res. 34:86, 2006).*

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Treatment of small lower pole calculi – SWL vs. URS vs. PNL?

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Summary

According to current guideline recommendations extracorporeal shock wave lithotripsy (SWL) remains the first choice treatment for small and mid-sized renal calculi. However, the results of SWL treatment for lower pole stones can be disappointing whilst more invasive endoscopic modalities, such as flexible ureterorenoscopy (fURS) and percutaneous nephrolithotomy (PNL) are often considered more effective.

This article summarizes a point-counterpoint discussion at the 9th eULIS symposium in Como, Italy, and discusses the potential advantages and disadvantages of the different therapeutic approaches.

KEY WORDS: Urinary calculi; Lower pole; Extracorporal shock wave lithotripsy; Ureterorenoscopy; Percutaneous nephrolithotomy.

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INTRODUCTION

Since its introduction in the early 80ies, extracorporeal shockwave lithotripsy (SWL) has become the method of choice for treatment of most upper urinary tract calculi and has replaced open and percutaneous procedures (1-3).

Recent guidelines have confirmed SWL as the primary method of choice for small and mid-sized urinary calculi. Today, urologists and patients have however become more critical about SWL when contemplating the best treatment for a stone. This is mainly due to the limited results of SWL, even after repeated treatment sessions, in particular for stones in the lower pole or for hard stones, i.e. calcium oxalate monohydrate. On the other hand endourological techniques and skills, especially in flexible ureterorenoscopy (URS) have advanced significantly, making URS a very efficient, competitive, and safe procedure (4-6). Likewise, percutaneous nephrolithotomy (PCNL), established in the 70ies, has re-gained increasing importance in the treatment of mid-sized stones due to excellent stone free rates.

This article summarizes a point-counterpoint discussion that was held at the *9th eULIS symposium in Como, Italy*. It discusses the advantages and disadvantages of the above mentioned therapy options.

EXTRACORPORAL SHOCK WAVE LITHOTRIPSY

Today, about 80% of all stones are treated by extracorporeal shock wave lithotripsy (SWL). Whereas this is by far the most successful stone treatment modality, its limitations are most apparent in the treatment of lower pole calyceal renal stones. There is widespread consensus within the urological community that stones > 2 cm in diameter in the lower pole of the kidney should be approached by percutaneous nephrolithotomy (PNL). But what about the stones smaller than 2 cm? One could opt for more invasive PNL, less invasive flexible ureterorenoscopy (fURS), or minimal invasive SWL. However, minimal invasiveness has a price. The pay-off is a reduced stone-free rate (SFR) which for small stones in the lower pole calyces has been reported between 25 and 84%, dependent on the experience of the operator and the machine used. A variety of other factors also have to be considered when making the choice of an optimal treatment modality: body habitus, renal anatomy, treatment costs, patient preference, and local infrastructure in terms of expertise and equipment (7).

Factors for successful SWL treatment

The gravity-dependent position of the lower pole calyces is considered as the main factor for an impeded post-

SWL fragment clearance (8). Others found the stone size the most determining factor in the lower pole (9). From the late 90ies of the last century, several authors postulated a negative determining role of renal anatomical factors for the clearance of post-SWL lower pole fragments. In particular, these were an infundibulo-pelvic angle < 45°, an infundibular length > 30 mm, and an infundibular width < 5 mm (10-14). More recent studies could not confirm these findings however, neither in the adult (15) nor in the paediatric patient population (16). The skin-stone-distance (SSD) has likewise been postulated as a determining factor but this has not found widespread acceptance to date (17).
Thus, many factors have been implicated to hamper fragment clearance from the lower pole but the discussion is ongoing and remains controversial.

Efficacy

When it comes to SFR after SWL of lower pole stones, most authors quote stone-free status at 3 months after treatment. Most authors differentiate between stones < 10 mm, 10-20 mm, and > 20 mm. SFR in these groups range from 64-84%, 38-66%, and 25-49%, respectively (Table 1) (11, 18-20). Patients need on average 1.05 treatments to stone freeness (19), meaning that the overwhelming majority of those patients rendered stone-free with SWL will have undergone a single treatment. Notably, SWL is not helpful in asymptomatic calyceal stones (21).

Complications

SWL treatment failures in small lower pole stones are reported in 1.2 % of cases (22). In other words, in > 98% SWL can be delivered more or less successfully. The most common complications are Steinstrasse and pyelonephritis, both in < 1% of patients. As to adjuvant procedures, 8.6% of patients may need a JJ stent insertion, and < 2% an adjuvant URS to help with fragment clearance (19). Serious or life-threatening complications are very rare (Table 1).

Flexible ureterorenoscopy

Technical advances such as miniaturisation of instruments, better optical quality, efficient intracorporeal lithotripsy, and availability of flexible scopes have increased the frequency of ureteroscopies. Modern scopes with outer diameters less than 9F allow direct access to the upper urinary tract without dilatation of the ureteric orifice in almost all cases (5, 6, 23, 24). Flexible URS (fURS) can access difficult stone localisations such as the lower pole. The introduction of the Holmium:Yttrium-Aluminium-Garnet (Ho:YAG) laser into endourology can be seen as a milestone (25). It is highly effective for all stone compositions and can be used in both semirigid and flexible scopes (26-28). Furthermore, the latest generation of flexible scopes seem to be a step in the right direction to overcome the problem of constant technical defects and may further support a wider use of fURS (26, 29-31).
According to international guidelines for kidney stone management, flexible URS (fURS), or retrograde intrarenal surgery [RIRS], figures as second choice in calculi < 1 cm, or third choice in stones 1-2 cm. There is no recommendation for fURS in calculi > 2 cm (32).

Table 1.

SWL for lower pole stones – the options compared.

	PNL	URS	SWL
Efficacy	very good	fair/good	fair/good
SFR	> 90%	as SWL	25-84%
Invasive	quite	moderate	minimal
TC *	14%	8-20%	8%
MC **	4.5-5.8%	rare	very rare
mortality	0.5%	very rare	very rare
Anaesthetic	GA/SA	GA	none
OP time	~ 120'	~ 99'	~ 88'
OP Rx	no	possible	yes
Pat pref	-	-	favoured ***

** Including access- and treatment failure.*
*** Re-intervention and/ or life threatening.*
**** Pearle et al. J Urol 2005.*

Legend: PNL = percutaneous nephrolithotomy; URS = ureterorenoscopy; SWL = extracorporeal shock wave lithotripsy; SFR = Stone Free Rate; TC = total complications; MC = major complications; GA = general anaesthesia; SA = spinal/ epidural anaesthesia; OP time = operating time; OP Rx = outpatient treatment; pat pref = patient preference.

Percutaneous nephrolithotomy

Percutaneous nephrolithotomy (PNL) was established as minimally invasive treatment option for kidney stone removal in the 70ies, and was further developed over the following years (33). Percutaneous stone therapy competes not only with SWL but also with URS and, in selected cases, with open procedures.
Whilst SWL has proven its value in the treatment of kidney stones < 1 cm when the renal anatomy favours the clearance fragments, endourological procedures have gained increasing importance in most other situations. However, whilst the frequency of URS was increasing, the use of PNL kept decreasing over time. Only more recently, as clinical experience with SWL and URS revealed their limitations, the role of PNL in the treatment of urolithiasis has been re-defined. Urologists did realized that in some situations PNL offers advantages over SWL and URS, respectively. PNL is especially superior in the treatment of lower pole calculi and complex stone situations.
The introduction of so-called "*Mini-PERC*" has further contributed to a wider use of percutaneous techniques, even for stones < 2 cm (34-36). The term is not exactly defined but is commonly used for small diameter accesses (mostly 18F compared to 24-30F in conventional PNL). Potential advantages are lower morbidity due to less bleeding and pain. However, its value is still under discussion (37). Treatment time is prolonged while stone free rate seems to decrease with larger stone size. In

experienced hands, complications in conventional PNL are also rare (35, 38, 39).
Lahme et al. raised the question whether Mini-PNL would lead to an extension of the indications for percutaneous treatments (35, 40). *Nagele et al.* reported the successful and safe use of Mini-PNL even for smaller stones 8-15 mm (41). Others did not agree with the benefits of such approaches and recommend the use of standard nephroscopes if URS or SWL have been ineffective (37). Further studies are needed to clear whether there is a definite advantage in Mini-PNL.

Discussion

SWL, especially in an anaesthesia-free outpatient setting can still be considered as the first line treatment option for most stones (42). This includes small isolated lower pole renal stones where it delivers an acceptable SFR, few complications, and a low recurrence rate (11, 19). Despite its minimal invasiveness, we do know that shock waves do induce transient damage to the renal parenchyma. Recently, a new treatment strategy has been tested which reduces tissue trauma by low-energy shock wave pre-treatment, followed by the usual high-energy therapeutic treatment. This induces parenchymal vasoconstriction during SWL, rather than afterwards as it is the case without pre-treatment (43). This may make SWL even more minimally invasive and more attractive even in situations where a better clearance rate might be achieved by more aggressive approaches such as small lower pole calyceal stones.
How do these results and complications compare with other treatment modalities? The most serious competitor these days for the treatment of small lower pole renal stones is perhaps fURS. It has been shown that the efficacy of both, SWL and fURS, is not significantly different (7, 44). Also, post-operative complication rates are similar. In contrast, intra-operative complication rates are far lower in SWL (1.5%) as compared to fURS (10%) (22). Further favouring SWL are a significantly shorter operating time, a better patient acceptance, and a shorter convalescence (22, 44). fURS however has an undisputed role in obese patients, patients with bleeding diathesis, patients with complicated renal anatomy, and SWL treatment failures (45).
As to PNL, this is recommended for larger stones (18). It is the most effective treatment modality, but the most invasive too, with longer operating time, need for general anaesthesia, few but possibly severe complications, longer hospitalization, and longer convalescence than the other two (Table 1) (44). Only one paper reported the application of PNL for smaller lower pole calculi (41).
In 2009, *Srisubat et al.* published a Cochrane analysis of SWL vs. URS vs. PNL for the treatment of renal calculi (44). The authors criticized the low data quality of the available literature; only three studies could be included for meta-analysis. Based on this data, SWL had the lowest efficacy while PNL and URS did not differ significantly. Hospital stay was shorter with SWL.
All three treatment modalities offer a good chance to render the patient stone-free in one single session (19).

Conclusions

Small stones < 10 mm are usually successfully treated by shock wave lithotripsy. With increasing stone size, stone free rate decreases but still reaches up to 70% for 25 mm calculi. SWL therefore remains the method of choice for most kidney stones which is in accordance to recommendations in international guidelines. Larger stones, especially within the lower pole are more efficiently treated by PNL (3, 46, 47). Flexible URS is recommended as 2nd line treatment for smaller lower pole stones. Despite this recommendations it is however already used as the primary method of choice for such stones by many urologist. Further studies will have to confirm its superiority.

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Ureteral stones: SWL treatment.

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Summary

When stone removal is indicated SWL (Shock Wave Lithotripsy) and ureteroscopy (URS) are the two most commonly offered interventional procedures and they are both acceptable as first-line treatment. The choice of the procedure depends on several factors, including local experience, patient preference, available equipment, and associated costs.
The meta-analysis by the EAU/AUA Guideline Panel in 2007 analysed SWL stone-free results for three locations in the ureter (proximal, mid, distal) and reported an overall stone-free rate for proximal ureteral stones of 82%, with no difference in stone-free rate from URS results. However, for stones < 10 mm SWL, at 90%, had a higher stone-free rate than URS and even for mid and distal ureter it reached a stone-free rate of 84% and 86% respectively. It does appear that SWL may be more effective in the paediatric subset than in the overall population, particularly in the mid and lower ureter with a stone free rate of 82% and 80% respectively. In fact, children appear to pass stone fragments after SWL more readily than adults. SWL is a safe method to treat ureteral stones and serious complications occur very rarely when proper indications are followed. A few published studies addressed the role of SWL in acute renal colic. The available data suggest that is a safe procedure, with an overall success of 70-80% and a need for further intervention in 2-20%. In choosing the optimal therapy for an individual patient, several factors that might affect the outcome should be considered to identify the best candidate for SWL.
A superior success rate for proximal ureteral stones was reported in the EAU/AUA meta-analysis but stone size over 10 mm appears negatively correlated with the stone-free rate. About composition, calcium oxalate monohydrate, brushite, cystine and matrix are unfavourable compositions for SWL. Finally, impacted stones are often more resistant to fragmentation. Whether hydronephrosis affects the outcome of SWL remains controversial. A body mass index of over 30 has been found to be an independent factor in predicting failure of SWL treatment in ureteral stones.
A number of treatment strategies have been proposed to increase SWL efficacy: a promising suggestion to improve SWL outcome is to reduce the shock wave rate. There have also been attempts to improve shock wave efficiency of stone fragmentation with new shock wave lithotriptor devices. But although these innovation are promising, no advantage in stone-free rate or retreatment rate have yet been proven. Acoustic coupling is a key factor affecting the efficacy of shock wave lithotripsy. An accurate pre-treatment assessment of stone burden and composition with unenhanced CT scan provides useful information to discern which treatment strategy should be favoured and may reduce SWL failure. The real cost for SWL and URS varies considerably from one centre to another, as a result of different internal organisations and also due to the principles of reimbursement from the health care system.
Conclusions: SWL is the first treatment choice for stones smaller than 1 cm in the proximal ureter. With a lower grade of invasiveness and the possibility to complete the treatment with only analgesics and sedation on an outpatient basis, SWL still appears an excellent alternative for removing ureteral stones and these properties compensate for the higher need for repeated treatments. An accurate pre-treatment assessment of stone and clinical factors to select the best candidates for SWL could improve the stone-free rate and reduce retreatments.

KEY WORDS: *Shock Wave Lithotripsy (SWL); Ureteroscopy (URS) ureteral stones; Ureteral calculi*

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INTRODUCTION

The array of technologies currently available allows almost any symptomatic patient to be considered as a candidate for stone removal, although many patients will pass the stone spontaneously.

Stone size and initial stone location influence the likelihood of spontaneous stone passage and treatment recommendation. In fact 68% of the patients would pass spontaneously ureteral stones less than 5 mm in diameter and 47% would pass spontaneously ureteral stones larger than 5 mm and smaller than 10 mm; in addition medical expulsive therapy may be offered to facilitate stone passage.
On the contrary, stone removal is indicated in the presence of persistent obstruction, failure of stone progression, increasing or unremitting colic and in patients with ureteral stones > 10 mm.
When stone removal is indicated SWL (Shock Wave Lithotripsy) and ureteroscopy are the two most commonly offered interventional procedures, both acceptable as first-line treatments (Recommendation 2007 EAU/AUA guideline) (1).

Table 1.

Stone free rates for SWL - Overall population: Primary procedures or first treatment.

Ureter Location			N° Procedures procedures	N° Additional per patient
Proximal Ureter	overall	82% (79-85%)	1.31	0.62
	< 10 mm	**90%** (85-93%)	1.26	
	> 10 mm	68% (55-79%)	1.49	
Mid Ureter	overall	73% (66-79%)	1,11	0.52
	< 10 mm	**84%** (65-95%)	1.29	
	> 10 mm	76% (36-97%)	1.76	
Distal Ureter	overall	74% (73-75%)	1.22	0.37
	< 10 mm	**86%** (80-91%)	1.34	
	> 10 mm	74% (57-87%)	1,44	

EAU/AUA 2007 Guideline for the management of ureteral calculi

The choice of the best procedure depends on several factors, including local experience, patient preference, available equipment, and associated costs.
SWL treatment is less invasive but has some limitations, such as a high retreatment rate and lack of availability in many centres. Ureteroscopy requires general or regional anesthesia and might be associated with a greater risk of complications (2).
The patient's expectations must also be considered in recommending different treatment modalities. Although SWL is less invasive, a patient may be reluctant to select this modality because it entails an often lengthy follow-up until fragment clearance, as well as the risk of unplanned additional invasive procedures and retreatments. Conversely, patients may select SWL due to fear of the anaesthesia associated with ureteroscopy or the possibility of a temporary ureteral stent.
Moreover, in an outpatient setting, the low complication rates, low analgesia requirements, and high fragmentation rates may support patients' acceptance to undergo a further SWL session.
A patient must be informed about the existing active treatment modalities, including the relative benefits and risks associated with each modality (1).

SWL TREATMENT RESULTS IN ADULTS

The meta-analysis performed by the EAU/AUA guideline panel in 2007 analysed SWL stone-free results for three locations in the ureter (proximal, mid, distal) and reported an overall stone-free rate for proximal ureteral stones of 82%, with no difference in stone-free rate from URS results (Table 1) (1).
However, for stones < 10 mm, SWL, at 90%, had a higher stone-free rate than URS and even for mid and distal ureter it reached a stone-free rate of 84% and 86% respectively. For all distal stones URS yields better stone-free rates overall and in both size categories.
URS appears superior for all mid-ureteral stones, as well, but without reaching statistical significance.
There is a great variability between the success rate reports; this depends on the heterogeneity of lithotriptor devices and the lack of uniformity in reporting the stone-free status.
Success rates for SWL treatment have been reported to be machine dependent in clinical practice (*Graber '03*, *Kim '06*) with a consistently high stone-free rate, and low retreatment rate, with an HM3 lithotriptor (*Gerber '05*, *Portis '03*, *Nabi '09*).
However, some authors have recently reported that modern machines perform at least as well and often better than the Dornier HM3 (*Tailly '08*).) reaching a total stone free rate as high as 97% (*Tiselius '08*).

Table 2.

Stone free rates for SWL - Pediatric population: Primary procedures or first treatment.

Ureter Location			N° Procedures procedures	N° Additional per patient
Proximal Ureter	overall	**81%** (69-90%)	1.28	0.03
	< 10 mm	89% (72-98%)	1.19	
	> 10 mm	63% (21-94%)	1.38	
Mid Ureter	overall	82% (63-94%)	1.44	0.23
	< 10 mm	80% (41-96%)	1.50	
	> 10 mm	96% (67-100%)	1.33	
Distal Ureter	overall	80% (68-90%)	1.38	0.24
	< 10 mm	86% (78-92%)	1.42	
	> 10 mm	83% (58-97%)	1.42	

EAU/AUA 2007 Guideline for the management of ureteral calculi

Stone-free rates for SWL - Paediatric population

It does appear that SWL may be more effective in the paediatric subset than in the overall population, particularly in the mid and lower ureter with a stone free rate of 82% and 80% respectively (Table 2) (1). Children appear to pass stone fragments after SWL more readily than adults (5).
Possible explanations include the shorter length of the ureter which is more elastic and distensible and prevents ureteral impaction, in addition to the small loss of energy during the passage through body tissue (6).
Both SWL and URS are effective in this population. Treatment choice should be based on the child's size and urinary tract anatomy.The overall stone free rate and the small size of the paediatric ureter and urethra favour the less invasive approach of SWL (1).

Complications

SWL is a safe method to treat ureteral stones when proper indications are followed; serious complications occur very rarely. There are well known adverse effects related to stone fragmentation, infection, and effects on tissue. The Table 3 shows the most relevant adverse effects (1).

Table 3.

1	Sepsis	(3-5%)
2	Steinstrasse	(4-8%)
3	Stricture	(0-2%)
4	Ureteral injury	(1-2%)
5	Urinary tract infection	(4-6%)
6	Subcapsular haematoma	(0.5%)
7	Some gastrointestinal injury	(in case studies; Maker '04)

Emergency shock wave lithotripsy

There have been few published studies addressing the role of SWL in acute renal colic.
The available data suggest this is a safe procedure, with an overall success of 70-80% and a need for further intervention in 2-20% (7).
It seems that stones > 5 mm in the proximal ureter are favourable candidates for emergency SWL.
The rationale for emergency is to perform treatment before mucosal oedema associated with stone impaction develops, but definition of what constitutes emergency SWL remains to be established (e.g. 6-24 /96 h).
This should remain a valid alternative treatment option offered to patients, and its provision may be restricted by resource availability rather than clinical evidence.

Factors affecting stone fragmentation

In choosing the optimal therapy for an individual patient, numerous factors that might affect the outcome should be considered to identify the best candidate for SWL. A superior success rate for proximal ureteral stones was reported in the EAU/AUA meta-analysis.
Stone size over 10 mm appears negatively correlated with the stone-free rate (8) with more auxiliary procedures, more complications, and a higher retreatment rate. About composition: calcium oxalate monohydrate, brushite, cystine and matrix are unfavourable compositions. Finally, impacted stones are often more resistant to fragmentation. An impacted stone may be defined as a stone that cannot bypassed by a wire or catheter or a stone that remains at same site in the ureter for more than two months.
Whether hydronephrosis affects the outcome of SWL remains controversial, but recently no correlation with success rates for proximal and distal ureteral stones less than 15 mm was reported
A body mass index of over 30 has been found to be an independent factor in predicting failure of SWL treatment in ureteral stones as has difficult anatomy. In order to assess how many treatments are useful to achieve an ureteral stone fragmentation only a marginal success rate of 1% after a third shock wave application has been described, which has led some authors to suggest ureteroscopic treatment after initial SWL failure.

SWL Ureteral Treatment

Patients can usually be managed on an outpatient basis. There is no need for general or regional anaesthesia or to occupy an operating theatre for the procedure.
A combination of fluoroscopy and US can facilitate stone location and minimize radiation exposure. SWL is generally performed in situ (the use of a ureteral stent to improve stone-free rates is not warranted) (11). When stone manipulation is planned under regional or general anaesthesia or when a ureteral stent is to be placed before SWL, consideration should be given to immediate ureteroscopy.

Methods to increase SWL efficacy

A number of treatment strategies have been proposed to increase SWL efficacy:
A promising suggestion to improve SWL outcome is to reduce the shock wave rate, as the rate affects stone fragmentation in vitro and in vivo with improved efficiency at slower rates. Clinical studies have confirmed an increased fragmentation when lowering shock wave rate in ureteral stones (12).
A progressive increase in lithotripter output voltage during swl can produce greater stone fragmentation (13).
Treatment in rotated-supine or prone position was reported to achieve a superior outcome when treating ureteral stones.
There have also been attempts to improve shock wave efficiency of stone fragmentation with new shock wave lithotriptor devices. The twin pulse technique using two identical shock wave generators and dual focus lithotriptors with different focal sizes have been proposed. But although these innovation are promising, no advantage in stone-free rate or retreatment rate have yet been proven.
Acoustic coupling is a key factor affecting the efficacy of shock wave lithotripsy. Coupling between the treatment head of the lithotripter and the skin surface is inefficient and highly variable and can reduce the effectiveness of SWL treatment. The technique used to apply lithotripsy gel, as an excessive handling of the gel,creates air pokets on the coupling interface and can have a significant effect on the quality of coupling.

Identification of the best candidates for SWL

An accurate pre-treatment assessment of stone burden and composition with unenhanced CT scan provides useful information to discern which treatment strategy should be favoured and may reduce SWL failure.
Recent case series have reported Hounsfield Units on CT scans as a predictor of stone composition and potential fragmentation during SWL treatment.
Significant inverse relation between Hounsfield units (HU) and stone clearance has been reported (> 750 HU required more sessions and were less likely to achieve complete stone clearance (10). More recently was reported (In in vitro studies) than stone morphology assessed with CT imaging correlates with calcium oxalate monohydrate stone and Cystine stone fragility.

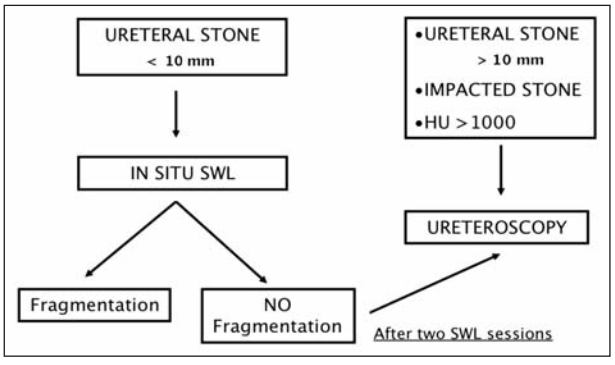
Costs of SWL vs URS treatment

The real cost for SWL and URS varies considerably from one centre to another, as a result of different internal organisations and also due to the principles of reimbursement from the health care system.
When outpatient ureteroscopic lithotripsy was compared with outpatient SWL monotherapy, SWL was more expensive due to the high cost of purchasing and maintaining a lithotriptor and to retreatments and auxiliary procedures.
However Some authors have reported that when ureteroscopy was performed on an inpatient basis, with an average hospital stay of 3 days, ureteroscopy, became more expensive than SWL (14).

Algorithm of ureteral stone treatment

To achieve stone clearance minimizing patient morbidity and hospital attendance in our institution we perform ureteral stone treatment following this therapeutic algorithm with a primary treatment with SWL for non impacted ureteral stones less than 10 mm in diameter (Figure 1).

Figure 1.
Algorithm of ureteral stones treatment.



Conclusions

1. SWL is the first treatment choice for stones smaller than 1 cm in the proximal ureter.
2. With a lower grade of invasiveness and the possibility to complete the treatment with only analgesics and sedation on an outpatient basis, SWL still appears to be an excellent alternative for removing ureteral stones and these properties compensate for the higher need for repeated treatments.
3. An accurate pre-treatment assessment of stone and clinical factors to select the best candidates for SWL could improve the stone-free rate and reduce retreatments.

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Predicting five-year recurrence rates of kidney stones: An artificial neural network model.

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Summary

Objective: *Due to high recurrence rates of urolithiasis, many attempts have been performed to identify tools for predicting the risk of stone formation. The application of Artificial Neural Networks (ANNs) seems to be a valid candidate for reaching this endpoint. The aim of this study was to find a set of parameters able to predict recurrence episodes immediately after clinical and metabolic evaluation performed at the first visit in a 5-year window.*

Material and methods: *Data were collected from 80 outpatients who presented idiopathic calcium stone disease both at baseline and after 5 years; patients underwent treatment including both general measures and medical therapy. After 5 years, patients were classified into two subsets, namely SSFs (without recurrence episodes), consisting of 45 subjects (56.25%) and RSFs, with at least one episode of recurrence after the baseline, consisting of 35 subjects (43.75%). Helped by conventional statistics (One-way ANOVA and three Discriminant Analyses: standard, backward stepwise and forward stepwise), an Artificial Neural Network (ANN) approach was used to predict recurrence episodes.*

Results: *An optimal set of 6 parameters was identified from amongst the different combinations in order to efficiently predict the outcome of stone recurrence in approximately 90% of cases. This set consist of serum Na and K as well as Na, P, Oxalate and AP(CaP) index from urine. The results obtained with ANN seem to suggest that some kind of relationship is present between the identified parameters and future stone recurrence. This relationship is probably very complex (in the mathematical sense) and non-linear. In fact, a Logistic Regression was built as a comparative method and performed less good results at least in terms of accuracy and sensitivity.*

Conclusions: *The application of ANN to the database led to a promising predicting algorithm and suggests that a strongly non-linear relationship seems to exist between the parameters and the recurrence episodes. In particular, the ANN approach identifies as optimal parameters serum concentration of Na and K as well as urinary excretion of Na, P, Oxalate and AP(CaP) index. This study suggest that ANNs could potentially be a useful approach because of their ability to work with complex dynamics such as recurrent stone formation seems to have.*

KEY WORDS: *Idiopathic calcium lithiasis; Recurrence rate prediction; Artificial neural networks.*

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INTRODUCTION

Kidney stone formation is a common disease in the general population with a prevalence in the world ranging between 5% and 15% (1). Idiopathic calcium stone disease is the commonest form of calcium urolithiasis and calcium oxalate is the main component in about 60-80% of the stones. Due to high recurrence rates of urolithiasis (about 50%) (2), several tools for predicting the risk of stone formation have been researched by many authors, either in patients with single (SSFs) or recurrent episodes of stone formation (RSFs). Several studies have been per-

formed to predict the recurrence of stone formation in patients with idiopathic calcium stone disease and various urine parameters and/or combinations of these risk factors have been shown to have some value for distinguishing calcium oxalate SFFs from RSFs (3, 4). Urinary risk factors are those urinary analytes recognized as influencing the likelihood of calcium stone formation or recurrence, and are routinely measured as a part of the metabolic investigation of calcium stone formers. The more common urinary risk factors include: urinary calcium excretion of calcium, phosphate, magnesium, sodium, potassium, oxalate, citrate, together with urine volume and pH. Some of these factors, such as hypercalciuria and hyperoxaluria, play a direct role in stone formation; other factors work in an indirect manner: for example, sodium increases urinary calcium excretion. Regular risk factor evaluation and risk monitoring during stone therapy are recommended measures to ensure reduction of crystal formation recurrence . Nevertheless, the evaluation of risk factors both at baseline and during the follow-up does not allow the future course of the stone disease to be understood exactly (5, 6). For these reasons, several authors have performed research to identify more selective indices of stone recurrence by means of contemporary determination of several urinary risk factors (3, 7, 8) or evaluating in-vitro crystallization or saturation tests of patients' urine (9-11), or finally by means of crystalluria study (12). Some authors have shown that a saturation/inhibition index allows stone formers to be distinguished from healthy subjects, but not SSFs from RSFs (9); moreover, the extension of these tests to clinical practice was excessively complex. The more common risk indices of recurrence together with their sensitivity and specificity are reported in Table 1 (4). Of the indices proposed above, the BRI (13) seems to show two major advantages in comparison to the other risk indices based on urine analysis. In fact, BRI has evaluated a ratio between calcium concentration [Ca++] expressed as mmol/L -1 in a fresh urine sample and the oxalate amount (mmol/l) that must be added to 200 ml of patient urine to make a beginning of the crystallization process. Thus this method accounts for the influence of minor and macromolecular substances on the crystallization process, which may significantly influence stone formation processes.

Furthermore, BRI determination is less time-consuming and easier to perform than the calculation of APCaOx and RSCaOx (14, 15). This latter index, which is the current gold standard, requires measurement of at least 15 parameters prior to calculation (11, 15). In 2005, *Daudon et al.* (12) proved that crystal presence in morning fresh urine samples was a strong predictor of stone recurrence; crystals were observed in 87.5% of RSFs but only in 15.6% of SSFs. According to literature data, our previous observations have shown that observation not only of crystals but also of their characteristics (type, number and size) and above all the crystal aggregate presence represents a strong indicator of stone disease activity (11, 12, 16). All these previous methods are not easy to apply in clinical practice and at the same time do not present sufficient predictive power for supplying concrete help in clinical decision-making (17). The majority of previous works analyse the data by means of linear statistical tools. The natural history of calcium stone disease seems to suggest that the relationship, if present, could be very complex in terms of strong non-linearity and thus the application of Artificial Neural Networks (ANNs) seems to be a valid candidate for overcoming this problem and reaching the endpoint of predicting episodes of stone formation recurrence.

Selecting from our database a group of 80 patients with a follow-up longer than 5 years, and thus knowing who the SSF and RSF patients were, the differences between the two groups before therapy (at their first visit) were studied in order to predict recurrences of stone forma-

Table 1.

The one-way ANOVA results of plasma (P) and urinary excretion (UE) of parameters evaluated in SSFs and RSFs; p < .05 indicates a significant difference.

Parameters	SSF			RSF			p
	Mean	±	SD	Mean	±	SD	
UVolume	2.32	±	0.95	2.31	±	1.02	0.974
PCr	0.97	±	0.19	0.96	±	0.17	0.766
PNa	140.29	±	3.43	138.46	±	3.81	0.027
UENa	150.86	±	65.32	200.60	±	82.47	0.004
PK	4.09	±	0.37	4.25	±	0.34	0.053
UEK	51.97	±	18.94	58.82	±	21.87	0.138
PCl	103.95	±	4.00	103.83	±	4.75	0.903
UECl	150.51	±	57.33	181.80	±	75.76	0.039
PCa	9.38	±	0.49	9.40	±	0.51	0.856
UECa	217.87	±	93.87	263.00	±	124.59	0.068
PP	3.51	±	0.60	3.46	±	0.63	0.714
UEP	758.42	±	269.45	926.96	±	276.04	0.007
PMg	1.98	±	0.15	1.94	±	0.17	0.282
UEMg	78.93	±	23.11	91.56	±	35.33	0.058
PUric Ac.	4.67	±	1.80	4.27	±	1.42	0.282
UEUric Ac.	535.48	±	180.05	609.01	±	143.65	0.052
UECitrate	484.67	±	229.29	538.06	±	250.32	0.324
UEOx	29.61	±	12.32	29.57	±	10.77	0.988
pH	5.57	±	0.56	5.85	±	0.62	0.037
AP(CaOx) index	0.99	±	0.74	1.00	±	0.54	0.949
AP(CaP) index(s2)	40.53	±	24.52	55.10	±	32.26	0.024
Crystalluria	1.96	±	0.21	1.91	±	0.28	0.456

tion in the next 5 years. The aim of this paper was to find a set of parameters able to predict recurrence episodes immediately after clinical and metabolic evaluation performed at the first stone episode in a 5-year window.

Materials and methods

Data were collected from 80 outpatients selected from a 542-patient database who presented idiopathic calcium stone disease and who had never followed any kind of therapy. There were 54 males and 26 females. They presented a mean age ± SD of 46.4 ± 13.5 years and 39.4± 13.0 years, respectively. Patients started a therapy including both general measures and personal therapy according to metabolic abnormalities; patients were recalled to the medical centre for periodical controls and metabolic evaluation at least once a year. Their health status and correct adherence to the prescribed therapy were checked each time. After 5 years, patients were classified into two subsets according to their behaviour over this time: the first subset, called Single Stone Formers (SSFs), were patients who never presented a recurrence episode, and was made up of 45 subjects (56.25%) and the second, called Recurrent Stone Formers (RSFs), those who presented at least one episode of recurrence after the baseline, was made up of 35 subjects (43.75%). Knowing who the SSF and RSF patients were, the differences between the two groups before therapy (at their first visit) were studied in order to predict recurrences of stone formation in the next 5 years.

All the subjects had normal renal function with a plasma creatinine concentration lower than 110 µmol/l. All the patients underwent a metabolic study including plasma (P) and urinary excretion (UE) measurement of creatinine, uric acid, sodium (Na), potassium (K), chloride (Cl), calcium (Ca), phosphate (P) and magnesium (Mg). Urinary excretion of citrate (Cit) and oxalate (Ox) was evaluated together with pH and urinary volume. Metabolic studies were performed while the patients were on their home diet. Patients made 2 consecutive twenty-four-hour urine collections; the urine of each day was divided into 2 plastic bottles containing hydrochloric acid and thymol. Urinary pH was determined on a morning urine sample, immediately after voiding, using a pH-meter (Orion). Oxalate and citrate were evaluated by the enzymatic method (Sigma and Boehringer-Mannheim Kits) and sulphate by the turbidimetric method. Analytical methods used for the measuring of other parameters have been reported previously (18). Finally, the activity product index for calcium oxalate [AP(CaOx)] and calcium phosphate [AP(CaP)] according to the Tiselius method (14) was evaluated.

Table 2.

Standardized coefficients for canonical variables. The standardized coefficients for canonical variables of the 3 DAs show in a peculiar position urinary sodium excretion (UNaE) and plasma concentration of sodium and potassium (PNa and PK).

		Discriminant Analysis	
Parameters	**Standard**	**Backward stepwise**	**Forward stepwise**
UENa	-1.07	-1.06	-0.99
PNa	0.84	0.88	0.79
PK	-0.66	-0.86	-0.71
UECl	0.56	0.61	0.53
PUric Ac.	0.53	0.53	0.56
PCa	-0.46	-0.39	-0.42
PMg	0.41	0.56	0.46
UEOx	0.39	0.32	0.32
UEMg	-0.37	-0.42	-0.31
UEP	-0.34	Out	-0.29
PCr	-0.32	-0.42	-0.35
pH	-0.28	Out	-0.23
PP	0.27	Out	0.23
UECa	0.16	Out	out
AP(CaOx) index	-0.10	Out	out
Volume	0.09	Out	0.12
AP(CaP) index(s2)	0.08	Out	out
PCl	-0.06	Out	out
UECitrate	-0.04	Out	out
UEK	0.01	Out	out
UEUric Ac.	0.00	Out	out
Crystalluria	0.15	Out	out

Statistical methods

Changes in measurements were computed between SSFs and RSFs at their baselines. Because data could be considered normally distributed, a one-way ANOVA was used to determine statistically significant levels of change. $p < 0.05$ was used for statistical significance. Then, 3 Discriminant Analyses (DAs) were performed: standard, backward stepwise and forward stepwise. The standardized coefficients for canonical variables were analysed in order to find the parameters in peculiar positions. Analyses were performed using STATISTICA (*STATISTICA, StatSoft Italia S.r.l., Italy*).

Artificial neural network methods

No commercial simulation software was used, and so an ad-hoc simulation program was built, starting from a general purpose computer language, C/C++ (*C++ Builder Professional, Borland International, Inc., Scotts Valley, CA, USA*). This way of working allows ANNs to be interacted with and trimmed in a deeper

and more detailed manner (19-21). A Multilayer Perceptron (MLP) or rather a Multilayer Feedforward Neural Network with Backpropagation algorithm was built up. The Delta Rule was used, in particular:

$$\begin{cases} \dfrac{\partial E_p}{\partial w_{ji}} = (t_j - u_j)\cdot f'(Net_j)\cdot u_i, & \text{for the output layer} \\ \dfrac{\partial E_p}{\partial w_{ji}} = r\cdot \sum_k \left\{(t_k - u_k)\cdot f'(Net_k)\cdot w_{ki}\right\}\cdot f'(Net_i)\cdot u_i, & \text{for hidden layers} \end{cases}$$

(Net = Net Input; w = connection weight; E = error; r = learning rate; t = target value; u = single node output). The Activation Function chosen is the sigmoid (S-shaped) function:

$$f(Net_j) = \frac{1}{1+e^{-Net_j}} \quad \text{giving a} \quad f'(Net_j) = f(Net_j)\left[1 - f(Net_j)\right]$$

Network weights are updated according to:

$$w_{j(n+1)} = w_{j(n)} + \Delta w_j + Momentum_{j(n)} \text{ with } Momentum_{ij(n)} = k\cdot \Delta w_{ij(n-1)}$$

The input layer was made up with the serum and urine parameters chosen from the database. The output layer had a single neuron expressing the presence of recurrence of stone formation (Figure 1). The 80-patient database was divided into a Training Set, with 75% used to let the ANN understand the problem, or rather, the dynamics of the problem and a Testing Set of 25%, used to verify the correct prediction ability developed by the network.

Several ANNs were built, changing the input parameters chosen from among those considered relevant from the conventional statistics point of view. Once we found the optimal input parameters, we trained different ANNs, changing the different settings, mainly the number of Hidden Nodes. Learning Rate and Momentum were not fixed but were changed in an adaptive way, that is, continuously corrected with a feedback algorithm.

A Logistic Regression (LR) was chosen as a comparison tool for ANN, as it is a good classical statistical method. LR was built and tested using the same Training Set and Testing Set as ANN. LR was constructed using SPSS statistical software (*SPSS, Inc., Chicago, Illinois*).

Results

The one-way ANOVA (Table 1) shows significant differences ($p < .05$) between SSFs and RSFs for plasma sodium concentration and urinary excretion of sodium, chloride and phosphate, urinary pH and AP(CaP) Index. The standardized coefficients for canonical variables of the 3 DAs show three parameters in a peculiar position, namely urinary sodium excretion (UENa) and plasma concentration of sodium and potassium (PNa and PK). Several ANNs were built, changing the input parameters chosen from amongst those suggested by conventional statistics. In fact, we tried to use the parameters resulting as significant according to the one-way ANOVA together with the most relevant from the three DAs. Best results were obtained using the value of serum Na and K concentration together with urinary excretion of Na, P and Oxalate and AP(CaP) index.

Once the optimal input parameters were found, the best result was obtained setting the Net with 4 hidden nodes (Figure 1). The final ANN reached an overall accuracy of almost 90% in both the Training Set and in the Testing Set, as shown in Table 3.

Moreover, the high level of sensitivity shown by the ANN means a low ratio of false negative predictions. Comparison between ANN and LR shows significantly better results for ANN in both Training and Testing Sets. Accuracy increases by roughly 20% from LR to ANN. While specificity does not change significantly, ANN

Table 3.

Comparison of accuracy, sensitivity and specificity results obtained by means of LR and ANN. ANN results were better in both Training and Testing Sets than those obtained by LR.

DATA SET	Accuracy (%)	Sensitivity (%)	Specificity (%)
LR			
Training	66.7	53.8	76.5
Testing	70.0	44.4	90.9
Total	67.5	51.4	80.0
ANN			
Training	88.3	100.0	79.4
Testing	90.0	88.9	90.9
Total	88.8	97.1	82.2

Figure 1.

The input layer was made up with the serum and urine parameters chosen from the database. The output layer had a single neuron expressing the presence of stone formation recurrence.

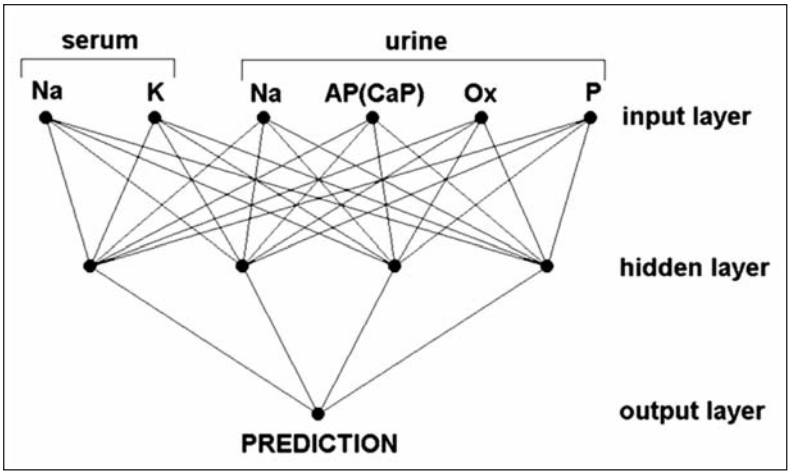
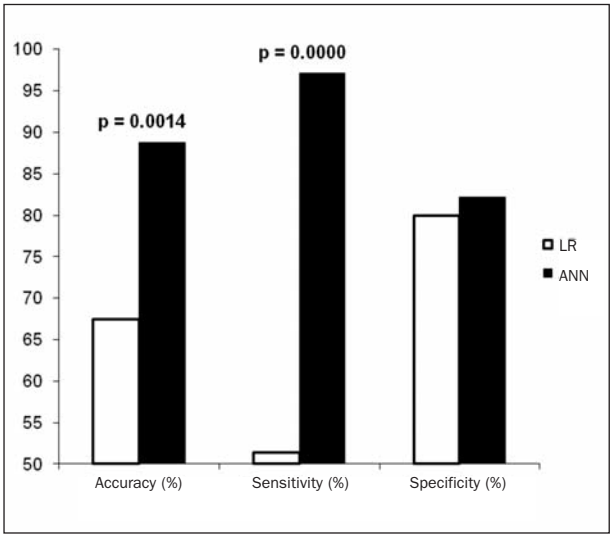


Figure 2.

Comparison between ANN and LR results. Percentage values of accuracy, sensitivity and specificity of both methods are shown.



almost doubles the sensitivity of LR (Figure 2). Moreover, the high level of sensitivity shown by the ANN means a low ratio of false negatives.

Discussion

Due to high recurrence rates of urolithiasis (about 50%) (2), several studies have been performed to predict the recurrence of stone formation in patients with idiopathic calcium stone disease. Various urine parameters and combinations of these variables have been shown to have some value for distinguishing calcium oxalate SFFs from RSFs (4), such as the BRI (22), relative urinary calcium oxalate supersaturation (RS) (15), urine activity product (AP)CaOx Index (14) and serial crystalluria in early morning urine samples (11, 12, 16). All these previous methods are not easy to apply in clinical practice and at the same time do not present sufficient predictive power for supplying concrete help in clinical decision-making. The majority of previous works analyse the data by means of linear statistical tools. The results reached by ANN seem to suggest that some kind of relationship is present between the identified parameters and the future recurrence of stone formation. This relationship is probably very complex (in the mathematical sense) and non-linear. In fact, LR led to very much lower results, at least in terms of accuracy and sensitivity (Table 3). Even if the ANN seems to "*understand*" the complexity of the problem and thus shows very good prediction ability, it cannot explain the differences between SSF and RSF in a simple way. In fact, the ANN approach works understanding a database, thus creating generalized rules based on all the inputs of the problem, considered simultaneously. Let us consider the architecture of an ANN: all the inputs are connected with all the nodes of the hidden layer and these with the output neuron. ANN gives its answer considering all the terms of a problem, and each input could have greater or lesser importance according to the others in a completely different way from one subject to another. ANN expresses the dynamic between input and output by means of a very complex mathematical function. This could appear as a limit of this approach, but if the real problem under investigation is ruled by complex laws by its nature, ANN could be one of the rare possible ways of studying it. In this case, asking for simple rules in order to understand the problem could be a nonsensical proposition. The ANN developed in this work gives no clear information about the biological role of the single parameter involved or its metabolic dynamic. Even if some conjectures are possible, they appear a little inadequate. In our opinion, the main result of this ANN seems to be the suggestion of a non-linear approach to this problem which appears very complex, at least in the mathematical sense. More studies need to be done employing different non-linear tools such as different ANNs or Support Vector Machines as well as Self-Organized Criticality. In conclusion, the application of ANN to the database led to a promising predicting algorithm and suggest that a strongly non-linear relationship seems to exist between the parameters and the recurrence episodes. In particular, the ANN approach identifies as optimal parameters serum concentration of Na and K as well as urinary excretion of Na, P, Oxalate and AP(CaP) index. The ANN built was embedded in software for Windows, which could be useful for clinical purpose to give an opinion about the future risk of recurrences of a particular patient. This software has been called "*RSF-Predictor*". Further studies are in progress to validate the ANN and to find better predicting markers by means of larger databases together with the use of different non-linear mathematical tools.

Table 4.

Evaluation of sensitivity and specificity of some risk indices. RS_{CaOx}: relative urinary supersaturation. API_{CaOx}: Activity $Product_{CaOx}$ Index. BRI: BONN Risk Index. SRPS: stone recurrence predictive score (10).

	Sensitivity (%)	Specificity (%)
RS_{CaOx}	65	78
AP_{CaOx}	55	87
BRI	80	70
SRPS*	62	75

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Prognostic estimation of chemical composition of recurrent urinary stones.

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Summary

We conducted a retrospective study of the course of recurrent urolithiasis in 127 patients (63 women, 64 men aged from 27 to 58) who were under close and regular outpatient follow-up for up to 15 years and who did not receive conservative prophylactic therapy due to different reasons. The group consisted of 33 patients with uric acid lithiasis, 52 patients with calcium oxalate lithiasis, 42 patients with magnesium-ammonium-phosphate lithiasis. By the start of follow-up not a single patient had had urinary stones detected by ultrasound and X-ray. For the period of observation there were up to 7 recurrences diagnosed in each patient and we studied the chemical composition not only of the primary stones but also of 352 recurrent stones by means of infrared spectrophotometry and X-ray diffraction. In our investigation we also performed biochemical and microbiological analysis and urinalysis. We established the chance and we found prognostic factors of changes in the type of stone formation in patients with different chemical forms of the disease. In patients with uric acid lithiasis recurrent stones can be composed of calcium-oxalate or phosphate, in patients with calcium-oxalate lithiasis recurrent stones could be composed of phosphate, and patients with magnesium-ammonium-phosphate stones may develop stones of uric acid or calcium-oxalate.

KEY WORDS: *Urinary calculi; Infrared spectrophotometry; X-ray diffraction.*

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INTRODUCTION

The problem of recurrent urinary stones attracts attention of researchers in many fields: urology, nephrology, biochemistry, genetics, physics and others (1). The approach to study this problem by different scientific fields is justified and a great experience says in favor of this. Solutions for the problem open up the ways of prophylaxis of urine stone disease. However, taking into account the prevalence of biochemical mechanisms in pathogenesis of this disease (2), it can be assumed that risk factors of recurrent urinary stones in most cases may lie in alterations of metabolism. This is also confirmed by a positive international experience in the prophylaxis of recurrent stones, directed to correct metabolic changes that lead to the stone formation. It should be noted that the choice of treatment depends on the results of biochemical blood tests and on chemical composition of the removed or spontaneously passing stones. Long-term (up to 15 years) regular outpatient follow-up of the patients with relapsing course of urinary stone disease made it possible to find out that the chemical composition of the stones may change. All this gave us a basis to conduct our investigation.

The purpose of the study was to define the possibility for change of chemical type of urinary stone disease and estimate the prognostic factors according to chemical composition of recurrent urinary stones.

METHODS

The considered group of patients with recurrent urolithiasis consisted of 127 subjects: 63 women and 64 men aged from 27 to 58 years at the time of investigation. By that time all patients had been under regular follow-up for 1 to 15 years after: 1) spontaneous passage of the stone, 2) ESWL, 3) PCNL, 4) open surgery, 5) combination of open surgery and spontaneous passage of the stones and 6) litholitic therapy (patients with uric acid stones). The absence of urinary stones in the patients at

the time of investigation was confirmed by ultrasound scan and the X-ray. Those methods were later used to detect recurrent urinary stones in patients. At the time of primary examination 33 patients had uric acid lithiasis, 52 patients had calcium-oxalate or mixed form of lithiasis with prevalence of calcium-oxalate (mono- or dihydrate), 42 magnesium-ammonium-phosphate lithiasis. During the investigation period the patients did not receive any metaphylactic therapy due to different reasons. The amount of recurrences in each patient varied form 1 to 7. In total 352 recurrent urinary stones were analysed. To define the chemical composition of stones either primary, or recurrent, we used infrared spectrophotometry (by device "*Hitachi-260-30*", Japan) and X-ray diffraction. Functional state of kidneys was within normal limits (blood levels of urea, creatinine, creatinine clearance). Biochemical blood and 24 hour urine determination were done with special test kits and automatic analyzers ("*Polimak*", Italy and "*Labsystem*", Finland). The total calcium levels in urine were defined by using complex-measuring method. Serum levels were determined in fasting venous blood. For microbiological analysis and microscopic urinalysis standard techniques were used. Statistical analysis of the data was made with use of Student's t-criterion.

Results

It has been found out that patients with uric acid lithiasis can change to calcium-oxalate and phosphate forms of lithiasis. During the analyses of 68 recurrent stones in 33 patients with uric acid lithiasis we found that 23 (69.7%) patients had recurrent urinary stones of the same chemical composition, 7 (21.2%) patients had calcium-oxalate stones or combined stones consisting of uric acid and calcium-oxalate (mainly monohydrate). Three (9.1%) patients were found to have phosphate stones: 2 of them had carbonate apatite stones, 1 patient had struvite'stone. The results of multiple biochemical investigation of the 7 patients with recurrent calcium-oxalate stones in comparison with the 23 patients with recurrent uric acid stones showed the absence of differences in uric acid serum levels and 24 hour uric acid renal excretions in these groups. It has been found that mean 24 hour renal excretion of total calcium in the group of patients with recurrent calcium-oxalate stones was higher than in the group of patients with recurrent uric acid stones (8.24 + 0.38 mmol/24 hrs vs. 5.88 + 0.49 mmol/24 hrs ($p < 0.01$). Analyzing the data of urine culture in patients with recurrent uric acid and calcium-oxalate stones we found the growth of non-urea-splitting strains of E.coli with counts of 103-104 CFU/ml only in 2 patients with recurrent uric acid stones. In patients with recurrent phosphate stones urine culture showed the growth of Pseudomonas aeruginosa with count of 106-108 CFU/ml (if carbonate apatite) and Proteus mirabilis with count of 106 CFU/ml (if magnesium-ammonium-phosphate).

The analysis of 142 recurrent stones in 52 patients with calcium-oxalate urolithiasis showed changes of their primary chemical composition in 11 (21.2%) patients: in 10 (19.2%) cases the composition was struvite or a combination of carbonate apatite and struvite, in 1 (2%) case was carbonate apatite. Recurrent calcium-oxalate stones (whewellite, weddellite, or their combination) were found in 41 (78,8%) patients. Urine culture in all patients with recurrent calcium-oxalate stones was sterile. In all patients with recurrent phosphate stones urine culture showed the growth Proteus mirabilis and Pseudomonas aeruginosa with counts of 105-108 CFU/ml. but in the patient with recurrent stone consisting of carbonate apatite urine culture was sterile in several testings. However, pH of the morning urine sample ranged between 6.7-6.9 and there were no leucocytes on microscopy. We have to note that the course of the disease was predictable but unstable: small (0.3-0.4 cm) stones were regularly passed and had different chemical composition.

Studying the course of urolithiasis in 42 patients with magnesium-ammonium-phosphate stones it has been shown that 39 (92.9%) patients had recurrent stones of the same chemical composition. Only 3 (7.1%) patients were found to have changes in the composition of the stone: in one case there was a change to calcium-oxalate lithiasis. In 2 other cases (after ESWL and open surgery) we observed a characteristic metabolic condition that was also observed in other patients with uric acid lithiasis: serum level of uric acid was 0.254-0.310 mmol/l, 24 hour renal excretion of uric acid and total calcium were 3.98-4.20 mmol/24 hrs and 4.08-5.91 mmol/24 hrs, respectively, anf pH of morning urine sample was 5.0-5.2, although at the time of the current investigation there were no recurrent urinary stones detected. However, from the history of these patients disease it was known that the 2 female patients had uric acid stones before the period of the current investigation, when phosphate stones were demonstrated. Analyzing urine cultures in all 42 patients both with recurrent stones and without the growth of Proteus mirabilis, Proteus vulgaris and Pseudomonas aeruginosa was diagnosed only in patients with recurrent magnesium-ammonium-phosphate stones. In aforementioned 3 patients urine cultures were sterile and morning urine sample microscopy was within normal limits.

Discussion

Present results show that the chemical form of urine stone disease can change in the same patient. One of the factors that leads to the transformation of uric acid lithiasis to calcium-oxalate is probably related to profound changes in calcium metabolism that are reflected in increased 24 hour total calcium excretion, as there were no differences in other biochemical values of lithogenic substances in patients with recurrent uric acid and calcium-oxalate stones. We previously investigated to prove that the level of excretion of total calcium in urine more than 7,25 + 0,31 mmol/24 hrs is a characteristic feature of recurrent Ca-oxalate urolithiasis (3). The transfomation of uric acid urolithiasis to phosphate is caused by the association with urinary urea-splitting infection. The formation of recurrent magnesium-ammonium-phosphate stones in patients with calcium-oxalate urolithiasis was probably sustained by the presence of urinary infec-

tion caused by urea-splitting strains (*Proteus mirabilis, Pseudomonas aeruginosa*) according to the modern concept of the genesis of struvite stones (4). The presence of recurrent calcium-phosphate stone (carbonate apatite stone) in sterile urine in one case with normal level of leucocytes on microscopy may be associated with metabolic changes of unknown origin that led to the alkalinisation of urine or with infection that was not detected in urine. The chances of recurrent uric acid or calcium-oxalate stone formation in patients with magnesium-ammonium-phosphate forms of the disease are probably caused by 2 factors. One of them is the removal of the main cause for struvite stone formation, that is urea-splitting infection, and, as a result of that, the decrease of urine pH. In fact there are cases of formation of the primary struvite urinary stone in pregnant women, that did not relapse after stone removal. Therefore it should be considered, as a second factor, the presence of urinary stones of different composition in the past medical history of the patient.

Conclusion

1. Recurrent urinary stones may have different chemical composition than primary stones.
2. Uric acid urolithiasis can be associated with:
   a) recurrent calcium-oxalate stones on the background of profound changes in calcium metabolism and increased 24 hour excretion of calcium in urine not less than 7,25 + 0,31 mmol/24 hrs.
   b) recurrent phosphate stones in the presence of urinary urea-splitting infection.
3. In patients with calcium-oxalate form of urolithiasis there could be conditions for the formation of recurrent phosphate stones associated with urinary urea-splitting infection.
4. Magnesium-ammonium-phosphate urolithiasis can transform into calcium-oxalate or uric acid urolithiasis after full eradication of urinary urea-splitting infection and in presence of the same chemical form of the disease in the patient past medical history.

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The role of long-term loading of cholesterol in renal crystal formation.

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Summary

We studied the effects of cholesterol load on urinary stone in rats receiving a standard diet or a high fat diet. Sixty male rats were randomized to two groups and were fed either a standard diet (SD group) or a high fat diet (HFD group) for 8 weeks. Then the two groups were further divided into four groups. SD group, HFD group, SD + EG group (with standard diet + ethylene glycol administration for two weeks), and HFD + EG group (with high fat diet + ethylene glycol administration). The starting date of EG administration was considered to be week 0. Twenty-four-hour urine samples were collected in week 0, week 1, and week 2, and oxalate excretion and citrate excretion were measured by capillary electrophoresis analyzer. The excretion of phosphorus, magnesium, and creatinine for 24 hours was measured using an automated analyzer. Serum sodium, potassium, chloride, calcium, phosphate, magnesium, creatinine, total cholesterol, triglyceride, HDL-cholesterol and glucose were determined using an automated analyzer. The kidney tissues were obtained to perform hematoxyline-eosine staining and Pizzolato's staining to detect oxalate-containing crystals. The average body weight in HFD groups and HFD + EG group in week 0 was significantly higher than that of SD group and SD + EG group. The calcium oxalate crystal deposition was not observed in all groups in week 0. HFD + EG group in week 1 had sporadically calcium oxalate crystal deposition in renal distal tubular cells and tubular lumens. In week 2, the number of crystal deposition in HFD + EG group was increased remarkably. The crystals were slightly observed in SD + EG group in week 2. The excretion of urinary calcium and phosphate in HFD group and HFD + EG group was significantly higher than that of the SD group and SD + EG group in week 0. The amount of urinary citrate excretion in the SD group and SD + EG group showed a significantly higher value compared with that of the HFD group and HFD + EG group in week 0. The level of serum total cholesterol in the HFD group and HFD + EG group was higher compared to that in the SD group and SD + EG group. The serum triglyceride level was not significantly different in the four groups in week 0. Interestingly, the level of triglyceride of EG administration groups (SD + EG and HFD + EG group) was significantly higher than that in EG no-administration groups (SD group and HFD group) in week 1 and week 2. The serum glucose level in the HFD group and HFD + EG group was significantly higher than that in the SD group and SD + EG group in week 0. In week 2, the glucose level of EG administration groups (HDF + EG group and SD + EG group) was significantly lower than that of EG no-administration groups (HFD group and SD group). In conclusion, this result suggested that long-term loading of cholesterol could increase renal calcium stone formation.

KEY WORDS: Urolithiasis; Cholesterol; Metabolic syndrome.

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INTRODUCTION

Calcium urolithiasis is a quite frequent disease in developed countries. Approximately 10% of men and 5% of women will experience a symptomatic urolithiasis by the age of 75 years and about 85% of urolithiasis contains calcium (1). Recently, some researchers reported that cholesterol plays an important role in urolithiasis formation. In patients with urolithiasis, the mean daily intake of fat was significantly higher than that in controls. Meanwhile, in

patients, it is reported that the daily intake of fat was higher in men than women and the difference was statistically highly significant (2), which is one of the reasons that explains the significantly higher incidence of urolithiasis in men than that in women. Thus, a close relationship may exist between pathological renal calcifications and lipids. The aim of the present work was to test whether long-term exposure to a dietary excess of cholesterol and lipids results into urinary calcium stone formation.

Materials and methods

1. Animals used

Eight-week old male SD rats weighing 280-320g (CLEA Japan, Inc.) were used for the experiment. All experimental procedures were performed with the approval of the Animal Care Committee of the Faculty of Medicine, Nagoya City University Graduate School of Medical Sciences.

2. Diet

Standard diet (CE-2, CLEA Japan, Inc., 4.4% crude fat, 0.09% cholesterol) and high fat diet (High Fat Diet 32, CLEA Japan, Inc. 32.0% crude fat, 0.75% cholesterol) were used.

3. Experimental protocol

Sixty rats were divided into two groups; a SD group (standard diet group), in which rats were provided with a standard diet and a HFD group (high fat diet group), in which rats were provided with a high fat diet, and they were administered with their respective diet for eight weeks from the age of eight-weeks. From the age of 17-weeks, the two groups were further divided into four groups. SD group, SD + EG group (with standard diet + oxalate precursor, ethylene glycol, Wako, Tokyo, Japan), in which rats were provided with a standard diet and 0.5% EG as drinking water ad libitum, HFD group and HFD + EG group (high fat diet + ethylene glycol), in which rats were provided with a high fat diet and 0.5% EG as drinking water. The starting date of EG administration was considered to be week 0.

The kidney tissue sample was collected and fixed in 4% paraformaldehyde, and embedded in paraffin. Frozen slices of the kidney tissues were obtained to perform hematoxyline-eosine staining and Pizzolato's staining to detect oxalate-containing crystals.

4. Statistical process

The four groups were compared by using statistical analysis software. Student's t-test for equality of variance was used for correlation after conducting dispersion analysis. Risk rates were less than 5% for both cases, which suggested that they were significant.

Results

1. Body weight

The average body weight in HFD groups and HFD + EG group at starting date of EG administration, week 0, was 410 ± 27g, which was significantly higher than that of SD group and SD + EG group (384 ± 28g) and indicated the body weight was increased due to the high fat diet.

2. Histological Findings

In the HFD group and HFD + EG group in week 0, internal fat of the abdomen clearly increased more compared to the SD group and SD + EG group.

HFD + EG group in week 1 has sporadically calcium oxalate crystal deposition in renal distal tubular cells and tubular lumens. In week 2, the number of calcium oxalate crystal deposition in HFD + EG group was increased remarkably. And the renal tubular lumen was faintly extended and renal tubular cells became flat and collapsed slightly. The crystals were slightly observed in SD + EG group in week 2.

3. Urine chemistry

The amount of urinary oxalate excretion showed no significant difference between the four groups before and after EG administration. The amount of urinary calcium excretion in HFD group and HFD + EG group was significantly higher than that of the SD group and SD + EG group in week 0, whereas there were no difference of urinary calcium excretion among four groups in week 1 and week 2. The amount of urinary phosphate excretion in HFD group and HFD + EG group was significantly higher than that of the SD group and SD + EG group in week 0, which remained slightly higher after EG administration. The amount of urinary citrate excretion in the SD group and SD + EG group showed a significantly higher value compared with that of the HFD group and HFD + EG group in week 0, which also remained slightly higher after EG administration. The amount of urinary magnesium excretion showed no difference between four groups in week 0, week 1, and week 2.

4. Blood chemistry

The serum calcium level in week 0 showed no significant change in four groups. The serum calcium level in HFD + EG group was significant higher than that of SD + EG group in week 2. The serum phosphate level indicated no difference in four groups. Serum creatinine, sodium, potassium, chloride, and magnesium also showed no difference in four groups.

5. Lipid metabolism related materials

The level of serum total cholesterol in the HFD group and HFD + EG group was higher compared to that in the SD group and SD + EG group. The serum triglyceride level was no significant in four groups in week 0. Interestingly, the level of triglyceride of EG administration groups (SD + EG and HFD + EG group) was significantly higher than that in EG no-administration groups (SD group and HFD group) in week 1 and week 2. There was not significant difference of serum triglyceride level between SD group and HFD group, and between SD + EG group and HFD + EG group. The serum HDL level showed no difference among four groups (data not shown). The serum glucose level in the HFD group and HFD + EG group was significantly higher than that in the SD group and SD + EG group in week 0. In week 2, the glucose level of EG

administration groups (HDF+EG group and SD + EG group) was significantly lower than that of EG no-administration groups (HFD group and SD group).

Discussion

The aim of the present study is to examine the association between the urinary calcium stone formation and the long term exposure to a dietary excess of cholesterol, with the hypothesis that cholesterol play a role of the urinary stone formation. Our findings confirm the hypothesis by showing that calcium oxalate crystal deposition in the renal tissues was increased by the administration of a high-cholesterol diet in urinary stone rat model.

This study examination protocol was to administer a precursor of oxalate such as ethylene glycol after feeding a high cholesterol diet for eight weeks. The dosage of ethylene glycol was adjusted to a small amount so that little renal calcium oxalate crystal deposition could be formed to observe the effect of cholesterol. This was set to investigate if renal calcium oxalate crystal deposition could be increased by loading cholesterol.

As a result, in the group of rats administered with ethylene glycol after been given a high-cholesterol diet for eight weeks, renal calcium oxalate crystal deposition was slightly observed during the first week of the administration, which significantly increased during the second week compared to the control group without cholesterol loading. This result suggested that long-term loading of cholesterol could increase renal calcium stone formation.

Taylor et al. (3) conducted over 46 years of follow-up investigation on 4,827 urolithiasis patients to adjust age, dietary factor, fluid intake and thiazide usage and reported that the risk of urolithiasis was higher in the male patients weighting 68.2 kg or more. It was also reported that the male patients who gained more than 15.9kg in weight since the age of 21 years had high risks. The report additionally explained that, for both men and women, the patients with a BMI more than 30 have higher risks than those with a BMI 21 to 22.9, and abdominal circumference can also be a risk factor.

It is considered that a mechanism by which urolithiasis can be easily formed due to cholesterol may be through increasing/decreasing the amount of urinary electrolyte excretion relating to urolithiasis formation. However, its effect is assumed to be little and the mechanism is not yet clear. Likewise, BMI, greater weight, larger waist circumference, and weight gain are associated independently with increased risk for kidney stone formation. However, the mechanisms underlying the relation between larger body size and increased stone risk are unknown.

It seems reasonable to conclude from these studies that long-term exposure to a dietary excess of cholesterol increases urinary calcium stone formation.

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Effect of sex hormones on crystal formation in a stone-forming rat model.

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Summary

Sex hormones have substantial effects on crystal formation in the rat kidney through oxalate metabolism and oxidative cell damage. Testosterone is a promoter and estradiol an inhibitor of such crystal formation. The development of new medications related to sex hormones or GO are anticipated for sufferers of recurrent urolithiasis.

KEY WORDS: Urinary calculi; Sex hormones; Testosterone; Estradiol.

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INTRODUCTION

Epidemiological research found that the incidence of upper urinary tract stones was 2.4-fold greater in men than in women, and stones were composed of calcium oxalate in 74.9% of males and 63.1% of females (1). Although the pathogenesis of urolithiasis appears to be multifactorial and intricate, sex hormone-derived gender differences are thought to influence its incidence.

In the present study, we evaluated the effects of sex hormones on endogenous oxalate synthesis, focusing on two important liver peroxisomal enzymes in this pathway: glycolate oxidase (GO) and alanine glyoxylate aminotransferase (AGT). In addition, we evaluated the effects of sex hormones on kidney crystal deposition due to oxidative stress (OS).

MATERIALS AND METHODS

Animal study groups and treatments

Sprague–Dawley rats were divided into seven groups (n = 6 each group) as follows: intact male rats as male controls (M-1); orchiectomized (ORX) male rats (M-2); intact male rats subcutaneously implanted with 60-day sustained release testosterone pellets (25 mg) (M-3); intact male rats subcutaneously implanted with 60-day sustained release estradiol pellets (2.5 mg) (Innovative Research of America, Sarasota FL, USA) (M-4); intact female rats as female controls (F-1); ovariectomized (OVX) female rats (F-2); and intact female rats subcutaneously implanted with 60-day sustained release testosterone pellets (25 mg) (F-3). The rats in groups M-1 and F-1 were obtained at 9-weeks-old, and allowed 1 week of acclimation to our animal facilities. At 10-weeks-of-age, they were given 0.5% ethylene glycol (EG) in drinking water and force-fed 0.5 µg of 1,25-dihydroxy vitamin D_3 (Chugai, Japan) every other day for 1 week. Rats in groups M-2 and F-2 were obtained at 6-weeks-old and gonadectomized 1 week later; rats in M-3, M-4, and F-3 were also obtained at 6-weeks-old and subcutaneously implanted with hormone pellets 1 week later. From 10 weeks of age, all experimental groups were treated the same as the control groups. Following treatment, the rats were euthanized with an excessive dose of anesthesia, and the kidneys and livers were immediately excised. The right kidneys were cut longitudinally, fixed in 4% paraformaldehyde, and embedded in paraffin, whereas the left kidneys and livers were frozen in nitrogen at -70°C. All specimens were stored for later examination.

Urine collection and determination of urinary oxalate

Twenty-four-hour urine samples were collected twice from each rat using metabolic cages, once on the day before starting the EG treatment and again on the day before euthanasia. A portion of each sample was acidified with concentrated HCl to a pH < 3.0 for measurement of urinary oxalate. Urinary oxalate was determined using capillary electrophoresis (SRL, Tokyo, Japan), whereas urinary 8-hydroxy-2-deoxyguanosine (8-OHdG), a marker of oxidative stress, was determined with a competitive enzyme-linked immunosorbent assay (ELISA) kit (8-OHdG Check; Japan Institute for the Control of Aging, Fukuroi, Japan).

Verification of kidney crystal deposits
The excised kidneys embedded in paraffin were cut into 5-μm sections, stained with hematoxylin and eosin, and mounted on slides. Crystal deposits were visually examined under a polarizing microscope.

Immunofluorescence of 8-OHdG in kidneys
Localization of 8-OHdG in the kidneys was analyzed by immunofluorescence. The slides were incubated with an anti-8-OHdG mouse monoclonal antibody (5 μg/ml) (Japan Institute for the Control of Aging) overnight at 4°C, washed in phosphate-buffered saline containing Tween 20 (PBS-T), probed with a fluorescent antibody (Alexa 567; anti-mouse IgG, Invitrogen, Carlsbad, CA, USA), and stained with 4', 6-diamino-2-phenylindole. The slides were examined by fluorescence microscopy, and the numbers of total and positive nuclei were counted.

Real-time quantitative polymerase chain reaction for AGT and GO in rat liver
AGT and GO expression was measured using real-time reverse transcription-polymerase chain reaction (RT-PCR). Total RNA was extracted from each liver using a Total RNA Purification System (Invitrogen) and reverse-transcribed into cDNA using an RT Reagent Kit (Takara, Tokyo, Japan). We performed real-time RT-PCR using a Thermal Cycler Dice system and SYBR premix kit (Takara). Messenger RNA expression was reported as the ratio of the gene of interest to β-actin.

Real-time quantitative PCR for SOD1, SOD2, and CAT in rat kidney
Of the three SOD isozymes, SOD1 (Cu, Zn-SOD) and SOD2 (Mn-SOD) are detectable in the kidney. The levels of SOD1, SOD2, and CAT expression in the kidneys were determined using real-time RT-PCR.

Results

Urinary oxalate excretion and detection of kidney crystal deposits
Prior to treatment, 24-hour excretion of oxalate in intact males was significantly greater than that in intact females. In ORX males and males administered estradiol, the level was significantly lower than that in intact males, whereas the level in testosterone-administered males was significantly greater than that in intact males. In contrast, in OVX females and testosterone-administered females, 24-hour oxalate excretion was significantly higher than in intact females. Following treatment, oxalate excretion was significantly higher in all groups except for intact females (Figure 1A). Extensive crystal deposition was observed in intact males and testosterone-administered males, whereas very few crystals were detected in intact females. In the male groups, ORX and estradiol administration inhibited crystal deposition, whereas OVX and testosterone administration enhanced crystal deposition in the female groups (Figure 1B).

Urinary 8-OHdG excretion and 8-OHdG immunofluorescence in kidneys
The 24-hour excretion of 8-OHdG in intact males was significantly greater than that in intact females. No significant difference was observed in the ORX males and OVX females compared to their respective control groups. However, the 8-OHdG level was significantly higher in testosterone-administered males and females and significantly lower in estradiol-administered males, compared to their respective control groups (Figure 2A). Intensely stained nuclei were detected in tubular cells in intact males, whereas only a few stained nuclei were found in intact females. ORX and estradiol-administered males

Figure 1A.
Prior to ethylene glycol (EG) treatment, 24-hour oxalate excretion in intact males was significantly greater than that in intact females. Oxalate excretion increased in the testosterone-administered and OVX groups, but decreased in the estradiol-administered and ORX groups. Following EG treatment, only the intact female group showed no significant difference compared to the control group.

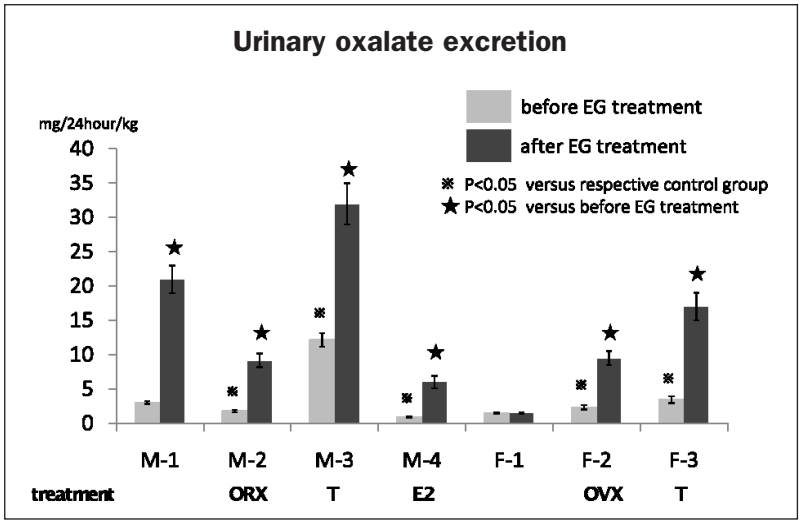


Figure 1B.
Representative microscopic photographs showing crystal deposits in kidneys from each group (hematoxylin and eosin stain, polarizing microscope).

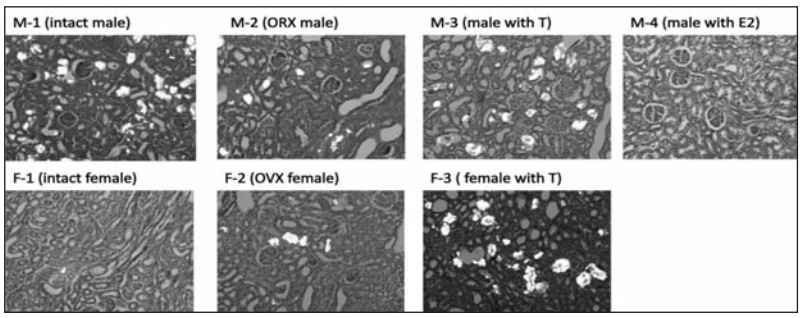


Figure 2A.

Twenty-four-hour 8-hydroxy-2-deoxyguanosine (8-OHdG) excretion in intact males was significantly greater than that in intact females. It was significantly higher in the testosterone-administered groups and significantly lower in the estradiol-administered group.

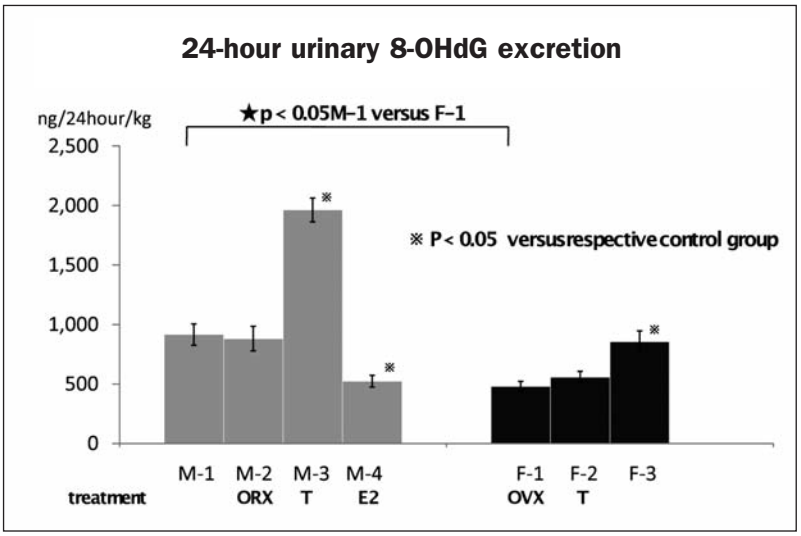


Figure 2B.

Representative fluorescence microscopic photographs showing 8-OHdG-positive nuclei in the tubular cells of kidneys from each group.

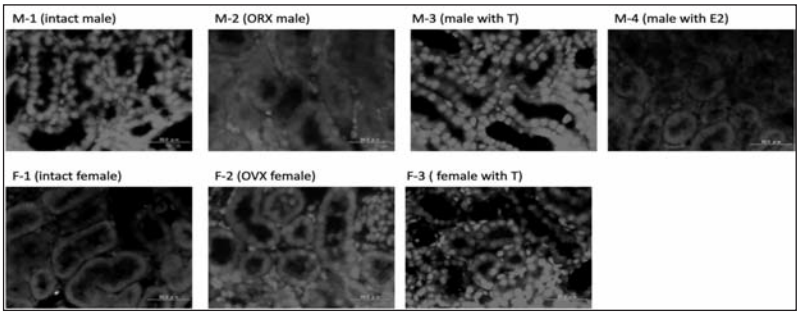
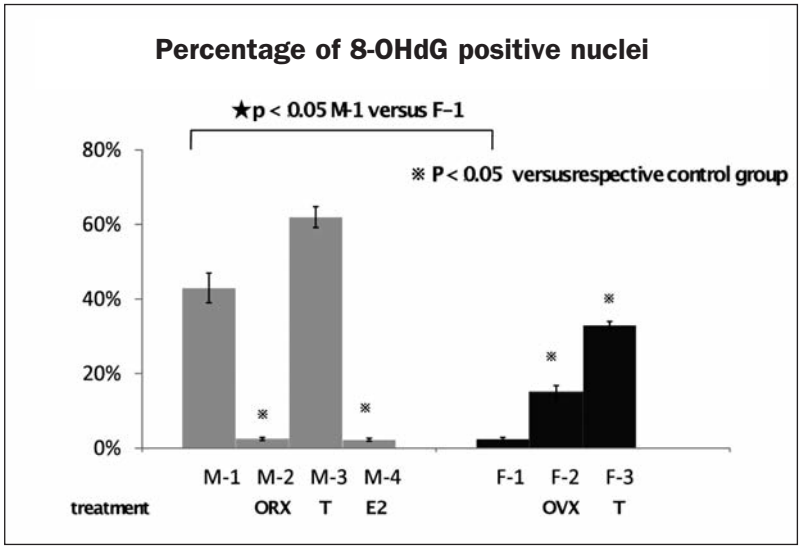


Figure 2C.

Numbers of positive nuclei are summarized.



showed fewer positive nuclei than intact males, whereas the numbers of positive nuclei were greater in OVX and testosterone-administered females (Figure 2B). The numbers of positive nuclei are summarized in Figure 2C.

GO and AGT mRNA expression in the liver

The relative expression of GO in intact males was significantly greater than that in intact females. In contrast, ORX males showed significantly lower GO expression, and testosterone-administered males showed significantly higher expression compared to the control group. In addition, GO expression tended to be lower in estradiol-administered males, although this difference was not significant. GO expression increased significantly in OVX and testosterone-administered females compared to the control group (Figure 3A). No difference in relative AGT expression was observed among groups (Figure 3B).

SOD1, SOD2, and CAT mRNA expression in kidneys

The relative levels of SOD1, SOD2, and CAT expression in intact females were significantly greater than those in intact males. In OVX and testosterone-administered females, the levels of SOD1, SOD2, and CAT expression were significantly lower than those in the control group, whereas the expression of all three genes increased significantly in estradiol-administered males. The levels of SOD2 and CAT increased significantly in ORX males. In testosterone-administered males, no significant change was observed (Figures 4A-C).

Discussion

In the present study, we found extensive crystal deposition in intact male rats and testosterone-administered males and females, whereas relatively few crystal deposits were observed in intact females, OVX females, ORX males, and estradiol-administered males. Our findings show that testosterone is a promoter and estradiol an inhibitor of crystal deposition. We also evaluated the contribution of sex hormones to stone formation by focusing on oxalate synthesis, GO expression, oxidative stress, and antioxidant enzyme expression.

Following EG treatment, 24-hour urinary oxalate excretion increased and was accompanied by an increase in kidney

crystal deposition. Oxalate is a useless end product of metabolism that must be excreted in urine. Glycolate is metabolized to glyoxylate in liver peroxisomes, which is then metabolized to oxalate by GO, which is the most important enzyme in the pathway (2). Because EG is a precursor of glycolate, EG administration leads to hyperoxaluria, while important enzymes in the pathway are GO and AGT, which are peroxisomal enzymes. Glyoxylate is also metabolized to glycine by AGT activity. GO is a promoter and AGT an inhibitor of oxalate synthesis. In the present study, GO mRNA expression increased significantly after testosterone administration, and decreased significantly following testosterone deprivation; in contrast, AGT mRNA expression was not influenced by either sex hormone. We concluded that the differential modulation of GO in response to sex hormones affects urinary oxalate excretion and may contribute to gender-based differences in urolithiasis.

8-OHdG is widely used as a marker of oxidative DNA damage. We found that urinary 8-OHdG increased significantly with testosterone administration and decreased significantly with estradiol administration. This marker is thought to be present on the nuclei of tubular cells with oxidative damage, and this was supported by our immunofluorescence results. In addition, kidney-localized 8-OHdG was greater in groups with extensive crystal deposition than in groups with few of these deposits.

The degree of OS in a cell is dependent on the balance between ROS anabolism and catabolism. NADPH oxidase is the most important source of ROS production. Superoxide anion (O_2-) is converted to hydrogen peroxides (H_2O_2) via the action of cytosolic SOD1 and mitochondrial SOD2, whereas H_2O_2 is converted to water and oxygen by CAT. Thus, all three enzymes are important in the cellular antioxidant system (3). Estradiol increases antioxidant activity by promoting the mRNA expression of these three antioxidant enzymes, which was low in the testosterone-administered females examined here. We concluded that OS in the kidney and relatively low levels of estradiol contribute to crystal deposition.

The development of medications to prevent recurrent urolithiasis is expected in the near future. Our group previously reported that atorvastatin, a competitive inhibitor of 3-hydroxy-3-methylglutaryl enzyme A, attenuates oxidative damage to renal tubular cells and inhibits renal crystal deposition. The present results indicate that sex hormone-modulating medications may be effective for the prevention of kidney crystal deposition through the modulation of oxalate metabolism and OS. The development of medications for urolithiasis is anticipated to involve few serious side effects because it is a benign disease.

For example, saw palmetto, which is widely given to benign prostatic hyperplasia patients in Europe, may be useful, because this drug is a 5-α reductase inhibitor and does not influence sex hormone levels. Estrogen replacement therapy may also be useful for postmenopausal women with recurrent urolithiasis. In addition, research regarding the structure of GO is progressing towards substrate specificity and drug design. If a medication that mediates the activity of GO can be developed, it may also be effective for urolithiasis

Figure 3A.

The relative expression of glycolate oxidase (GO) in intact males was significantly greater than that in intact females. Testosterone administration and OVX enhanced expression, whereas ORX and estradiol administration weakened expression.

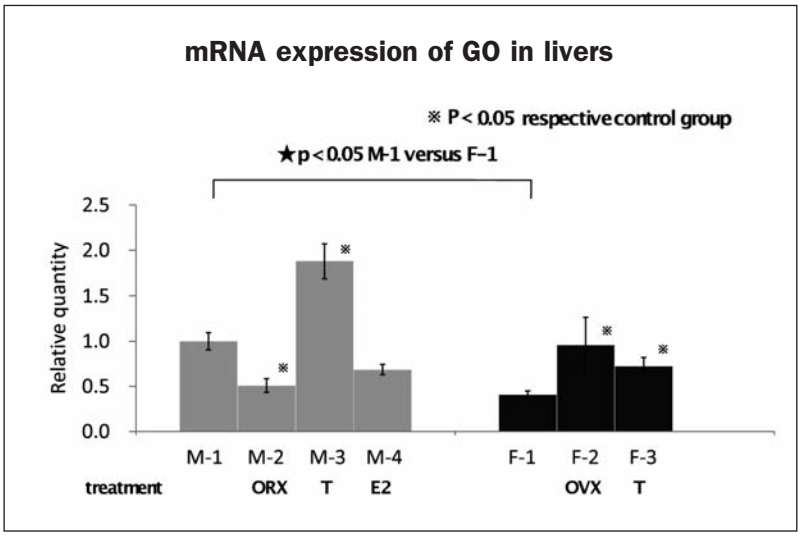


Figure 3B.

No significant difference in AGT expression was observed among groups.

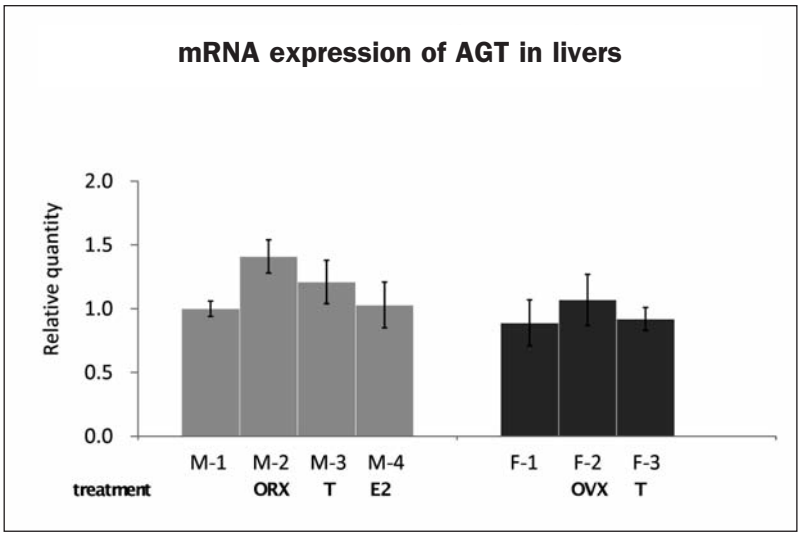
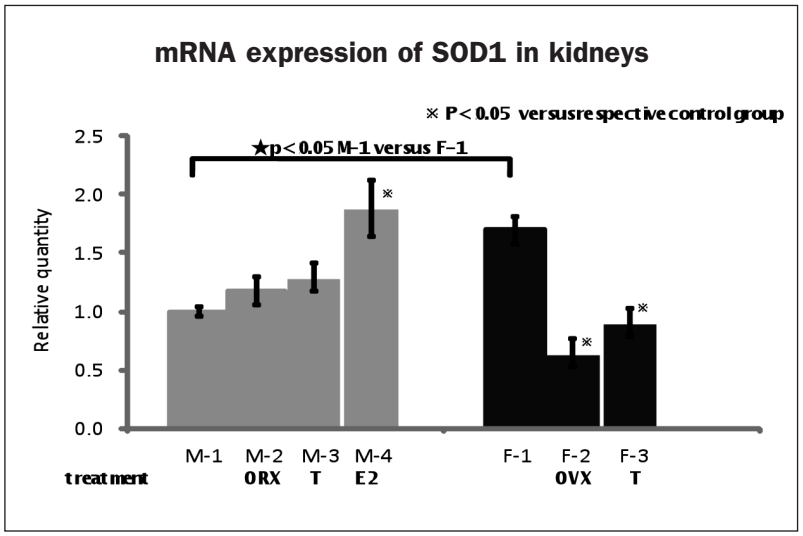
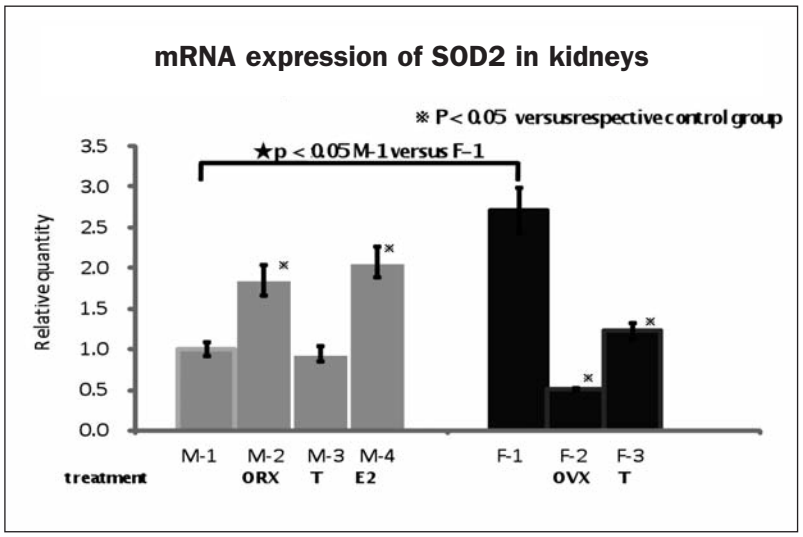
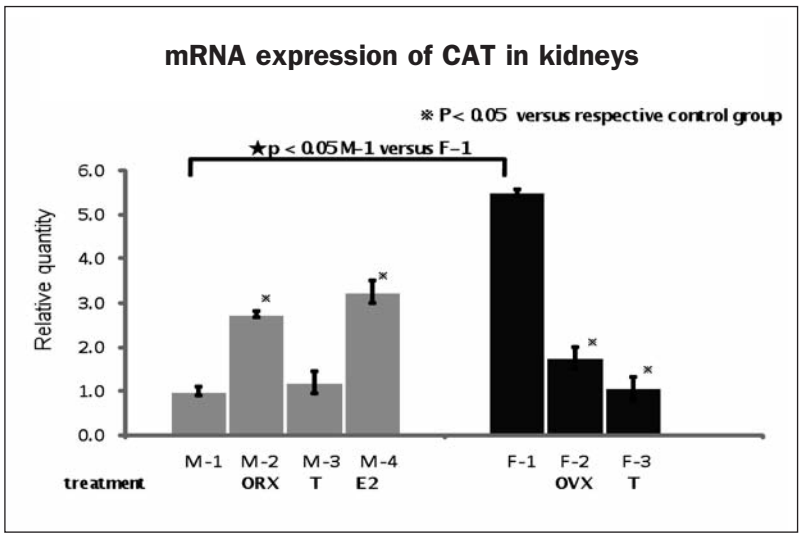


Figure 4A-C.

The levels of relative super oxide dismutase (SOD) 1, SOD2, and catalase (CAT) expression in intact females was significantly greater than in intact males. Estradiol administration enhanced expression and OVX weakened the levels of expression.







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The role of functional urodynamic disorders in the pathogenesis of urolithiasis.

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Summary

Objective: The aim of this study was to analyze the functional urodynamic parameters, which affect renal function and can promote stone formation.
Materials and Methods: We examined sixty consecutive patients with renal and ureteral stones and indication to urinary diversion by nephrostomy tube or indwelling catheter. In upper urinary tract, urodynamics was assessed with the help of electromanometry and multichannel impedance ureterography. To measure ureteral peristalsis, a probe equipped with 9 successively incorporated electrodes was indwelled retrogradely into distal ureter through a urethroscope. The documented data included renal pelvic pressure (RPP) and the number of ureteric contractility parameters such as peristalsis amplitude, peristalsis rate, the ureteral wall tone, the characteristics of contractile waveform and its direction (antegrade or retrograde). Urinary biochemistry and enzymuria were studied in order to characterize the lithogenic activity and renal function. The patients were divided into three groups: group 1 included patients with acute pyelonephritis caused by unilateral stone obstruction (n = 24), group 2 patients with stones and non-acute latent chronic pyelonephritis (n = 31) and group 3 unobstructed patients without signs of inflammation (n = 5).
Results: In the three groups of patients, the mean baseline RPP values were, respectively 28.7 ± 2.6 (range 20.0-32.4); 15.6 ± 1.9 (range 3.5-29.0); and 3.6 ± 1.4 (range 0-8) cm H_2O. The ratio of GGT to urinary creatinine changed similarly: it was elevated during acute inflammation, moderately enhanced during the chronic process, but significantly decreased after stone removal and resolution of inflammation (11.5 ± 3.2; 8.1 ± 2.0, and 1.6 ± 0.5 unit/L). Biochemical evaluation revealed 54% patients with enhanced lithogenic activity assessed by elevated calcium and oxalates in the urine (4.95 ± 0.25 mM and 504 ± 35 μM, correspondingly) and low level of citrates (2.5 ± 0.1 mM). In a subgroup of 11 patients with urolithiasis the baseline RPP values were assessed in relation to ureteral contractile activity in the distal region of ureter. Low RPP was found in a patient (9%) with strong ureteral contractions and a low tone while RPP was moderately higher in another patient (9%) with moderate mean peristaltic amplitude value but with elevated tone of ureteral wall. In the majority of examined patients with significantly elevated mean RPP value (45%), peristalsis of distal ureter was characterized by weak long-term and frequent contractions as well as increased tone with respect to the patients with normal RPP. The patients (36%) with moderately increased RPP demonstrated strong frequent contractions in the distal ureter and low ureteral wall tone. Changes in urodynamic parameters in patients examined before and immediately after ureteroscopy and lithotripsy procedures were observed. Factors affecting the ureteral wall tone were duration of stone disease, location and disposition of stones.
Conclusions: Our clinical observations obtained with the help of physiological methods revealed various factors modulating the urodynamic disorders in renal pelvis: temporary or persistent elevation of pelvic pressure; peculiarities of contractile function in distal ureter manifested by the tonic changes and variations in contractile amplitude, and certain abnormalities in propagation of contractile wave in the upper urinary tract. The reported urodynamic changes in patients with stone disease can be supplementary pathogenic factors causing deterioration of renal function probably followed by stone formation.

KEY WORDS: *Urinary calculi; Upper urinary tract; Urodynamic; Pelvic pressure; Ureteral peristalsis.*

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Introduction

Metabolic, inflammatory, and urodynamic abnormalities are considered as the pathogenic factors in stone formation. The primary factors favouring the development of urolithiasis are stone-promoting urine chemistries, Randall's plaques, and the defects in the crystallization-inhibiting system. It is unlikely that any single defect could explain the development of this disease in the majority of cases. The clinical data ensure that urinary tract inflammation and anatomical anomalies may play individual role in the genesis of stone disease. The urodynamic changes are usually explained in the terms of structural alterations within the upper urinary tract manifested by its dilation and/or anatomical anomalies. However, little attention was paid to the functional disturbances in renal pelvis and ureter, especially to its contractile activity. The technique for assessment of ureteric function in experiment is described, although the relative clinical data on the upper urinary tract are scarce (5, 6). Since renal pelvis machinery plays a special role in concentrating urine in the papilla (2) we suppose that disorders in the urinary tract peristalsis can upset urine outflow from renal tubular-caliceal system thereby favouring the development of urolithiasis.

The aim of this study was to analyze the functional urodynamic parameters, which affect renal function and can promote stone formation.

Material and methods

We examined sixty consecutive patients with renal and ureteral stones and indication to urinary diversion by nephrostomy tube or indwelling catheter. In upper urinary tract, urodynamics was assessed with the help of electromanometry and multichannel impedance ureterography. To measure renal pelvic pressure, a gauge (746, E2150 "*Siemens-Elema*", Germany) was connected hydraulically to nephrostomy tube via a three-way stopcock providing rapid renal drainage in the case of drastically increased pressure. To measure ureteral peristalsis, a probe equipped with 9 successively incorporated electrodes was indwelled retrogradely into distal ureter through a urethroscope. The impedance waveforms were acquired with an impedance converter "*RPKA2-01*" ("*Medass*", Russia). Original software (MCDP 32) provided simultaneous 6-channel monitoring of the instantaneous impedances between the interelectrode segments of the ureter. Pressure was recorded with the help of PC or Mingograf-804. The documented data included renal pelvic pressure (RPP) and the number of ureteric contractility parameters such as peristalsis amplitude, peristalsis rate, the ureteral wall tone, the characteristics of contractile waveform and its direction (antegrade or retrograde). Urinary biochemistry and enzymuria were studied in order to characterize the lithogenic activity and renal function. Ca2+ and oxalates were assessed in urine with Boehringer analyser (Bayer). The urine samples drawn via the nephrostomy tube were assayed for gammaglutamyl transferase (GGT), alkaline phosphatase (AP), lactate dehydrogenase (LDH), choline esterase (ChE), and N-acetyl-glucosaminidase (NAG) expressed as a ratio of enzyme concentration to urinary creatinine.

The groups of patients

The patients were divided into three groups depending on functional examinations and their combinations that could be carried out in the course of treatment.

Group 1: The patients with acute pyelonephritis caused by unilateral stone obstruction (n = 24). In these patients, RPP measurements and clinical urine analysis for leukocyturia and enzymuria were carried out.

Group 2: The patients with stones and non-acute latent chronic pyelonephritis (n = 31). Most of these patients had unilateral nephrostome (1 patient had bilateral nephrostomes) necessitated by 5 unilateral and 3 bilateral renal stones or 9 proximal and 14 distal ureteral stones. Twenty-three of these patients were comprised subgroup *2a*, where RPP measurements were performed simultaneously with extended enzymuria analysis via nephrostomy tube. Of them, five patients were studied in the course of a 2 week treatment (at the beginning and the end). The subgroup *2b* patients (n=19) were subjected to one of the following procedures: extracorporal shock wave lithotripsy ESWL (n = 10) or contact lithotripsy (n = 9). In this subgroup, 12 patients with indwelled nephrostome tube were examined only with RPP measurements. In subgroup *2c* patients (n = 11) RPP was assessed in parallel with assessment of distal ureteral function.

Group 3: The unobstructed patients without the signs of inflammation (n = 5). They were examined after successful stone removal with RPP measurement performed prior to removing of the nephrostome tube.

The data were analysed statistically using Student's test ($p < 0.05$) for paired and unpaired samples.

Results

In the three groups of patients, the mean baseline RPP values were, respectively 28.7 ± 2.6 (range 20.0-32.4); 15.6 ± 1.9 (range 3.5-29.0); and 3.6 ± 1.4 (range 0-8) cm H_2O. In all groups, the ratio of GGT to urinary creatinine changed similarly: it was elevated during acute inflammation, it was moderately enhanced during the chronic process, but it significantly decreased after stone removal and resolution of inflammation, respectively: 11.5 ± 3.2; 8.1 ± 2.0, and 1.6 ± 0.5 unit/L. The elevated values of urinary GGT levels in the groups 1 and 2 compared to group 3 indicated damage to renal tubules. In group 1, we revealed correlation between leukocyturia and enzymuria which was most pronounced between leukocyturia and urine GGT enzyme (the correlation coefficients were 0.46 and 0.63 for GGT and GGT/creatinine, respectively). RPP and urinary GGT levels changed individually. In average they were moderately increased in group 2. In patients of this group leucocyturia as well as RPP values differed markedly.

In majority of group 2 patients (73%) who had the recurrent stones more than a year without the signs of acute inflammation during examination period, RPP values measured in resting supine position were higher than 10 cm H_2O. However, biochemical analysis revealed 54% patients with enhanced lithogenic activity assessed by elevated Ca2+ and oxalates in the urine (4.95 ± 0.25 mM and 504 ± 35 μM, correspondingly) and the low level of

Table 1.

Enzymuria in patients with low (n = 6) and elevated (n = 17) pressure in the renal pelvis (RPP).

RPP cm H_2O	AP	GGT	LDH	ChE	NAG
5.6 ± 0.6	0.7 ± 0.4	3.4 ± 0.4	2.6 ± 0.9	7.3 ± 1.1	4.2 ± 2.1
15.7 ± 0.4	2.3 ± 0.3	5.9 ± 0.5	9.0 ± 1.9	34.7 ± 8.9	4.2 ± 0.4
P < 0.001	P < 0.01	P < 0.001	P < 0.01	P < 0.01	

RPP – renal pelvic pressure; GGT - gammaglutamyl transferase; AP - alkaline phosphatase; LDH - lactate dehydrogenase, ChE - choline esterase, NAG - N-acetyl-glucosaminidase expressed as a ratio of enzyme concentration to urinary creatinine.

Figure 1.

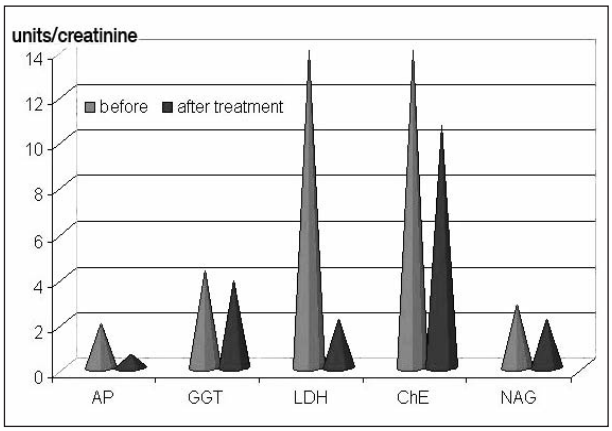


Figure 2.

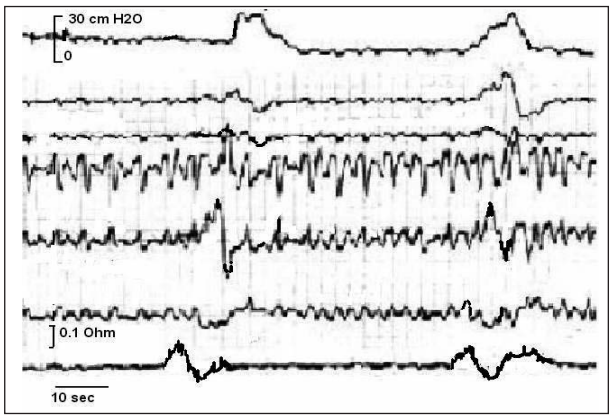


Table 2.

Characteristics of the upper urinary tract urodynamics with respect to contractile function of distal ureter.

RPP cm H_2O		Characteristics of ureteral contractile activity			Tone of ureteral wall (Ohm^{-1})	Patient distribution
Baseline	Peristaltic	Amplitude (Ohm)	Duration (sec)	Peristalsis rate (min^{-1})		
5.4	9.6	1.01	8.3	1.5	2.9	9%
6.6	11.4	0.62	5.8	1.2	9.8	9%
14.0 ± 2.5	17.2 ± 2.4	1.04 ± 0.06	7.08 ± 0.28	3.2 ± 0.2	3.4 ± 0.2	36%
16.4 ± 1.3	23.1 ± 3.4	0.29 ± 0.03	7.68 ± 0.61	3.1 ± 0.6	6.8 ± 0.7	45%

citrates (2.5 ± 0.1 mM). Therefore, these data favour the view that metabolic and urodynamic pathogenic factors may be independently involved in pathogenesis of urolithiasis.

To test this hypothesis, an extended enzyme urinary excretion was observed in 23 patients of subgroup 2a, which had no metabolic disorders, according to the mean baseline RPP values.

Of this subgroup, 17 patients demonstrated the mean basal RPP higher than 10 cm H_2O. There was a positive correlation between elevated RPP values and increased levels of urinary AP, GGT, LDH, and ChE excretion (Table 1).

Moreover, the levels of urine enzyme excretion and RPP decreased simultaneously in 5 patients examined before and at the end of 1-2 week course of successful treatment (Figure 1).

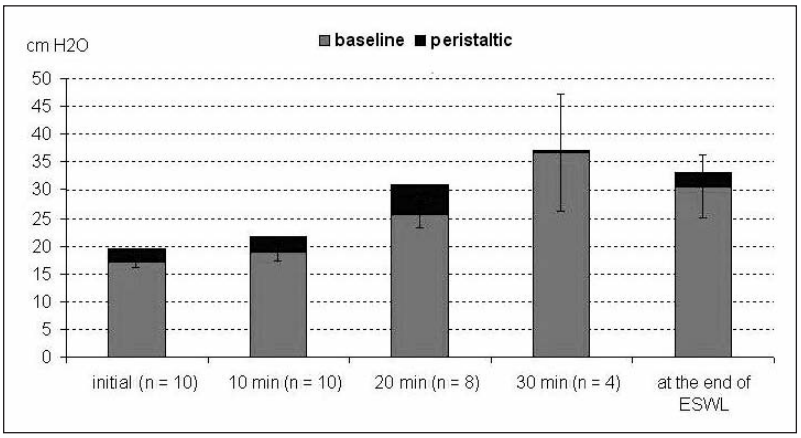
As the signs of harmful affect of elevated RPP to renal function were observed, further study was directed to reveal the parameters of ureteral peristalsis responsible for elevation of RPP and the factors affecting them. In 11 patients with urolithiasis (subgroup 2c) we assessed the baseline RPP values in relation to ureteral contractile activity in distal region of ureter (Table 2). Low RPP was found in 1 (9%) patient with strong ureteral contractions and a low tone; RPP was moderately higher in another patient (9%) with moderate mean peristaltic amplitude value but with elevated tone of ureteral wall. In the majority of examined patients with significantly elevated mean RPP value (45%), peristalsis of distal ureter was characterized by weak long-term and frequent contractions as well as increased tone, as compared to the patients with normal RPP. The last 36% patients with moderately increased RPP demonstrated strong frequent contractions in the distal ureter and low ureteral wall tone.

The high-amplitude retrograde ureteric contractions provoked urine refluxes into the renal pelvis and elevation of RPP as Figure 2 demonstrated.

Similar rapid elevations of RPP up to 70-80 cm H_2O were observed during some phases of contact lithotripsy, namely during insertion the catheter into ureteral orifice and also at the end of lithotripsy procedure, when the residual stone fragments were removed mechanically by endoscopic tools and liquid irrigation.

Also, the urodynamic effects of pronounced ureteral contractions resulting in RPP elevation were well documented in 2 of 10 patients (subgroup 2b) examined during ESWL session. On ESWL minute 7, periodic peristaltic pressure increments up to 30 cm H_2O occurred at the rate of 2 per minute. Evidently, these RPP oscillations resulted from ureteric contractions, and they were observed until the end of ESWL session and empting the urinary bladder. In subgroup 2b patients, a gradual increase in the mean baseline and peristaltic RPP was observed during lithotripsy procedure. This reaction was especially pronounced

Figure 3.



during ESWL prolongation when the patients received massive saline transfusion and Lasix. RPP rose gradually while the baseline pressure reached the value of the peristaltic pressure reflecting diuretic mode of urine transport (Figure 3).

The differences in urodynamic parameters were obtained in patients (group 2c) examined before ureteroscopy (URS) and lithotripsy procedure or immediately after it; they are summarized in Table 3.

After URS&lithotripsy the mean baseline RPP tended to be higher while the work of distal ureter was characterized with low peristaltic amplitude and a high tone compared to the patients observed before this procedure.

In this study, we tried to reveal the factors underlying the changes of two important characteristics of ureteral peristalsis (ureteral wall tone and amplitude of contraction) in patients with stone disease. Table 4 shows the factors affecting the ureteral wall tone: duration of stone disease, location of stones, and their disposition. The largest values of the mean ureteral wall tone were observed in patients with prolonged urolothiasis history especially when the stones were located in the distal part of ureter.

A special analysis of factors affecting the amplitude of ureteral contractions revealed the importance of the upper urinary tract dilation (Table 5). Peristalsis amplitude was increased in patients with dilated pelvicaliceal system compared to those without such dilatation.

Both quantitative urodynamic indices (ureteral wall tone and the amplitude of contraction) should be evaluated in relation to the qualitative characteristics of ureteral peristalsis. In this study, the greatest RPP values were observed in patients with high-amplitude retrograde contractions or simultaneous contractions in lower cystoid of ureter in the dilated upper urinary tracts. Generation of retrograde peristaltic waves and deformed cystoid contractions in ureter were often observed in patients with urolithiasis.

Table 3.

Functional parameters of ureteric peristalsis in patients before and after contact lithotripsy sessions.

Patient groups	RPP cm H_2O		Characteristics of ureteral contractile activity			Tone of ureteral wall (Ohm^{-1})
	Baseline	Peristaltic	Peristalsis amplitude (Ohm)	Duration of concentration (sec)	Peristalsis rate (min^{-1})	
Before lithotripsy	15.6 ± 1.9	20.3 ± 2.2	0.81 ± 0.07	7.4 ± 0.2	2.4 ± 0.1	4.7 ± 0.3
After lithotripsy	18.9 ± 2.6	20.3 ± 2.1	0.50 ± 0.07	8.4 ± 1.6	3.3 ± 1.2	6.8 ± 1.4
			P < 0.001			P < 0.05

Table 4.

Factors affecting ureteral wall tone.

Renal colic	Urolithiasis history		Stone location		Location&Hystory
	< 5 years	> 10 years	Pelvis&upper ureter	Middle&lower ureter	Middle&lower ureter > 10 years
4.7 ± 0.9	4.4 ± 0.9	5.6 ± 0.6	4.5 ± 0.4	5.4 ± 0.8	6.5 ± 1.2

Table 5.

Effect of upper urinary tract dilation on urodynamic parameters.

Dilation	RPP cm H_2O		Parameters of ureteral contractile function			Tone of ureteral wall (Ohm^{-1})
	Baseline	Peristaltic	Amplitude (Ohm)	Duration (sec)	Peristalsis rate (min^{-1})	
1.5-5.5 cm	19.0 ± 1.6	23.8 ± 1.0	0.81 ± 0.06	8.06 ± 0.33	2.6 ± 0.1	4.99 ± 0.37
< 1.5 cm	11.4 ± 0.6	15.5 ± 0.9	0.52 ± 0.10	6.18 ± 0.68	1.8 ± 0.2	5.31 ± 0.81
	P < 0.001	P < 0.001	P < 0.01	P < 0.01	P < 0.001	

Discussion

Urolithiasis is a complex condition resulting from a number of interchangeable and mutually complementary factors. Our study did not try to encompass the whole spectrum of mechanisms involved in pathogenesis of stone formation. We attempted to evaluate the functional urodynamic disorders in the upper urinary tract in patients with renal and ureteral stones by physiological methods because we consider such disorders as urine stagnation, refluxes, and inflammation can contribute to stone consolidation and urolithiasis.

Physiological data indicate rhythmic contractions of the renal pelvis and calyces as the prerequisite to urine concentration (2). Ureteral peristalsis is closely coupled with renal pelvis function via various

reflexes (5). Although physiology of urinary tract in general and electrical and contractile activity of renal pelvis and ureter in particular count almost a hundred years, the methods of their evaluation are little used as the potent clinical tools.

In this study, we measured pressure in the upper urinary tract in patients who had nephrostomy tube and assessed function of distal ureter by indwelling of special probe during ureteroscopy. The changes in impedance waveform from consecutive ureteral regions are determined by bioelectrical and contractile activity in the ureteral wall, and by the form and direction of ureteric contractions and urine boluses propelling. Although pressure and ureteral impedance measurements are not easily made in all cases, it is reasonable to perform them because they yield useful data on contractile function of urinary tract.

Surprisingly, RPP exceeded 10 cm H_2O in the large majority of patients (73%) despite persistent urinary diversion. Under normal conditions, it ranged 0-5 cm H_2O. One can expect that RPP to decline after adequate renal pelvis drainage, but it did not happen in all cases. Thus, the mean value of RPP can be considered as an independent index reflecting the state of pelvicaleceal urodynamics. It is likely that elevated RPP can disturb pelvic muscular contractions in such a way that pelvic wall could be paralyzed impeding the process of urine concentration (2).

During acute inflammation, the mean value of RPP was high, although it was only moderately increased in the patients with chronic pyelonephritis and stones. In these patients, the levels of urinary enzyme excretion differed similarly. Correlation between leukocyturia, which is the major index characterizing inflammatory process in kidney, and enzymuria was well documented. At the end of successful treatment, the patients demonstrated normal mean RPP and correspondingly low levels of enzyme excretion. These finding ensured that during stone disease, both inflammation and elevated RPP are the major damaging factors to renal tissue, which in turn can promote stone formation.

The unfavourable effect of elevated RPP to renal function was proved by the extended enzymuria studies in the patients, who donated the urine samples during pressure measurements via the same nephrostomy tube (Table 1). In the course of treatment, excretion rates of urinary enzymes paralleled RPP values (Figure 1). The comprehensive individual role of each enzyme in tubular (proximal or distal) or glomerular disorders is a matter of discussion, although it is obvious that their increase indicates damage to renal cells (1, 3). Elevation of RPP can trigger an increase in tubular pressure accompanied by mechanical stretch (4) leading to tubulointerstitial and glomerular disorders that finally could modify urine composition by changes in renal transport thereby contributing to urolithiasis.

Since renal pelvis and ureter are closely interconnected by common hydrodynamics and neurogenic control, further study was aimed to reveal interrelation between RPP values and the parameters of ureteral contractile function. Our study carried out in the patients, in which simultaneous RPP and distal ureteral peristalsis recording was possible, reported that RPP was maintained within the normal limits in the cases with active peristaltic activity of ureter indicating importance of ureteric contractile function for urine propelling into the bladder. The ureteric antegrade contractile waves monitored in these patients by impedance method showed that urine was propelled toward the bladder in boluses. The cystoid (or simultaneous) contractile waves in their distal ureters indicated increasing length of the boluses.

Two modes of peristalsis could be distinguished in patients with elevated RPP (Table 2). Most of them (45%) demonstrated weak contractions and increased ureteral wall tone. As a rule, the low peristaltic activity in their distal ureters consisted of chaotic contractions. Probably, such weak peristalsis of ureter was insufficient to develop the necessary pressure gradient to propel urine. In such a case, enhanced ureteric wall tone could impede urine evacuation along the ureter and promote elevation of RPP. Another mode of peristalsis was found in 36% patients. It was characterised with strong fast contractions and a low ureteral wall tone in the distal ureter. In these patients, elevation of RPP resulted from ureteroureteral refluxes provoked by contractile retrograde waves in the distal ureter. They were obviously demonstrated during simultaneous pressure and ureteric impedance recording (Figure 2). The high-amplitude ureteric contractions evoking peristaltic waves of retrograde direction provoked urine refluxes into renal pelvis accompanied by a short-term elevation in RPP.

So, our study showed that the individual peristalsis characteristics of the ureter can be the reason of RPP elevation in patients with stone disease. Moreover, general and individual reactions to operative stone treatment can induce pressure increase, too.

In patients subjected to ESWL, an elevation in mean basal renal pressure caused by hydrodynamical reasons was observed during this procedure (Figure 3). The urodynamic reaction depended on the time of the session and was more pronounced in the patients who received a massive intravenous saline infusion and furosemide. In addition to elevation of RPP by hydrodynamic reasons, the strong ureteric contractions could evoke reflex pelvicaliceal irritation contributing to RPP increment via neurogenic pathways. In literature such ureteropelvic excitatory reflex was reported occasionally (5). Our measurements have documented the sharp increments in RPP during catheterization of the ureteric orifice. The nature of the pressure elevations triggered by stretching of ureteral wall by shock waves or instrumental manipulations is probably neurogenic.

The immediate changes in ureteral function were demonstrated by comparing the urodynamic parameters in patients examined before and after the ureteroscopic procedures. The patients subjected to operative stone extraction demonstrated lower peristaltic amplitude and a higher tone in distal ureter in comparison with the patients examined prior to lithotripsy (Table 3). These quantitative indices of ureteral peristalsis together with elevated baseline RPP can be considered as the signs of aggravated evacuation of urine via the ureter.

The consequences of short-term RPP elevations were manifested by the changes in upper urinary tract urodynamics. We consider that regular transient increments in

RPP can contribute to persistent pressure rise in renal pelvis by affecting the wall tone in urinary tract via smooth muscle cells and perhaps by inducing the changes in their structure. During ureteral colic, the origin of abnormalities in ureteral wall tone is probably neurogenic. Therefore, they should be treated by corresponding pharmacological tools. In the course of time, the wall of ureteral muscle can be impregnated with connective tissue substituting muscle cells, which can degrade or eliminate its functional abilities. Assessment of ureteral functional parameters can evaluate the range of regulatory abilities of the upper urinary tract and help to choose the adequate treatment.

The current study showed that acute renal inflammation (group 1 patients), as well as chronic inflammatory process in kidney (group 2 patients) lead to elevation of the pressure in renal pelvis, which in many cases can be maintained even after drainage of the renal pelvis. The disorders in ureter peristalsis such as strong retrograde contractions, as well as the low-amplitude chaotic contractile waves accompanied by elevated ureteral wall tone contribute to RPP elevation via neurogenic and hydrodynamic mechanisms. Increased RPP and inflammatory process together with reflux nephropathy harmfully affect the proximal tubules, damaging them with release of a large amount of various enzymes. The major consequence of this injury would be deterioration of the functional ability of the proximal tubules affecting metabolism of electrolytes. In addition, increased RPP can elevate tubular and glomerular pressure and therefore diminish the rate of glomerular filtration leading to deterioration of urine transport as well as to deposition and precipitation of the salts. Playing together, these factors can provoke stone formation. It is not clear whether disorders of urodynamics in the upper urinary tract resulted from urolithiasis or they appeared prior to it because we examined the patients with stones. However, these changes surely promote stone formation.

In this study, we could demonstrate that the tone of ureteral wall and amplitude of ureteral contractions (Table 4 and 5) are the key urodynamic parameters describing efficiency of urine evacuation from renal pelvis. To understand physiological implications of these quantitative urodynamic parameters and their importance for restoration of normal urine flow in patients with urolithiasis, they should be assessed together with qualitative urodynamic parameters, especially with the direction of contraction wave propagation along the ureter. In our opinion, the risk of urodynamic damage to renal function is enhanced when the high-amplitude contractions assume the retrograde direction.

Conclusion

Acute or chronic inflammation, functional urodynamic disturbances, the changes in urine enzymes are considered as important risk factors of stone development when they are present alone or in combination. Our clinical observations obtained with the help of physiological methods revealed various factors modulating the urodynamic disorders in renal pelvis: temporary or persistent elevation of pelvic pressure; peculiarities of contractile function in distal ureter manifested by the tonic changes and variations in contractile amplitude, and certain abnormalities in propagation of contractile wave in the upper urinary tract. The reported urodynamic changes in patients with stone disease can be supplementary pathogenic factors causing deterioration of renal function probably followed by stone formation.

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Evaluation of methods for urine inhibitory potential for precipitation of calcium oxalate.

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Summary

Renal lithiasis is a significant medical and social problem. Worldwide recurrence is anywhere from 3% to 5%. Objective of this paper is to evaluate two methods for distinguishing between stone formers and non-stone formers. Urine samples were titrated with calcium and seed crystals were added to facilitate precipitation. Ionic calcium levels were monitored and compared between the two groups. Stone formers showed impaired tolerance to the calcium added and increased precipitation on seed crystals. Both methods discriminated between stone formers and non-stone formers. Further evaluations are needed to establish the better of the two for wider clinical use.

KEY WORDS: Urinary calculi; Calcium oxalate; Inhibition.

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INTRODUCTION

Renal lithiasis is a significant medical and social problem with still a high recurrence rate (3%-5%) worldwide. In Croatia urinary and/or renal stones are composed predominantly of calcium oxalate and phosphate stones (about 75%), uric acid (about 10%-12%) and less frequently of struvite in the case of so-called infection stones (about 10%-15%). Major factors contributing to renal stone formation are urine supersaturation and various metabolic factors. However urine can contain various substances which by different mechanisms can inhibit the precipitation process. The objective of this paper is to evaluate and compare several chemical methods for distinguishing between stone formers and non-stone formers.

MATERIALS AND METHODS

This study was done on two informed groups – recurring stone formers and healthy individuals. Each group had 20 participants (both male and female) ranging from 24 to 66 years of age.

Whole urine samples of both 24 h urine and morning urine were used in the experiments. The methods in question are based on testing the inhibitory capacity of urine with respect to precipitation of calcium salts.

a) Seed method (initiation of precipitation from whole urine by addition of calcium oxalate monohydrate seed crystals)
This method monitors ionic calcium concentration in samples with seed crystals of calcium oxalate monohydrate (COM) (initiated precipitation) and without (spontaneous precipitation) after 3 h and 24 h incubation at 37°C (1). Results are shown according to the following equation: $\Delta c\ (Ca) = c\ (Ca)_{spont.\ prec.} - c\ (Ca)_{init.\ prec.}$
Low values of Δc (Ca) mean that added seed crystals very slightly promote precipitation, higher value of Δc (Ca) represents stronger promoting effect of seed crystals on the precipitation in urine, while negative value of Δc (Ca) (for non-formers) indicates dissolution of added seed crystals.

b) Titration method (testing the capacity of urine for calcium

complexing by adding calcium solution to samples of first morning urine) (2).

Samples are titrated with calcium chloride solution ($c(CaCl_2)$ = 0.1 mol dm-^{3}) and ionic calcium concentration is observed. The discriminating criterion for the results is the slope of the titration curve. Low value of the slope means that urine complexes added calcium, while higher value of the slope shows that urine does not form complexes. All Ca^2 + ion concentrations were measured by PVC matrix Ca-ion selectrode suitable for urine measurements. Artificial urine (3) was used as a control and for standardization.

Results

Results are summarized in Figure 1 and 2.

Both methods were also repeated on daily urine samples (data not shown) and gave similar results that did not show any significant difference when compared with morning urine samples.

Discussion

Results show that both methods have potential in discriminating between stone formers and healthy individuals. Titration method seems to be better giving a more noticeable difference. Seed method has failed to confirm the initial hypothesis. There are great differences in the response between male and female samples so other factors should be considered.

Both methods require further evaluations on larger and more defined subgroups that will allow for other varying

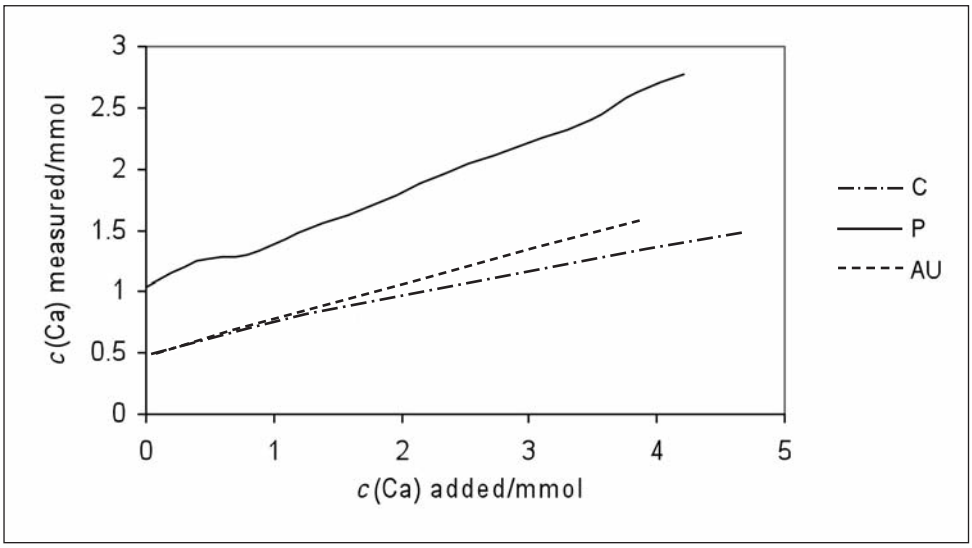

Figure 1.

Titration curves of first morning urine samples (C, control; P, patients) and artificial urine (AU).

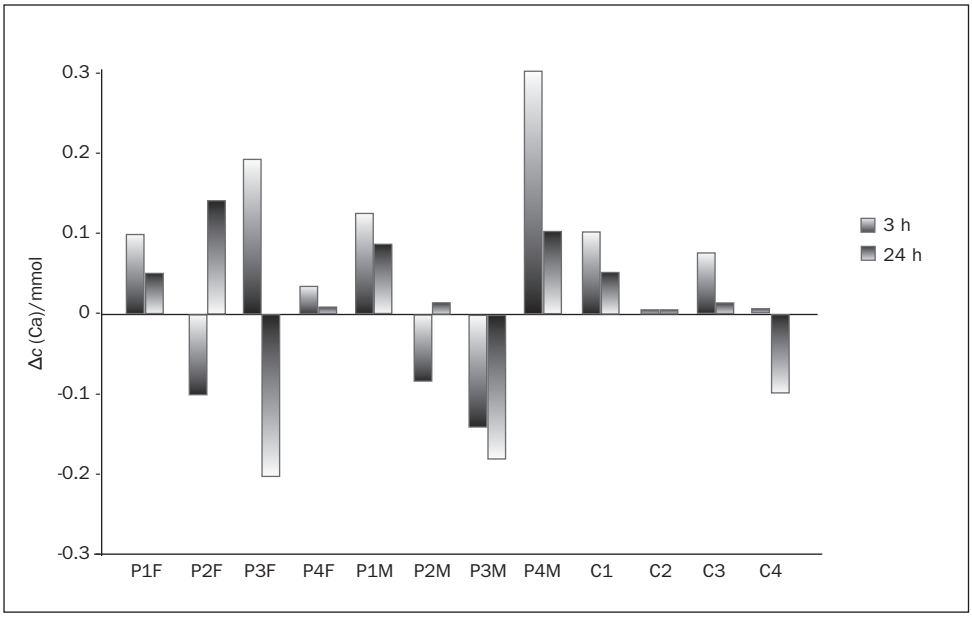

Figure 2.

The values of Δc (Ca) for stone-formers (P, patients) and non-formers (C, control) by seed method.

factors to be taken into considerations (hormonal status, related illness, genetic predisposition, etc.).
First morning urine showed no significant difference when compared with daily urine, so all experiments shown were done on those samples that are easier to collect. Many daily urine samples have to be rejected because they are not collected according to instructions.

Acknowledgement

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Nephrolithiasis in medullary sponge kidney.

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Summary

Seventy-one patients with documented Medullary Sponge Kidney (MSK) and nephrolithiasis underwent complete metabolic evaluation. These patients constituted 7.3% of our calcium stone-forming population Metabolic anomalies (hypercalciuria, hyperoxaluria, hypocitraturia and hyperuricosuria) were observed in 82% of patients. No patient was hypercalcemic and none had hyperparathyroidism. Thus the patients with medullary sponge kidney and renal stones had the same spectrum of metabolic anomalies as the overall population of idiopathic stone formers. Although these patients may have anatomic anomalies which determine stasis of urine and infection causing stone formation, they should be evaluated and treated appropiately for any metabolic defect.

KEY WORDS: Nephrolithiasis, Medullary sponge kidney (MSK).

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INTRODUCTION

Medullary sponge kidney (MSK) is a congenital abnormality of the renal medulla characterized by the precalyceal collecting tubules ectasia. These changes were first described radiologically in 1939 by *Leonarduzzi* and a decade later Cacchi and Ricci confirmed these findings histopathologically. The diagnosis of MSK is made by excretory urograms according to radiographic criteria that include the characteristic "*paint brush*" appearance of the dilated tubules draining into flattened calyces, which may favour salt crystallization and precipitation with consequent stone formation (1). The frequency of MSK in patients with nephrolithiasis is highly variable. Differences in radiologic criteria may account for the various prevalence reported. Furthemore, previous works have reported a variety of metabolic anomalies, including hypercalciuria, hypocitraturia, hyperuricosuria and distal renal tubular (RTA) that, together with papillary collecting ducts dilatation, could play a role in stone formation. Also the concurrence of hyperparathyroidism and MSK has been reported, which suggests that renal hypercalciuria from irregular nephron function may lead to parathyroid hyperplasia and adenoma.

This study was carried out to obtain additional information about underlying metabolic anomalies of patients with MSK and nephrolithiasis.

MATERIALS AND METHODS

From January 1984 to December 2008 in the Urologic clinic of Treviso General Hospital among 974 patients with recurrent calcium stones 71 (7.3%) (42 males and 29 females, mean age 37.4+/-17.1 and 35.8+/-16.4 yrs respectively) had the characteristic features of MSK on intravenous pyelograms. These features included radial distribution of calcification around enlarged papillae, flattened calyces and dilated collecting tubules with or without cystic deformities. No case was diagnosed as MSK unless both urologist and radiologist agreed that the condition was present after reviewing the pyelograms. In 68 patients the defect was bilateral and in all cases tubular ectasia involved three or more papillae. Of 974 with recurrent calcium stones, 567 could be fully evaluated metabolically, of whom 71 were patients with MSK and and 496 were idiopathic stone formers. Three 24 h urine samples were collected on an out basis, while maintaining the usual diet. Urine was analyzed for levels of oxalate, uric acid, citrate, creatinine, sodium and potassium. After at least 10 hours fasting, venous blood was drawn for calcium, phosphate, uric acid, creatinine, sodium, potassium, chloride, PTH; morning spot urine was also collected for urine analysis and culture. Passed or removed stone were analyzed whenever possible.

Idiopathic hypercalciuria was defined as 24-h urine calcium excretion greater than 300 mg, normocalcemia and exclusion of other hypercalciuric state. Hyperuricosuria was defined as 24-h uric acid excretion above 800 mg, hypocitraturia as less than 350 mg and hyperoxaluria as more than 40 mg. Distal renal tubular acidosis (RTA) was diagnosed when at least 2 of these conditions was observed: morning urinary pH higher than 5.5 in sterile

Table 1.

Frequency of metabolic anomalies.

	MSK stone formers (n = 71)		Idiopathic Stone Formers (n = 496)		
	N°	%	N°	%	P
Low urine volume	29	41	221	44	n.s.
Hypercalciuria	31	44	202	40	n.s.
Hyperoxaluria	16	22	181	36	n.s.
Hypocitraturia	33	46	201	40	n.s.
Hyperuricosuria	20	28	158	32	n.s.

urine, systemic acidemia and urinary citrate excretion lower than 100 mg/24h. When only one of above three conditions was observed, an oral ammonium chloride test was performed to reveal incomplete renal tubular acidosis. A fasting urine pH after acid load higher than 5.6 associated with systemic acidemia indicates RTA.
Data are presented as mean ± SE and Student's t-test was used for statistically analysis. The frequency of metabolic anomalies was compared by chi-square statistical analysis. P less than 0.05 was considered as significant.

Results

Frequency of metabolic anomalies in MSK and idiopathic stone formers are listed in Table 1. Hypercalciuria was present in 31 (44%) patients, hypocitraturia in 33 (46%), hyperuricosuria in 20 (28%) and hyperoxaluria in 16 (22%) of patients with MSK. Frequency of low urine volume (< 1,500 mL/24 h) and metabolic anomalies (hypercalciuria, hypocitraturia, hyperoxaluria and hyperuricosuria) were similar between the MSK and idiopathic stone formers. Hyperuricosuria was found in 20 MSK stone formers, 16 of whom also have hypercalciuria. Twelve patients were both hypercalciuric and hypocitraturic and 6 hypercalciuric, hypocitraturic and hyperuricosuric. Renal tubular acidosis was present in 3 (4%) of MSK patients. No differences were found in creatinine, calcium, phosphate, uric acid, potassium, sodium and chloride serum values between the two groups. No patient could be classified as having primary hyperparathyroidism (Table 2). Urinary tract infections were present in 18 patients and were treated by adequate antibiotic therapy. In 13 (18%) of MSK stone formers no metabolic anomalies were present. The chemical analysis of stones passed or removed was calcium-oxalate and/or calcium phosphate in all patients but 5 who had mixed (calcium oxalate - uric acid).

Table 2.

Serum parameters in MSK and idiopathic stone formers.

	MSK stone formers (n = 71)	Idiopathic stone formers (n = 496)	P
Creatinine (mg/dL)	0.95 ± 0.03	0.95 ± 0.04	n.s.
Calcium (mg/dL)	9.35 ± 0.06	9.37 ± 0.05	n.s.
Phosphate (mg/dL)	3.21 ± 0.09	3.17 ± 0.05	n.s.
Uric acid (mg/dL)	5.22 ± 0.21	5.56 ± 0.19	n.s.
Potassium (mEq/L)	4.25 ± 0.16	4.19 ± 0.08	n.s.
Sodium (mEq/L)	139.3 ± 0.4	140.1 ± 0.7	n.s.
Chloride (mEq/L)	109.2 ± 0.05	108.5 ± 0.03	n.s.
PTH (pg/mL)	46.5 ± 5.7	44.2 ± 2.1	n.s.

Discussion

Patients with MSK usually come to the attention of physicians because of kidney stones. The diagnosis of MSK is made using intravenous urography, which demonstrates the characteristics linear or spherical tubules filled with contrast medium in renal papillae; in fact during retrograde pyelography the dilated ducts do not fill with contrast medium or they do not fill completely (1). Ultrasonography, arteriography and computed tomography have little if any role in diagnosis.
The frequency of radiographic features of MSK reported in the medical Literature is highly variable. Most of the reports make no distinction between patients with and without nephrolithiasis and give a global prevalence of MSK found on urograms that ranges between 0.5 and 3.5%. The frequency of MSK may vary from to 2.3 to 21% of patients with renal calculi.
Differences in radiologic criteria may account for the various prevalences reported; indeed Palubinskas performed the diagnosis of MSK only if all the papillae of both kidneys were involved, whereas Parks diagnosed it when half of the papillae of both kidneys were involved. Furthemore, Yendt made the diagnosis of MSK if three papillae in one or both kidneys were involved (2).
Therefore, because it is now agreed that MSK may be a unilateral and even a local lesion, Ginalski made the diagnosis even if only one single papilla was involved. The incidence of MSK in our patients with recurrent calcium nephrolitihiasis was 7.3% according to Yendt's radiographic criteria.
Metabolic disorders were associated with MSK stone formers, as well as with idiopathic stone formers (3). Hypercalciuria was present in 44% of our MSK stone formers according to previous studies. In fact, *Parks et al.* reported the frequency of hypercalciuria in 42% of MSK patients, *Jungers et al.* in 44% and Yendt in 30%. These findings could suggest that increased calcium excretion from impaired calcium reabsorption, which tend to occur in diseased tubules, is closely associated with hypercalciuria in MSK stone formers.
Whether primary hyperparathyroidism is related to stone formation in patients with MSK is unknown. The association of hyperparathyroidism and MSK has

been reported and it has been suggested that hyperparathyroidism resulted from prolonged parathyroid stimulation due to a primary renal tubular calcium leak. These reports, however, do not provide proof that there is a higher incidence of hyperparathyroidism in patients with MSK than in stone patients who do not have this disorder. The only report of an abnormality high incidence of hyperparathyroidism in MSK is that of *Maschio et al.* who found seven cases with surgically proven hyperparathyroidism during the investigation of 28 patients with MSK, while in other studies the incidence ranged from 9.1 to 2%. Our study shows that no patient had hyperparathyroidism. Intact PTH levels were similar between MSK and idiopathic stone formers and there were not differences in serum calcium phosphate levels and urinary phosphate and calcium excretion between the groups. These results could suggest no special relationship between calcium metabolism and stone formation in patients with MSK.

The incidence of hypocitraturia in our MSK stone formers was high (46%), such as the reports from *Kinoshita* and *Lahme et al.* that found a hypocitraturia rate of 58.3% and 44.4% respectively. With few exceptions, such as the report from *Ginalski et al.* that found hypocitraturia in only 2.9% of MSK stone formers, several studies reported hypocitraturia to be an important risk factor for stone formation in patients with MSK. Renal tubular acidosis (RTA) has been reported in several studies as a cause of hypocitraturia. *Osther et al.* observed RTA in 40% of MSK stone formers, *Higashihara et al.* in 36.4% and *Lahme* in 33.3%, while the incidence of RTA was low in *Ginalski's* study (2.9%). Also in our experience the incidence of RTA in patients with MSK was low (4.%). The acidification defects have been suspected in several reports as cause of hypocitraturia and the could also form the background for the high urinary excretion of calcium in MSK stone formers. The urinary excretion of citrate in all our patients with MSK was of the same order as in the distal type of RTA although most of the had normal acidification. Further investigations regarding renal tubular function and hypocitraturia in patients with MSK should be performed to clarify the link between two parameters involved in stone formation.

Hyperuricosuria was present in 20 of MSK stone formers and 80% of them have also hypercalciuria. This association could be etiologically related to calcium oxalate stone formation, as well in idiopathic stone formers.

Furthemore, additional anomalies, such as prolonged urinary transit time in dilated ducts, are probably important. Urinary infection is probably not an important factor in the majority of patients, because most of whom have sterile urine. Therefore, even when infection is present, it could be more apt to be the result rather than the cause of the stones, as *Parks et al.* reported. In our study urinary infection was present in 18 MSK patients with very high rates of intracorporeal and/or extracorporeal treatment for stone removal. However, there is no doubt that the combination of MSK and urinary infection may occasionally lead to an explosive progression of the stone disease, so that careful monitoring of patients and prompt treatment of urinary infection are important.

In conclusion, a metabolic evaluation of 71 patients with MSK nephrolithiasis has disclosed the same pattern underlying anomalies causing stone formation as is seen in idiopathic stone formers. In addition, the features of MSK such as dilated tubules with or without cystic deformities may increase the risk of stone formation by promoting stasis and thereby favour precipitation of stone material; it is possible that this conjectural mechanism played a role in the patients with medullary sponge kidney without metabolic disorders (18% in our series). Since correction of the underlying metabolic anomalies may reduce the incidence of nephrolithiasis, a careful metabolic evaluation and appropriate therapy may help to prevent stone formation in MSK patients.

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Increasing water intake by 2 liters reduces crystallization risk indexes in healthy subjects.

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Summary

Objective: *The objective of the present study was to evaluate the effects of drinking 2 additional litres of water/day on several urinary risk factors for lithiasis in healthy subjects, through measurement of crystallization risk indices (Tiselius CRI).*

Materials and methods: *48 healthy subjects, aged 25 to 50 were studied for urinary parameters including CRI in the laboratory ward, for 24 hours. After this first period, they were randomized either to a 2L/d additional water intake (treated group) or usual fluid consumption (control group) for a 6 days period, which ended by a second measurement period in the laboratory ward for 24 hours.*

Results: *Total additional water intake was actually 1.3L/d on average in treated subjects, because subjects decreased other usual sources of fluid intake. In 24 hour urine, Tiselius CRI varied differently among treated subjects and controls between the 2 periods; male controls subjects experienced much higher values (above 2 in average in first morning urine sample) in the second period ($p = 0.05$). Of interest, in a transversal analysis, we observed a positive relation between BMI or waist circumference on the one hand, and with 24 hour urea excretion or osmotic load on the other hand.*

Conclusion: *These results show a beneficial effect of a final 1.3L additional water intake on Tiselius CRI in healthy subjects.*

KEY WORDS: *Crystallization risk index; Urolithiasis; Water intake.*

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INTRODUCTION

Nephrolithiasis affects about 10% of the population of Western countries (1), and the prevalence of kidney stones has been increasing during the past decades (2). Calcium oxalate stones are prevalent, while uric acid stones are commonly found in overweight, obese and diabetic persons (3, 4). Medical expenditures for nephrolithiasis in the US were estimated at 2.1 billion $ in 2000, and costs continue to rise because of the increasing prevalence of kidney stones (5). Also, a history of kidney stone disease was recently shown to be associated with an increased risk of chronic kidney disease (6, 7).

In most cases, prevention of stone recurrence is based on dietary advices (8) and recommendations to increase water intake in order to maintain diuresis to above 2 liters/day. Indeed, an increased water intake has been linked to an increased urine volume and lower nephrolithiasis recurrence (9-11). Recently, the occurrence of crystalluria in serial urine samples was reported as the better marker of predicting stone recurrence in stone formers (12). In that study, the authors found that crystalluria was significantly less frequent in patients who experienced an increase in their urine volume above 2 liters/day, which was in agreement with previous reports providing evidence of a reduced stone recurrence in patients who maintained diuresis above 2 liters/day (10). However, there are very few data assessing the effect of an increase in water intake on the risk of crystal formation in urine and stone prevention in healthy subjects. On the other hand, measuring crystalluria is not easy, due to analytical considerations, in both stone formers and normal subjects. For this reason,

Table 1.

Stone characteristics.

	FontVella® Sigüenza source
Ca (calcium) (mg/mL)	78.4
Mg (magnesium) (mg/mL)	29.3
Na (sodium) (mg/mL)	5.4
HCO_3 (bicarbonates) (mg/mL)	300.0
Cl (chloride) (mg/mL)	11.4
SO4 (sulphates) (mg/mL)	27.8
Conductivity (µS/cm)	525.0
Total dissolved solids (mg/L)	323.0

some authors have proposed the determination of risk indices able to predict stone formation from urine parameters easily determined in routine practice (13, 14). Among the most popular indices, APCaOx index proposed by Tiselius is based on the measurement of the urine volume and of only four urine parameters, namely calcium, oxalate, magnesium and citrate. The author progressively refined his index and compared it with the calculation of urine supersaturation as determined with the EQUIL software (15). He concluded that the new APCaOx index EQ could be a good tool for assessing the crystallization risk in urine from both stone formers and healthy subjects.
The objective of the present study was to evaluate the effects of drinking 2 additional litres of water (*FontVella®*, Spain, composition in Table 1) per day on several urinary risk factors for lithiasis in healthy subjects through measurement of Tiselius cristallization risk indices (CRI) (13).

Methods

Subjects

48 healthy subjects were selected for the study according to the following criteria: age range between 25 to 50 years old, BMI (body mass index) between 20 and 30 kg/m²; urine osmolality > 450 mOsmol/L in women, > 550 mOsmol/L in men; osmotic load > 10 mOsmol/kg/day based on a 24-h urine collection; and creatinine clearance > 90 mL/min. Normal health was assessed at pre-study screening, based on medical history, physical examination and blood chemistry including glucose, creatinine and urea, uric acid, sodium, calcium, magnesium measurement.
Exclusion criteria included a past or recent history of lithiasis, cardiac or hepatic failure, or subjects taking any medication.

Procedure and evaluations

Subjects were all evaluated at baseline (period 1) and at the end of the study period (period 2), separated by one week. For each period, subjects arrived at the laboratory ward at 7:00 pm and had dinner. Standard meals were served at 7:00 pm, 9:00 am, 12:00 am. Subjects were instructed to consume fluids as they were used to and to record their exact fluid intake in the nutritional diary. They had free access to beverages they usually consumed (water, soda, coffee, alcoholic beverages, tea, milk…). 24 hour urine samples were collected from 10:00 PM (T0) to 10:00 PM the next day (experimental day). At T 0, subjects received a 274 mg oxalate load provided by 100 g black chocolate, a common practice in general population able to increase oxalate ions absorption by the gut and oxalate excretion in the first morning urine, which was reported at higher risk of calcium oxalate crystallization by comparison to 24 hour urine collection.
Urines were collected during all the period, at each required bladder emptying and natural micturition; collec-

Table 2.

Subject's physical characteristics.

	Mean ± SD	
	Males (n = 27)	**Females (n = 21)**
Age (y)	29.7 ± 4.6	29.1 ± 3.2
SBP (mm)	120.7 ± 8.2	110.0 ± 9.5
DBP (mm)	74.2 ± 6.9	70.0 ± 7.6
Height (cm)	176 ± 4.8	164.2 ± 4.3
Weight (kg)	77.7 ± 9.0	60.1 ± 7.7
BMI (kg/m²)	25.0 ± 2.2	22.3 ± 2.6

Table 3.

Urinary parameters at inclusion (24 hours urine).

	Males (n = 27)		Females (n = 21)	
	Concentration	**24 h excretion**	**Concentration**	**24 h excretion**
Osmolality (mosmol/kg or /24h)	467.6 ± 169.1	833.8 ± 247.0	438.6 ± 115.4	753.06 ± 176.94
Citrate (µmol/l or /24h)	1.5 ± 1.2	2.5 ± 1.7	2.2 ±1.3	3.5 ± 1.5
Mg (mmol /l or /24h)	2.08 ± 0.82	3.70 ± 1.20	1.79 ± 0.73	2.99 ± 1.02
Ca (mmol/l or /24h)	2.11 ± 0,90	3.72 ± 1,30	1.76 ± 1,02	2.92 ± 1.48
Oxalate (mmol/l or /24h)	0.25 ± 0.15	0.46 ± 0.27	0.27 ± 0.15	0.42 ± 0.15
Volume (l)	-	1.9 ± 0.6	-	1.82 ± 0.63
Sodium (mmol/l or /24h)	82.4 ± 35.7	148.0 ± 56.2	86.0 ± 25.4	147.9 ± 40.1
Potassium (mmol/l or /24h)	35.2 ± 14.3	63.3 ± 24.8	35.9 ± 13.2	61.7 ± 19.5
Chloride (mmol/l or /24h)	77.6 ± 35.8	139.8 ± 57.2	77.38 ± 21.47	134.9 ± 38.7
Tiselius CRI	0.49 ± 0.60	1.01 ± 0.60	0.48 ± 0.52	0.81 ± 0.56

Table 4.

24 h urine parameters (excretion, mmol/24 h), all patients.

		All patients				
			Baseline		Treatment	
		n	mean	SD	mean	SD
Sodium	C	24	152.9	40.4	192.0	60.2
	T	24	143.6	57.4	178.4	55.5
Calcium	C	24	3.43	1.43	4.25	1.84
	T	24	3.30	1.44	4.25	1.87
Citrate	C	24	2.81	1.83	3.38	1.71
	T	24	3.08	1.54	3.18	1.51
Creatinine	C	24	11.37	3.42	12.95	3.53
	T	24	12.74	3.44	12.53	3.42
Potassium	C	24	66.4	21.4	75.7	23.4
	T	24	58.8	23.3	59.1a	19.0
Magnesium	C	24	3.40	1.20	3.96	1.22
	T	24	3.31	1.14	4.13	1.30
Oxalate	C	24	0.419	0.131	0.561	0.265
	T	24	0.466	0.288	0.447	0.182
Uric acid	C	24	3.47	0.89	3.80	0.33
	T	24	3.45	0.87	3.65	0.79
Urea	C	24	407.3	116.0	456.7	96.4
	T	24	380.2	112.2	410.1	92.3
Tiselius CRI	C	24	0.84	0.37	1.29	1.06
	T	24	1.01	0.74	0.63	0.45[a]

C: Controls, T: Treated
[a] $p < 0.01$ vs control group.

tion times and urine weight were recorded and urines were sampled and deep frozen for further analysis performed in a central laboratory. Clinical and physical examinations (body weight, blood pressure) were performed at 8:00 AM. At the end of period 1, subjects were randomized into either a treatment group (additional water intake) or a control group (free water and fluid intake). Period 1 and 2 were separated by a "*water*" or a control phase (6 days), which included, according with subjects randomisation, the additional intake of a 2l/d load of *FontVella®* water in the treatment group.
This additional water intake was completed at the end of period 2.

Biochemistry - Urine parameters

First morning urine and 24 hour urine were analysed at day 2 for the following parameters: sodium, potassium and chloride (ion selective electrode); calcium and magnesium (inductively coupled plasma atomic emission spectroscopy); phosphate (colorimetry); oxalate and citrate (HPLC); creatinine (method of Jaffé rate blanked); uric acid and urea (enzymatic methods); osmolality (osmometer by freezing point depression); specific gravity (refractive index method). The CRI_T was calculated with the following formula: $CRI_T = A \times Ca^{0.84} \times Ox \times Mg^{-0.12} \times Cit^{-0.22} \times V^{-1.03}$ (15).

Statistics

Results are expressed as mean ± SD. Comparisons were done by analysis of covariance for quantitative variables

Table 5.

First Morning Urine parameters (concentrations, mmol/l), by gender.

		Males					Females				
			Baseline		Treatment			Baseline		Treatment	
		n	mean	SD	mean	SD	n	mean	SD	mean	SD
Sodium	C	13	73.1	37.4	101.5	38.9	11	49.5	15.4	67.0	35.9
	T	14	74.6	44.6	71.7[a]	26.3	10	58.4	17.3	60.0	23.4
Calcium	C	13	2.01	1.19	2.92	1.57	11	1.22	0.89	1.50	0.96
	T	14	2.04	1.66	2.01	0.80	10	1.50	1.00	1.36	0.53
Citrate	C	13	1.55	1.76	1.98	1.34	11	1.52	0.77	1.86	1.22
	T	14	1.58	1.45	1.29	0.83	10	2.47	1.94	2.59	1.81
Creatinine	C	13	9.73	4.40	14.05	6.21	11	4.44[c]	1.73	5.46[d]	1.82
	T	14	9.99	6.23	8.44[a]	4.02	10	5.30	3.50	5.52	2.82
Potassium	C	13	31.5	14.7	38.1	21.5	11	16.4c	7.4	24.8	13.1
	T	14	27.6	16.1	20.2	11.8	10	18.8	13.5	19.3	9.7
Magnesium	C	13	2.32	1.33	3.80	1.89	11	1.58	0.80	1.87[c]	0.75
	T	14	2.48	1.57	2.32	0.79	10	1.92	0.92	1.04	0.28
Oxalate	C	13	0.354	0.136	0.547	0.331	11	0.214[c]	0.070	0.317	0.182
	T	14	0.380	0.234	0.318	0.274	10	0.293	0.156	0.359	0.344
Uric acid	C	13	2.19	1.03	2.81	1.14	11	1.39	0.52	1.51[c]	0.56
	T	14	2.27	1.24	1.95	0.61	10	1.51	0.69	1.59	0.64
Urea	C	13	350	175	462	174	11	186c	104	233d	88
	T	14	315	177	253[b]	113	10	216	126	214	89

C: Controls, T: Treated
[a] $p < 0.01$;
[b] $p < 0.001$ vs group C for the same sex;
[c] $p < 0.01$;
[d] $p < 0.001$ vs males (same condition).

Table 6.

First Morning Urine parameters (excretion mmol/24 h), by gender.

		Males					Females				
			Baseline		Treatment			Baseline		Treatment	
		n	mean	SD	mean	SD	n	mean	SD	mean	SD
Sodium	C	13	34.8	11.8	44.5	22.4	11	30.3	9.5	34.3	12.7
	T	14	36.1	18.3	50.2	28.8	10	32.2	11.6	34.1	10.4
Calcium	C	13	0.93	0.47	1.26	0.70	11	0.67	0.34	0.78	0.47
	T	14	0.89	0.45	1.27	0.51	10	0.81	0.60	0.78	0.31
Citrate	C	13	0.62	0.59	0.79	0.54	11	0.89	0.39	0.96	0.50
	T	14	0.65	0.34	0.76	0.32	10	1.14[a]	0.38	1.32[a]	0.53
Creatinine	C	13	4.51	0.94	5.25	0.72	11	2.65[b]	0.65	2.90[b]	0.76
	T	14	4.37	0.87	5.00	1.10	10	2.67[b]	0.84	2.89[b]	0.59
Potassium	C	13	14.7	3.5	13.8	4.1	11	11.0	6.5	13.0	6.7
	T	14	12.8	3.6	12.2	4.8	10	9.11	2.7	10.4	3.9
Magnesium	C	13	1.08	0.43	1.42	0.40	11	0.93	0.36	1.01	0.41
	T	14	1.19	0.55	1.47	0.54	10	0.83	0.25	1.03	0.27
Oxalate	C	13	0.179	0.070	0.194	0.084	11	0.133	0.059	0.164	0.089
	T	14	0.180	0.083	0.184	0.110	10	0.149	0.047	0.174	0.105
Uric acid	C	13	1.01	0.17	1.09	0.22	11	0.83	0.21	0.80[a]	0.22
	T	14	1.06	0.34	1.22	0.34	10	0.81	0.28	0.86[a]	0.15
Urea	C	13	160	42	179	41	11	110	39	123[a]	34
	T	14	143	52	154	42	10	114	43	117	36
Tiselius CRI	C	13	1.24	0.64	2.16	1.71	11	0.54[a]	0.31	0.75a	0.50
	T	14	1.25	0.94	1.17	0.76	10	0.79	0.67	0.79	0.58
Volume	C	13	0.545	0.225	0.455	0.233	11	0.659	0.247	0.572	0.219
	T	14	0.579	0.280	0.680	0.240	10	0.607	0.280	0.605	0.183

C: Controls, T: Treated
[a] $p < 0.01$;
[b] $p < 0.001$ vs males.

Table 7.

24 h urine parameters parameters (concentrations, mmol/l), by gender.

			Males					Females			
			Baseline		Treatment			Baseline		Treatment	
		n	mean	SD	mean	SD	n	mean	SD	mean	SD
Sodium	C	13	86.0	28.6	103.1	24.2	11	80.3	22.4	94.2	28.9
	T	14	79.1	42.1	61.3[f]	15.1	10	92.3	28.1	53.2[b,e]	9.2
Calcium	C	13	2.10	0.95	2.44	0.97	11	1.66	0.75	1.94	0.94
	T	14	2.11	0.87	1.71	0.72	10	1.87	1.29	1.09[a]	0.75
Citrate	C	13	1.55	1.50	1.70	1.03	11	1.77	0.93	1.96	0.79
	T	14	1.43	0.78	0.82[d]	0.35	10	2.70[a]	1.60	1.51[a]	0.82
Creatinine	C	13	7.41	2.06	8.54	3.71	11	4.74[a]	1.22	5.20[a,b]	0.98
	T	14	7.56	2.38	5.01[d]	1.43	10	5.98	2.36	3.17[a,e]	0.91
Potassium	C	13	38.0	13.0	39.8	21.0	11	32.9	9.7	41.8	9.2
	T	14	32.7	15.4	17.7[b,e]	5.5	10	39.3	16.2	21.5[b,f]	4.0
Magnesium	C	13	1.94	0.75	2.24	0.93	11	1.73	0.53	2.01	0.68
	T	14	2.16	0.88	1.57	0.63	10	1.82	0.91	1.16[d]	0.35
Oxalate	C	13	0.220	0.068	0.348	0.258	11	0.242	0.094	0.274	0.138
	T	14	0.281	0.189	0.147[d]	0.061	10	0.289	0.203	0.146	0.056
Uric acid	C	13	2.06	0.65	2.31	1.07	11	1.71	0.53	1.79	0.36
	T	14	2.07	0.70	1.33[b,d]	0.37	10	2.08	0.68	1.08[c,f]	0.21
Urea	C	13	256	87	279	119	11	185	53	209	48
	T	14	229	83	148[b,e]	37	10	219	61	121[c,f]	29

C: Controls, T: Treated
[a] $p < 0.01$ vs males;
[b] $p < 0.01$;
[c] $p < 0.001$ vs baseline;
[d] $p < 0.01$;
[e] $p < 0.001$;
[f] $p < 0.0001$ vs control group.

Table 8.

24 h urine parameters (excretion, mmol/24 h), by gender.

		Males					Females				
			Baseline		Treatment			Baseline		Treatment	
		n	mean	SD	mean	SD	n	mean	SD	mean	SD
Sodium	C	13	156.5	41.6	205.4	68.2	11	148.6	40.6	176.1	47.4
	T	14	141.0	67.8	190.1	61.5	10	147.1	41.8	162.0	43.4
Calcium	C	13	3.79	1.42	4.74	1.89	11	3.01	1.37	3.64	1.64
	T	14	3.65	1.22	5.01[c]	1.26	10	2.81	1.65	3.16[a]	2.09
Citrate	C	13	2.57	2.19	3.12	1.76	11	3.09	1.33	3.70	1.66
	T	14	2.44	1.16	2.47	1.01	10	3.98[a]	1.59	4.18[a]	1.57
Creatinine	C	13	13.55	2.52	15.62	1.92	11	8.80[b]	2.40	9.80[b]	1.99
	T	14	13.17	2.96	14.89	2.31	10	9.04[b]	1.43	9.21[b]	1.10
Potassium	C	13	69.1	18.8	71.8	23.3	11	63.2	24.7	80.2	23.8
	T	14	57.9	29.0	54.5	19.3	10	60.0	12.8	65.5	17.6
Magnesium	C	13	3.56	1.15	4.14	1.08	11	3.21	1.28	3.76	1.39
	T	14	3.76	1.26	4.68	1.40	10	2.67	0.51	3.38[a,c]	0.61
Oxalate	C	13	0.415	0.132	0.610	0.292	11	0.424	0.136	0.503	0.228
	T	14	0.500	0.351	0.445	0.162	10	0.419	0.173	0.450	0.216
Uric acid	C	13	3.75	0.84	4.16	0.61	11	3.15	0.88	3.78[a]	0.71
	T	14	3.64	1.05	3.99	0.82	10	3.18	0.46	3.19[a]	0.45
Urea	C	13	460	88	512	71	11	346[a]	118	391[b]	81
	T	14	405	130	446	85	10	345	73	360	80
Tiselius CRI	C	13	0.91	0.34	1.57	1.22	11	0.76	0.40	0.97	0.76
	T	14	1.12	0.76	0.74	0.43	10	0.87	0.72	0.48	0.44
Volume	C	13	1.94	0.58	2.05	0.71	11	1.95	0.68	1.99	0.70
	T	14	1.87	0.58	3.13[d,f]	0.71	10	1.68	0.57	3.07[d,e]	0.78

C: Controls, T: Treated
[a] $p < 0.01$;
[b] $p < 0.001$ vs males;
[c] $p < 0.01$;
[d] $p < 0.001$ vs baseline;
[e] $p < 0.01$;
[f] $p < 0.001$ vs control group.

and chi-square test for qualitative variables. Correlations between several urinary parameters and anthropological parameters were calculated.

Ethical committee

The protocol was approved by the hospital ethical committee.

Results

Subjects baseline characteristics

Physical characteristics of the subjects are summarized in Table 2 and baseline urine parameters are presented in Table 3. Control and treated groups did not differ significantly. Interestingly, among the whole population, we found a positive correlation between 24 hour urea excretion and BMI ($r = 0.49$, $p = 0.0005$, Figure 1a) or waist circumference ($r = 0.37$, $p = 0.011$, Figure 1b) and between osmotic load and BMI ($r = 0.45$, $p = 0.01$, Figure 2a) or waist circumference ($r = 0.32$, $p = 0.026$, Figure 2b). However, 24-hour CRI_T or its components, i.e., oxalate, citrate, calcium and magnesium excretion were not significantly related to BMI or waist circumference.

Urine volume

In the treated group, subjects only increased their daily fluid intake by about 1.3 L, because they decreased their basal fluid intake in favour of the study water. Therefore, 24-hour urine volume rose by 1.4 L in treated subjects, from 1.8 ± 0.56 L at baseline to 3.21 ± 0.74 L after treatment ($p < 0,01$) whereas it remained stable in the control group (1.98 ± 0.60 L at baseline to 2.05 ± 0.68 L, NS).

Cristallization risk indexes

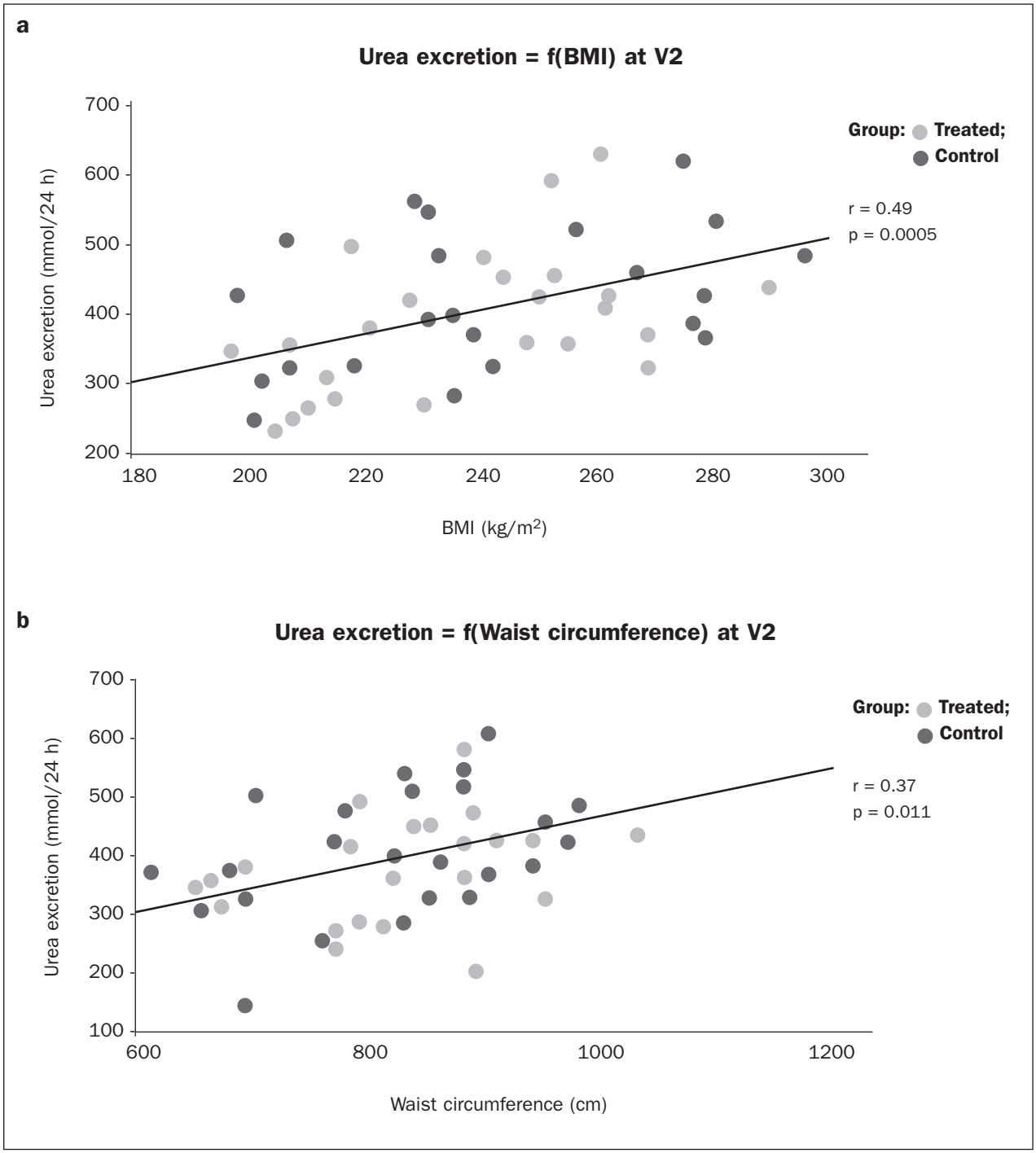
In 24 hour urine, Tiselius CRI varied differently between the 2 periods in treated subjects and controls (Table 4). Controls subjects experienced much higher values in the second period ($p = 0.05$). Tiselius CRI in treated subjects tended to decrease in tendency so that the difference between subjects and controls in the treatment period was significant ($p < 0.01$). Indeed, all subjects tended to increase the osmole excreted load in the treatment period, so it appears that Tiselius CRI increased in control subjects, whereas it was kept steady in the treated group. Such effects were observed for 24 hour Tiselius CRI whereas in first morning urine (FMU), no such significant differences were found. Notably, comparing men and women in control group, Tiselius CRIs in FMU were significantly lower in females in each period.

Other parameters

FMU parameters are shown in Table 5 (concentration)

Figure 1.

Urea according to BMI or waist circumference at baseline.

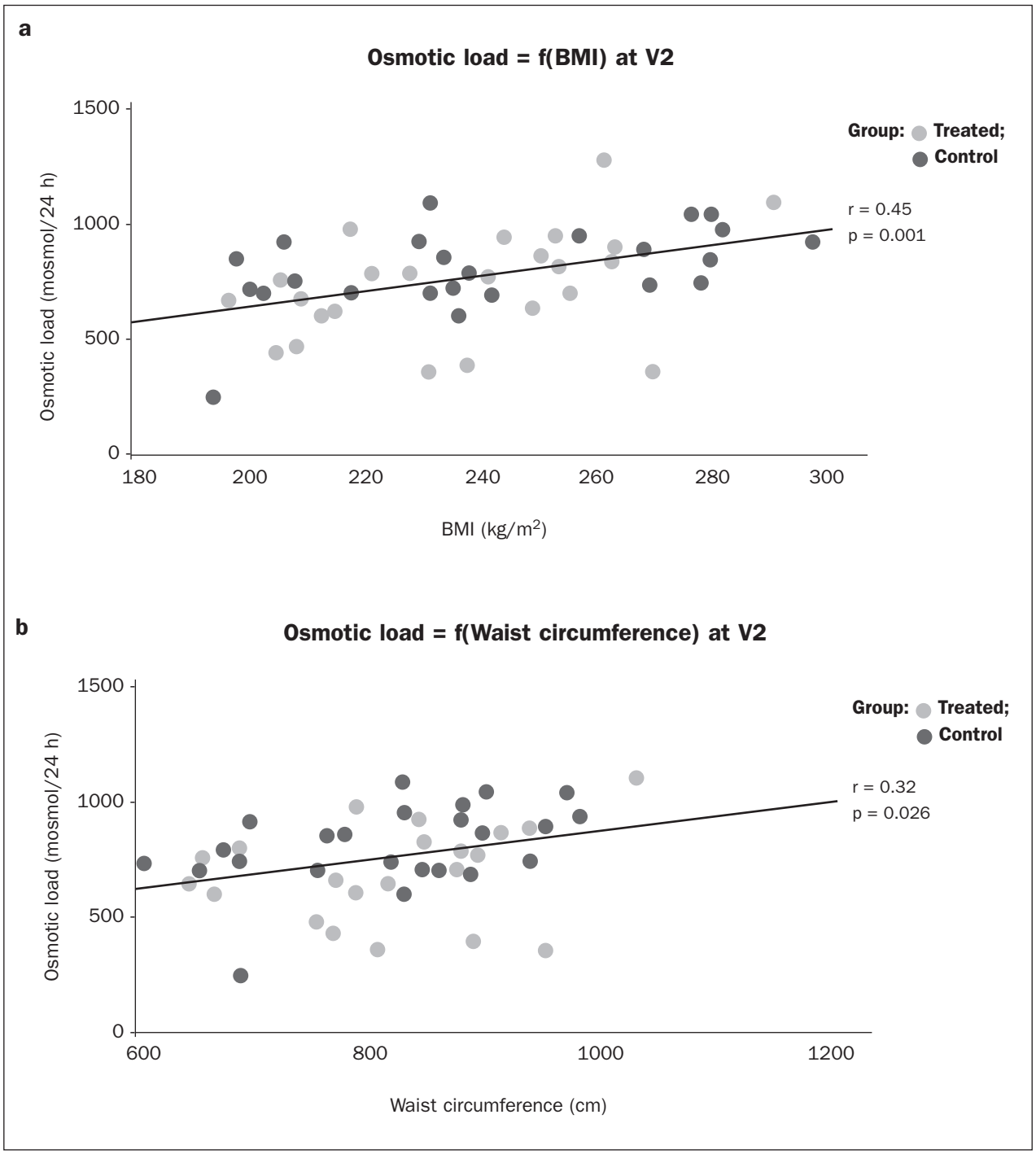


and Table 6 (excretion). Both men and women displayed high oxalate concentrations, which may have induced an increase in Tiselius CRI. Citrate excretion was significantly higher in females than in men in both groups and both periods. Conversely, creatinine was lower in females than in males. No other significant differences were observed.

The results of 24 hour urine analysis are given in Table 7 (concentration) and 8 (excretion). Creatinine, sodium, potassium, uric acid, urea and citrate concentrations were significantly decreased in both genders in treated subjects; oxalate concentration was reduced in treated men, while calcium and magnesium concentrations were reduced in treated women. Regarding 24 hour excretions, creatinine was significantly lower in females than in males in both groups for each period and citrate was significantly higher in treated females compared with males ($p < 0.01$).

Figure 2.

Osmotic load according to BMI or waist circumference at baseline (V2).



Discussion

In this study, at baseline, we observed a positive relationship between osmotic load and BMI, but we failed to find a correlation between either osmotic load or BMI and parameters involved in stone formation, such as calcium or oxalate excretion. This was in agreement with other studies showing an increased excretion of osmotic load in parallel with BMI while the excretions of lithogenic solutes were not clearly correlated with the body mass (16, 17). However, in these studies, either an increased calcium oxalate supersaturation or an increased risk of stone formation was reported. In the present work, we did not found any correlation beetwen Tiselius CRI and BMI, suggesting that, in healthy non lithiasic people, there is no direct relationship between an excess of food intake and an increased excretion of lithogenic factors in urine. However, we found a positive correlation between calcium excretion and either sodium

($r = 0.33$, $p < 0.01$) or urea excretion ($r = 0.37$, $p < 0.001$), suggesting that overweight subjects may be at risk to excrete higher lithogenic solutes. Because these subjects did not exhibit an increased diuresis by comparison to subjects with a normal BMI (data not shown), they could have a higher risk of urine supersaturation leading to stone formation. Indeed, if we examine more accurately the Tiselius CRI values found in our population, we find that males of the control group may exhibit relatively high values (period 2), especially in FMU, by comparison to females in the same group and to males and females in the treated group. According to recommendations of Tiselius as concern the value of the A parameter of the ApCaOx index, we used A = 3.2 for the calculation of Tiselius CRI in FMU and A = 1.9 in 24 hour urine. Such values allow a better estimate of the crystallization risk in stone forming subjects. When studying relationships between calcium oxalate crystallization and values of his Tiselius CRI, Tiselius found that in stone formers, a value of 2.0 could be considered as a threshold. Above that value, an increased occurrence of crystallization was observed, which expose the subject to a risk of stone recurrence. Tiselius also reported that normal subjects and stone formers exhibit different thresholds regarding the value of the Tiselius CRI predicting the risk of calcium crystallization in urine (15). However, in the general population, most stone formers present as normal before the first stone episode. Thus, the use of the same threshold value for stone formers and normal subjects seems to be pertinent. Based on a value of 2 for the threshold CRI, we found that a potential risk of crystallization exists in the normal male population. Increasing their fluid intake allowed to reduce the Tiselius CRI value under the threshold proposed by Tiselius. Actually, our results clearly show the beneficial effect of a final 1.3 L additional water intake on Tiselius CRI, which significantly decreased under 1.2 in FMU and 0.75 in 24 hour urine. Of interest is the variation in the Tiselius CRI values in treated subjects between FMU and 24 hour urine samples before and after treatment: in FMU, the decrease of Tiselius CRI was about 6% in males and 0% in females while in 24 hour urine, the decrease was 33.9% in males and 44.8% in females. Such a difference suggests a worse distribution in the water intake which should be better distributed all over 24 hours. This is a recommendation for stone patients, and it could be also useful in a normal subject in order to prevent the possible risk of becoming a stone former.

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Ureterolithiasis in children.

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Summary

The aim of our work is to present our own experience in the field of urolithiasis treatment in children using ureteroscopic lithotripsy.

KEY WORDS: Urolithiasis; Lithotrypsy; Children.

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INTRODUCTION

Urolithiasis is a state when concrements of different urinary components are formed in the urinary system. It is estimated that 5-10% of European population suffer from urolithiasis. In developed countries, children with urolithiasis account for approximately 1-3% of all the people suffering from this disease, which gives incidence at the level of 0.5‰.

Urolithiasis in children is connected with:

– metabolic disorders, among which the most important are hypercalciuria and oxaluria;
– anatomic malformations, among which the most frequent is pyelo-uretero junction stenosis;
– urinary tract infections, e.g. struvite lithiasis (magnesium-ammonium-phosphate) is formed when urine and kidney parenchyma get infected with bacteria producing urease (e.g. Proteus, Pseudomonas, Klebsiella, Serratia).

The size of stones ranges from microscopic foci of crystallization to concrements with the diameter of several centimetres. Big and, so called, cast stones can fill up the whole pyelocalyceal system.

Clinical symptoms of urolithiasis are not very specific. The most common are:

– non specified abdominalgia;
– erythrocyturia or haematuria;
– aseptic pyuria;
– dysuric symptoms like pollakiuria, oliguria or anuria.

Basic methods of operative treatment of urolithiasis are:

– extracorporeal shockwave lithotripsy (ESWL);
– endoscopic procedures such as percutaneous nephrolithotomy (PCNL) or ureterorenoscopic lithotripsy (URSL);
– open surgery treatment.

The choice of treatment depends on many factors such as size of the stone, its location, presence of concomitant anatomic malformations, patient's age, urinary stasis, infection of the urinary tract.

Among four above mentioned methods, ESWL has become the most popular method of treatment for urinary stones since 1980s. Its efficacy rate is about 54-97% Research shows that single procedure bears little risk of complications and their character is usually transitory. In cases where this method is inefficient or other contraindications exist, endoscopic methods are applied.

The essence of PCNL (percutaneous nephrolithotomy) is crushing and removing stones from pyelocalyceal system under the control of eyesight by using instruments inserted through a nephrostomy tract. Children with stones of diameter larger than 2 cm, with cast lithiasis or residual lithiasis after ESWL are suitable for this method. The efficacy rate ranges from 68% to 89%.

URSL enables endoscopy of the entire ureter up to the uretero-pelvic junction, crushing the stone with a lithotriptor, and after that extracting it with a basket or forceps. Due to technical progress and the development of equipment this method has become useful for extraction of stones from any part of ureter.

Indication for URSL in children are:

– impacted calculi in the ureter after ineffective ESWL;
– calculi in the mid part of ureter out of reach of shock waves;
– calculi which could not be crushed by shock waves;
– obstruction of urinary flow, such in case of the so-called "*stein strasse*".

Nowadays, only two absolute contraindications for URSL are considered: haemorrhagic diathesis and infection of urinary tract with fever.

The size of the child and the availability of miniaturized

equipment still remain a relative limitation to safely perform ureteroscopy. The efficacy rate of this method (according to various Authors) ranges from 78 to 100%. In medical literature concerning URSL in children, a small number of series have been presented to date.

The aim of our work is to present our own experiences of urolithiasis treatment in children using ureteroscopic lithotripsy.

Materials and methods

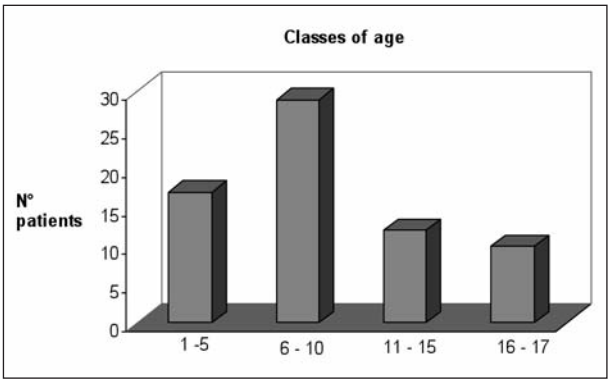
In the years 2006-2009 in the Paediatric Surgery Department of our Hospital 57 children underwent USRL. In this group there were 27 girls and 30 boys, aged from 1 (the youngest was 13 months old) to 17 years, all with body mass above 10 kg (Table 1).
For the majority of the patients the indication for URSL was presence of calculi located in the lower ureter, whereas in the remaining 10 children stones were located in the upper ureter. The size of stones ranged from 6 to 14 mm. The majority of procedures were performed on children under 10 years of age (46 in total) (Figure 1).
All patients underwent imaging examinations including ultrasonographic imaging of the urinary tract, urography, scintigraphy. The level of microelements in the urine was determined in order to define the type of lithiasis. Urinalysis and urine culture were monitored to exclude urinary tract infections. All the patients submitted to URSL according to ultrasonographic imaging had a dilated pyelocalyceal system together with the tract of the ureter above the concrement.
Sixty-one percent of patients underwent URSL because of urgent indications. In 7 children URSL was preceded by ESWL and, as a consequence of this procedure, residual stones obstructed the urinary flow. The remaining patients previously underwent conservative measures. They received pharmacologic treatment and intravenous fluids in order to increase diuresis.
Both ultrasonic and pneumatic lithotriptors with rigid and flexible ureteroscopes 4.5/6Ch and 6.5/8 Ch were used, under general anaesthesia in an operating theatre. Hospitalization time ranged from 3 to 11 days.
All the removed concrements were analysed to check their chemical composition so that further conservative therapy could be individually planned for every child.

Table 1.

	Boys	Girls
No of patients	27	30
Age	16 months - 17 years	13 months - 15 years
Weight (kg)	11-60	10-35
Localisation of stone in ureter	20% upper ureter	20% upper ureter
	80% lower ureter	80% lower ureter
Diameter of stone (mm)	6-10	6-14
Hospital stay (days)	3-11	5-9

Figure 1.

Age of patients.



Results

URSL was performed on 57 children A very good result was obtained in 51 of them. In these cases, the stones were completely removed or crushed into small pieces so that self-evacuation could take place, as was later on confirmed by ultrasonographic examination. Within a few days the dilatation of the pyelocalyceal system and of the ureter disappeared, and children in good condition and without any complaint were discharged from hospital.
In 3 patients URSL had to be repeated due to an incomplete evacuation of stones from the ureter. These children required an additional lithotripsy procedure in the lower ureter.
In 3 cases complications were observed. In the first case, ureter wall damage was suspected and a pig-tail catheter was inserted after the procedure and removed 2 weeks later: the postoperative course was uneventful without urinary leakage around the ureter. In the second case a small amount of irrigating liquid around the renal capsule was initially observed, although a subsequent ultrasonography on the second day after the treatment revealed no more fluid collection. The third case was a boy who had been conservatively treated for about a year after ESWL for the presence of a wedged stone in the orifice associated with a significantly dilatation of the pyelocalyceal system and of the entire ureter. The stone was removed but during this procedure extensive inflammatory changes were found in the ureter mucosa. A pig-tail catheter was inserted due to marked dropsy. In spite of this, after 7 weeks, a stricture of the ureter with associated alteration of the urinary flow from the upper urinary tract were found. The boy was submitted to operative treatment with excision of the stricture and simultaneous ureter implantation to the bladder according to Leadbetter-Politano method.

Discussion

In the last two decades urolithiasis treatment in children has changed, although it still remains a very difficult task. The main operative method of treatment is ESWL, particularly because of its reduced invasiveness and easy

accessibility. However, together with the progress in ureteroscope construction, the evolution of additional accessories and the improvement of endoscopy, URSL procedure in children is obtaining growing approval and recognition from the paediatric surgeons all over the world. This procedure can be performed in children weighing about 10 kg. A condition that must be fulfilled is to safely enter the orifice of the ureter. Sometimes width and elasticity of the ureteral orifice can be altered by the prolonged stay of the concrement in the area of the ureter orifice that can cause chronic inflammation and secondary stricture of the ureter.
In the presented series (57 children) a basic indication for URSL was a reduced or absent urinary flow in the pyelocalyceal system and in the ureter because of a concrement located in the ureter.
In 7 patients of this series URSL was preceded by an ESWL procedure, that resulted in the presence of crushed concrements blocking the urinary flow in the ureter (stein strasse).
The remaining patients were treated only by conservative therapy (extracorporeal lithotripsy was not indicated in a boy was on the basis of the composition of his stone).

A limitation to URSL might be a too narrow ureteral orifice (2 cases). According to different authors, the URSL efficacy when the concrement is located in the lower ureter is 78 to 100%. In 51 of our all patients concrements were removed completely. In 3 patients the procedure of crushing had required repetition.
The follow-up period was from 3 months to 2 years. The children were monitored by USG examinations.
Among the most frequent complications after URSL the following are listed: vesicoureteral reflux, perforation, disruption and abruption of ureter, ureter stricture, urinary system infection. Nevertheless, long-term observations show that if 4.5Ch and 6.5-7.2Ch equipment is used, there are no complications connected with mechanical injury of ureter. Authors reporting vesicoureteral reflux stress the fact that usually it had no clinical meaning and after 2 years its presence could no longer be detected.
In children URSL can be efficiently and safely employed in ureterolithiasis treatment. This method enables to cure a patient faster as well as reduce the pain. What is more, the miniaturization of endoscopic equipment led to considerable growth of URSL efficacy rate and reduction of the risk of complications.
Indications for URSL:
– concrements blocked in ureter after ESWL
– concrements located in the mid part of ureter out of reach of an ultrasonic wave
– concrements which could not be crushed by an ultrasonic wave
– blocked urea outflow, the so-called "*stein strasse*".

We believe that the technique of performing URSL procedures in small children slightly differs from those used in older children and adults.

Attention should be paid to details which facilitate an effective removal of concrements and, at the same time, improve the safety of performed procedures.

Conclusion

1. ESWL is a basic method of operative treatment of urolithiasis in children.
2. A surgeon performing URSL in children must be highly precise and experienced in endoscopic treatment of urolithiasis.
3. It is a safe method, with a high efficacy rate when 4.5 Ch and 6.5 Ch ureteroscopes are used.
4. URSL provides opportunity to avoid open surgery treatment.

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Diagnostic difficulties with estimation of the cause of nephrolithiasis. Case presentation.

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Summary

In some patients with recurrent urolithiasis we cannot identify the cause of stone formation.
A 18 years old girl was evaluated for recurrent urolithiasis. Analysis of her stones demonstrated: calcium oxalate and 10% cystine; calcium phosphate and traces of magnesium and chloride, calcium phosphate and traces of potassium and calcium oxalate and ammonium-magnesium phosphate.
We failed to make a correct etiological diagnosis despite of a very broad spectrum of laboratory investigations.

KEY WORDS: Urinary calculi; Recurrence; Calcium Oxalate; Cystine.

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INTRODUCTION

In some patients with recurrent urolithiasis we cannot evaluate the cause of stone formation. We present one such case picturing our failure of making correct diagnosis despite of a very broad spectrum of laboratory investigations.

CASE REPORT

A 18 years old girl with recurrent urolithiasis was admitted to the Department of Nephrology, Renal Transplantation and Hypertension of Children's Memorial Health Institute in November 1999 at the age of 9 years. There was a nephrolithiasis history in her father's family.
In our patient the disease was diagnosed while looking for the cause of recurrent urinary tract infections. There were many stones in both renal pelvises (Figure 1).
Bilateral pyelolithotomy and then shock wave lithotripsy (SWL) had been performed in a local hospital. The stone had been composed of calcium oxalate and 10% of cystine.

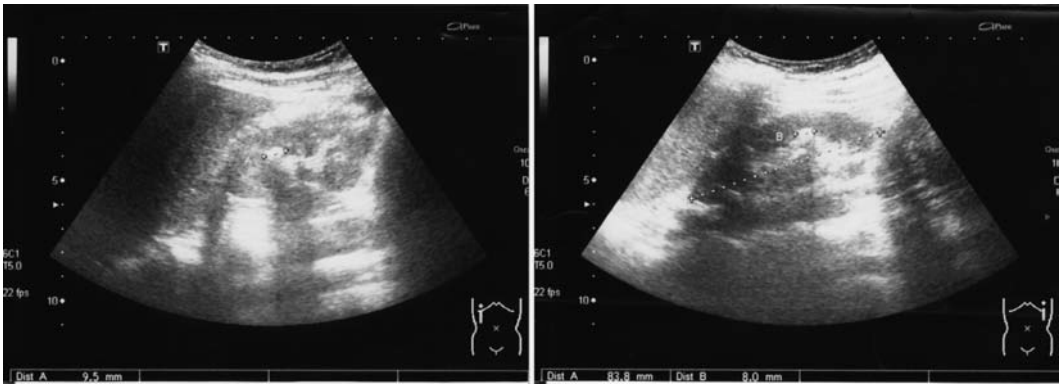


Figure 1.
USG examination - multiple concrements in both kidney.

Table 1.

24 hours urine excretion of calcium, phosphate, uric acid, magnesium, citrate, oxalate and mean urine pH.

24 hour urine excretion	Mean value
Calcium mg/kg/24 h	1,87 ± 1,0
Sodium mmol/kg/24h	2,05 ± 0,25
Phosphate mmol/24h	19,28 ± 1,73
Uric acid mg/kg/24h	6,0 ± 1,9
Magnesium mmol/1,73 m²/24h	3,69 ± 1,4
Citrate µmol/kg/24h	49,0 ± 9,8
Oxalate mg/1,73m²/24h	16,4 ± 2,3
Mean urine pH	6,52 ± 0,3

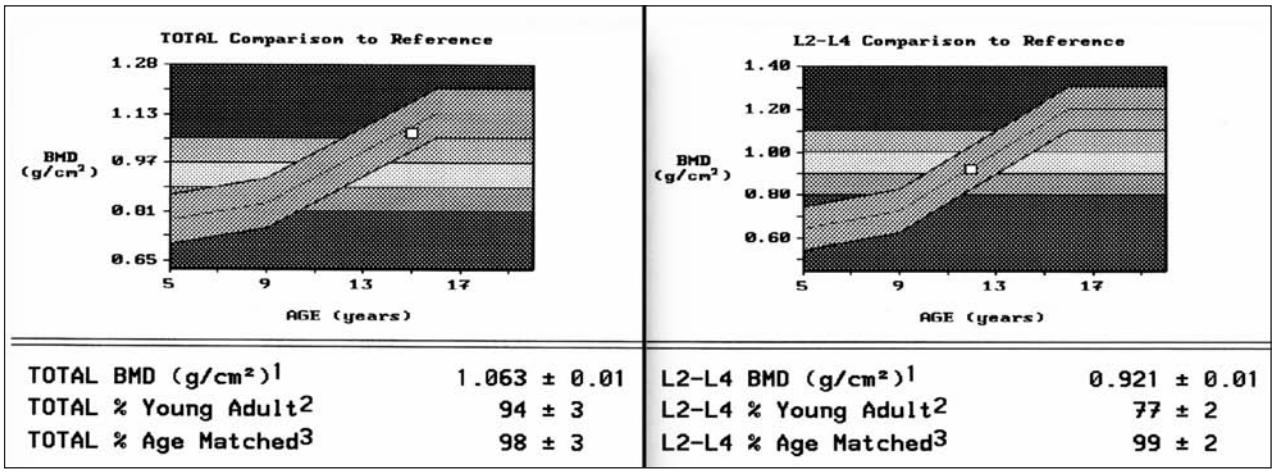
During ten years observation in Children's Memorial Health Institute we found in repeated examinations: normal renal function, hypomagnesemia without hypomagnesuria, hypocitraturia, normal levels of serum calcium and phosphorus, PTH ranging 53.6-72.3 pg/mL, 25OH-vitamin D3 ranging 3.6-23.8 ng/mL, normal excretion of calcium, phosphate, magnesium and uric acid in 24 hour urine collection (Table 1), normal excretion of free aminoacids in urine.

Metabolic diseases leading to stone forming were excluded; correct total bone mineral density (BMD) and L2-L4 BMD (Figure 2) were measured; bilateral renal scars in renal scintigraphy were demonstrated (Figure 3).

She excreted many concrements. The results of kidney stone analysis showed calcium oxalate and 10% cystine (Nov 1999); calcium phosphate and traces of magnesium and chloride (Nov 2000); calcium phosphate and

Figure 2.

Correct total BMD and L2-L4 BMD.



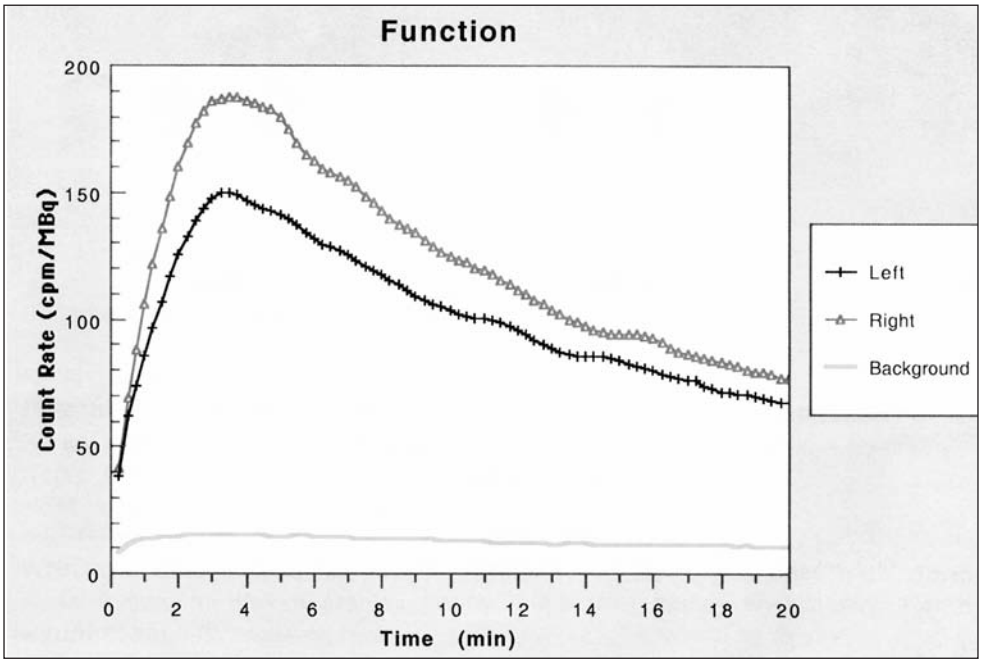


Figure 3.

Renal scintigraphy.

Bilateral renal scars
left kidney 43% ERPF
right kidney 57% ERPF

Efficient excretory function.

traces of potassium (Jan 2001) and calcium oxalate and ammonium-magnesium phosphate (Feb 2009).

Treatment

The patient has been on diet with limited amount of sodium chloride (NaCl), oxalate and purine and large amount of low-mineral water. She has also been treated with captopril and potassium citrate until cystinuria was excluded.

SWL has to be performed many times for recurrent stones.

Conclusions

In this patient with recurrent urolithiasis the reason of concrements forming was not found. It is possible that constant presence of stones made diagnosis more difficult. In such cases the choice of treatment can be made only by analyzing the composition of excreted concrements.

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Stenting after ureteroscopy for ureteral lithiasis: Results of a retrospective study.

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Summary

Objectives: *Routine ureteral stenting after ureteroscopy for stone removal is common. However ureteral stent negatively impact quality of life and can cause significant morbidity. This study was carried out to report our experience.*

Materials and Methods: *A total of 529 patients underwent ureteroscopy for the treatment of ureteral stones. In 436 pts (82%) a stent was placed, in 281 double J (removed within 2-4 weeks) and in 155 mono J (removed within 24 h). Ninethy-three did not received stenting. At 24 hour the mesaured outcomes were post operative pain, fever and hematuria, at 4 weeks need for hospital care (readmission or visit in the clinic) for lower urinary tract symptoms (LUTS), hematuria, fever or pain.*

Results: *No significant difference was observed between two groups regarding the complications at 24 hour after the treatment (pain p = 0.6, fever = 0.7, hematuria p = 0.8). At 4 weeks after the ureteroscopy the incidence of LUTS, hematuria, pain and fever requiring the need for hospital care (readmission or visit in the clinic) was higher in the group with double J stent respect to the group with mono J stent ($p < 0.05$). At 3 months follow-up no difference was oberved between the two groups regarding stone-free rate and incidence of ureteral stricture formation.*

Conclusions: *Routine stenting is necessary after ureteroscopy for ureteral lithiasis to prevent pain and fever without difference in stone free rate and incidence of stricture formation rate between the two groups. LUTS, hematuria and /or pain needing for hospital care were more frequent in the group with double J stent in spite of high stone free rate and low incidence of stricture formation. Further prospective randomized studies are needed to assess the role of using "short" and "long-term" stenting after ureteroscopy lithotripsy, considering that the choice actually depends on the surgeon's intraoperative judgment.*

KEY WORDS: *Ureteroscopy; Stenting; Ureteral lithiasis.*

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INTRODUCTION

For many years the routine placement of ureteral stents has been considered the standard of care after ureteroscopy for stone fragmentation (1). In fact, to reduce post operative ureteral obstruction at the impaction site and possible future stricture formation, surgeons traditionally placed a ureteral stent after ureteroscopy . However, the ureteral stent itself causes morbidity, including bladder irritation, loin pain, hematuria, encrustation, infection and even stent migration, that requires subsequent surgical removal. Indeed, because of the morbidity and added cost associated with indwelling ureteral stents, identifying patients who may not require routine stenting is necessary. Prior work has demonstrated that distal ureteral calculi can safely be managed without stenting in a prospective cohort and in a randomized population. Despite this aggregate of data, factors associated with morbidity following stentless ureteroscopy for urinary calculi remain unclear and identifying such factors would facilitate patient selection. This retrospective analysis was carried out to report our experience.

MATERIALS AND METHODS

Between June 1999 and December 2008 a total of 529 consecutive patients underwent ureteroscopy and intraco-

Table 1.

Stone characteristics.

	Double J (281 pts)	Mono J (155 pts)
Mean stone size (mm)	11 ± 0.8 (5-20)	10 ± 1.1 (5-19)
Stone location	**n° (%)**	**n° (%)**
Upper	60 (21)	29 (19)
Mid	77 (27)	34 (22)
Lower	144 (51)	92 (59)
Multiple stones	28 (9)	11 (7)
Anatomic narrowing on IVP	56 (20)	122 (14)
Hydronephrosis (nephrostomy tube or double J)	49 (17)	18 (11)

Table 2.

Complications at 24 h after ureteroscopy.

	DJ (281 pts)		MJ (155 pts)		
	n	%	n	%	
Pain	57	20	27	17	0.6
Fever	30	10	14	9	0.7
Hematuria	140	49	74	47	0.8

Table 3.

Complications at 4 weeks after ureteroscopy.

	DJ		MJ		p
	n	%	n	%	
LUTS	35	12	8	3.8	< 0.01
Hematuria	91	32	26	16	< 0.001
Pain/fever	33	11	8	5	< 0.05

poreal lithotripsy with Lithoclast and Holmium Laser Luminis 20W for the treatment of ureteral stones in our Department. In 436 (82%) patients a stent was placed, in 281 (152 males and 129 females, 18 to 73 years old, mean age 44) double J stent (removed within 2-4 weeks) and in 155 (82 males and 73 females, 19 to 71 years old, mean age 45) mono J stent (removed within 24). Ninety-three did not receive stenting. The two stented patients groups were comparable with the respect to mean stone size, stone location, number of stones, anatomic narrowing on IVP and rate of preoperative ureteral stent or nephrostomy drainage (Table 1). At 24 hour after ureteroscopy the outcomes measured were post operative pain, fever and hematuria, at 4 week need for hospital care (readmission or visit in the clinic) for LUTS (dysuria, urinary tract infection, frequancy/urgency), hematuria and fever and/or pain. Three months after ureteroscopy a radiogram of kidney, ureter and bladder (KUB) and a renal sonogram were performed. Excretory urography and computed tomography (CT) were performed in selected cases.

The chi-square test was used to determine the significance of differences of data between the 2 groups. $P < 0.05$ was considered significantly.

Results

Ureteroscopic access was successfully achieved in 87% of all cases, while in 35 patients with double J stenting and in 15 with mono J stenting balloon dilation of the ureter was required. There was also no significant difference in type of anesthesia, operative time, amount of laser energy used and use of basket between the 2 groups. No significant difference was observed between the two groups regarding the complications at 24 hour after the treatment (pain $p = 0.6$, fever = 0.7 and hematuria $p = 0.8$) (Table 2). At 4 week after the ureteroscopy the incidence of LUTS, hematuria, fever and/or pain requiring the need for hospital care (readmission or visit in the clinic) was higher in the group with double J stent with respect to the group with mono J stent ($p < 0.05$) (Table 3). At 3 months follow-up no difference was observed between the two groups regarding stone free-rate (97% and 96%) and incidence of ureteral stricture formation (0.7% and 0.6% respectively).

Discussion

Ureteral stenting after ureteroscopic lithotripsy is a common practice among urologists attempting to prevent postoperative complications (1). Some series report a stenting rate of almost 100%. However, as many as 49% of patients have reported stent related symptoms. Today, with use of small calibre ureteroscopes, routine balloon dilation of the ureter before introduction of the ureteroscope is seldom required. This fact, together with the smaller fragments that result from laser lithotripsy, indicates that the practice of routine post-operative ureteral stenting could be questioned.

The rationale for routine use of stenting after ureteroscopy emanates from supposition rather than evidence based medicine. This widely held belief is based on findings of acute ureteral obstruction after ureteral dilatation in a minipig model and on presumption that placement of a stent can eliminate clinically significant obstruction and prevent ureteral stricture formation. Consequently, in most historical and contemporary ureteroscopy series ureteral stents were routinely placed after treatment for urinary stone disease. Undeniably, there are clinical setting in which ureteral stenting is mandatory (eg following ureteral perforation, transplant or solitary kidney, history of renal failure), but the morbidity associated with indwelling ureteral stents is equally incontrovertible and current evidence support a more limited use of ureteral stenting.

Nonrandomized studies have shown that routine stent-

ing was not necessary after uncomplicated ureteral stone removal. Hollenbeck et al, after a retrospective case control study, expanded this conclusion to include upper ureter stones. A number of randomized, prospective studies in patients undergoing ureteroscopic stone removal investigated the effect of placing a stent at the end of the procedure and concluded that the complication rates in patients were non different in the nonstented cohort with respect to the stented cohort with no impact on stone-free rates. However, postoperative pain and irritative symptoms were reduced with the omission of the ureteral stent. These studies mainly included distal ureteral stones with small mean stone size (about 6 mm) and excluded patients with renal calculi or requiring ureteral dilation and preoperative indwelling ureteral stenting . In addition, subjective assessment of mucosa edema, tissue reaction, presence of mucosa injury excluded some patients from these studies.

A recent randomized study to identify the clinical characteristic affecting postoperative morbidity in unstented patients after ureteroscopy considered a number of factors (2). The longer operative times, especially more than 45 minutes when considered in conjunction with lithotripsy, were associated with postoperative complications in unstented patients. Renal pelvic location conveyed a greater risk of post operative complication compared to other renal stone locations, such us the lower pole. Bilateral procedures, a history of recent/recurrent infections and a history of recurrent stones were independent predictors of postoperative complications in unstented patients. However, if a patient had an indwelling stent preoperatively, stone location did not affect postoperative morbidity.

In our study, operative time and amount of laser energy were not different between the patients with "*short*" and "*long term*" stenting. Furthemore, no significantly difference was observed between the two groups regarding the complications at 24 hour after ureteroscopy (fever, pain and hematuria) and at 3 month follow-up (stone free rate and incidence of stricture formation rate). However, most of patients received adequate antibiotic therapy for long time after stone removal. In addition, patients with recurrent lithiasis received specific therapy for stone prevention according to underlying metabolic anomalies (allopurinol, potassium citrate, thiazides). Medical treatment could improve sand and/or small stone fragments clearance after ureteroscopy by reducing the growth or agglomeration and decreasing the risk for urinary infection . The ureteral strictures (2 in the group with double J stent and 1 in the group with mono J stent) resolved after repeat ureteroscopy with balloon dilation.

A meta-analysis of 4 randomized studies of the need for stent placement after ureteroscopy found that in the cohort receiving stent after procedure 2 of 134 patients (1.5%) were readmitted to the hospital, while 6 of 141 nonstented patients (4.3%) were readmitted. The reasons for readmission in stented group were sepsis and obstruction caused by stent migration. In nonstented patients readmisssion was due to unrelenting renal colic and pyelonephritis (3). In our experience, at 4 week after ureteroscopy the incidence of pain and/or fever requiring unplanned hospital visit or readmission was higher in patients with "*long*" stenting respect to patients with "*short*" stenting. Furthemore, patients with double J stent had frequent low urinary tract symptoms (dysuria, urinary tract infection, frequency/urgency) and hematuria requiring medical management with discomfort for the patient.

According to other studies, our analysis could confirm, that selective placement of ureteral stents following therapy for urolithiasis is warrented. Except for the patient with a small distal ureteral calculus (in our experience 78 of 93 patients nonstented and excluded by statistically evaluation), discerning the patients suitable for stentless ureteroscopy remains problematic because factors associated with morbidity have yet to be identified.

In conclusion, this retrospective analysis demonstrates that routine stenting after ureteroscopy for ureteral lithiasis improves draining of upper excretory tract and prevents pain and fever without difference in stone free and incidence of stricture formation rate between the two groups. LUTS, hematuria, fever and/or pain needing for hospital care were more frequent in group with double J stent in spite of high stone free rate and low incidence of stricture formation. Further prospective randomized studies are nedeed to assess the role of use of "*short*" and "*long*" stenting after ureteroscopy lithotripsy, considering that intraoperative decision actually depends on the surgeon's judgment.

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ORIGINAL PAPER

The management of erectile dysfunction: Innovations and future perspectives.

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Summary

Phosphodiesterase 5 (PDE5) inhibitors are recommended as first line therapy for the treatment of erectile dysfunction (ED). To date, three PDE5 inhibitors are on the market: sildenafil, vardenafil and tadalafil. These compounds are available as oral tablets; they are rapidly absorbed in the gastrointestinal tract and are excreted mainly in the feces and, to a lesser extent, in the urine. Recently, an orodispersible formulation of vardenafil (vardenafil ODT) has been developed, which is able to dissolve in the mouth within seconds, releasing a minty flavor, without the need of being swallowed with water. The clinical studies so far performed showed that vardenafil ODT has a bioavailability superior to the traditional film-coated tablet. Among the other PDE5 inhibitors under development we report mirodenafil, lodenafil carbonate, avalafil and SLx-2101 It is likely that in the future molecules that act on pathways other than the one of NO/cGMP will be available. Such as Rho-kinase inhibitors, which inhibit the mechanism that leads to smooth muscle contraction thus allowing erection and hydrogen sulphide (H2S), an endogenous molecule synthesized from cysteine that can be both a vasodilator and a vasoconstrictor according to its concentration.

KEY WORDS: *Erectile dysfunction; Sildenafil; Tadalafil; Vardenafil; Vardenafil ODT; Udenafil; Avanafil; Lodenafil carbonate; SLx-2101.*

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LIST OF ABBREVIATIONS:

AUC:	area under the curve
cGMP:	cyclic guanosine monophosphate
ED:	erectile dysfunction
NO:	nitric oxide
PDE5:	phosphodiesterase 5
PKG:	protein Kinase activated by cGMP
ROS:	reactive oxygen species

Phosphodiesterase 5 (PDE5) inhibitors are recommended as first line therapy for the treatment of erectile dysfunction (ED) (1). To date, three PDE5 inhibitors are on the market: sildenafil, vardenafil and tadalafil. These compounds are available as oral tablets; they are rapidly absorbed (t_{max} ranging from 0.7 in the case of vardenafil to 2h for tadalafil) in the gastrointestinal tract and are excreted mainly in the feces and, to a lesser extent, in the urine. Their epatic metabolism is accounted primarily by cytocrome P450, isoform 3A4 (2). While sildenafil and vardenafil share a common chemical structure, tadalafil has a different structure which accounts for its different pharmacokinetic profile (2, 3). Sildenafil, the first oral PDE5 inhibitor to receive market authorization, and vardenafil have an halflife of about 4h; instead, tadalafil has an halflife considerably longer (17.5h) which translates into a longer duration of action: 36h for tadalafil vs 8-10h for vardenafil and sildenafil (4, 5). Diet – especially fatty meals – affects sildenafil pharmacokinetics, but it alters minimally vardenafil and tadalafil pharmacokinetics (2, 3). The pharmacokinetic profile of these drugs is determinant in tailoring the therapy to the patient needs. Although many other therapeutic approaches are available for the management of ED, like the Vacuum Device, Alprostadil intracavernosal injections and mechanical prosthesis (1), the oral therapy is the most accepted one since it is non-invasive and easy to take. In the recent years, PDE5 inhibitors have improved ED management, thus drawing attention on a pathology that was previously considered as minor – although its prevalence in

the population is still underestimated (6). However, the medicalization of the sexual intercourse is a problem shared by all the therapies so far available. A successful sexual intercourse depends on the interplay among partners' behavior, participation and the environment surrounding the couple. Many feel uncomfortable when having a sexual encounter which is aided by a drug, these concerns being mainly of psychological nature. The patient wishes to be independent from the therapy, fearing addiction or psychological dependency. Sometimes this occurs when the treatment is not followed by a physician or an andrologist. Especially in the case of anxious patients, the specialist should give a psychological aid in addition to the pharmacological counseling. Moreover, the partner's attitude towards the pharmacological therapy plays a crucial role in the achievement of a positive outcome. Many reasons may lead to a negative attitude towards the pharmacological management of ED, however, most of times this is due basically to inaccurate information about the drug's mode of action: the partner often assumes that the tablet intake generates an unnatural erection in the male. Consequently, she believes that the role of seduction in the first phases of a sexual encounter is no longer needed and that the erection is not the result of the sexual arousal induced by the partner herself. The drug in the end becomes a barrier inside the couple: not an aid to restore a normal sexual activity, but the surrogate of partner's femininity itself.

In the last few years innovative pharmacological formulations able to overcome such psychological barriers linked to the ingestion of a tablet are under investigation. Recently, an orodispersible formulation of vardenafil (vardenafil ODT) has been developed, which is able to dissolve in the mouth within seconds, releasing a minty flavor, without the need of being swallowed with water. Thanks to this innovative formulation the preparation can be perceived as a sort of candy, with a pleasant taste, rather than an actual drug, thereby playing a more discreet role during the sexual encounter, interfering minimally with the psycho-physical balance of the encounter. The clinical studies so far performed showed that vardenafil ODT has a bioavailability superior to the traditional film-coated tablet, which is swallowed with water. Although the area under the curve (AUC; an index of the drug systemic exposure) was higher, the maximum plasmatic concentration (C_{max}) observed was similar to the one determined for the film-coated formulation, suggesting that the greater bioavailability of the ODT formulation is achieved within the therapeutic window (7). As a consequence, vardenafil ODT safety profile is substantially similar to the ones of the other PDE5 inhibitors. Its side effects are typical of these drugs, the most common ones being headache, nasal congestion, facial flushing, dyspepsia and back pain (8-10). Those side effects are due to the capillary vasodilatation induced by PDE5 inhibitors and are well characterized, short-lived and shared by all the compounds of this class. PDE5 inhibitors intake is not recommended in men with a recent history of ictus or myocardial infarction, unstable angina, severe hepatic or renal dysfunction. According to data collected both in Europe and North America, the recommended starting dose for sildenafil is 50 mg, while for vardenafil and tadalafil is 10 mg. In some subsets of patients (e.g. patients with mild to moderate epatic failure) the starting dose should be lower. These drugs have to be taken 1-2 hours before the sexual intercourse.

Phosphodiesterase 5 is the enzyme responsible for cyclic GMP (cGMP) degradation. Its inhibition leads to the increase of cytosolic levels of cGMP in the smooth muscle cells responsible for blood flow inside the corpora cavernosa, thereby prolonging its vasodilator effect. It is important to stress that the mechanism of action of PDE5 inhibitors requires sexual excitement to achieve erection. Moreover, the inhibition of PDE5 might have a role in preventing ROS damage in penile tissue, through the activation of the cGMP/PKG pathway, since this activity has been hypothesized for other tissues, namely the heart and spinal motor neurons (11-12).

Many pharmaceutical companies are trying to develop new molecules for the management of ED. More than 400 new molecules are under evaluation for their efficacy in inhibiting PDE5 (13). The last generation of PDE5 inhibitors, like Avanafil, Udenafil and Mirodenafil (14-16), seems to promise very good performances for ED patients as these molecules are very selective in targeting PDE5. Udenafil has been marketed in some Asiatic countries, but it is still not available in Europe and North America (14,17,18), in the USA its efficacy and safety are currently under investigation in a large cohort of patients. Udenafil pharmacokinetic profile requires intake at least 80 minutes before the sexual intercourse; the drug is highly effective and its action duration spans form 7 to 9 hours (19). Moreover, is shows a low degree of cross-reactivity with the other isoforms of PDE. The most common side effect observed with udenafil are facial flushing, nasal congestion, headache and visual disturbances. Among the other PDE5 inhibitors under development we report: mirodenafil, whose action duration is shorter than the udenafil one (14, 20). Lodenafil carbonate (21), which is administered as a dimer, with the two molecules linked by a carbonate bridge. The dimer shows an higher absorption in the gastrointestinal tract, and it is later converted in the active monomers by plasmatic esterases (22). Avalafil (TA-1790) acts rapidly and it seems to decrease less than sildenafil systemic blood pressure, however, it is also selective for PDE6 (14, 23, 24). SLx-2101 is among the last developed PDE5 inhibitors. It is transformed in the liver into a metabolite which is equally active. In addition to the indication for ED management, the use of SLx-2101 for the treatment of hypertension and Rynaud disease is under evaluation (14, 18). ICARIIN is a flavonoic glucoside isolated form Epimedii herba, an herbal medicine employed in traditional Chinese medicine to improve the sexual performance (25). Finally, other molecules able to increase cGMP in corpora cavernosa are the atrial natriuretic peptide and uroguanyllin (26), which are able to induce corpora cavernosa relaxation.

It is likely that in the future molecules that act on pathways other than the one of NO/cGMP will be available. Among these, it is noteworthy to remember Rho-kinase inhibitors, which inhibit the mechanism that leads to smooth muscle contraction thus allowing erection (27), and hydrogen sulphide (H2S), an endogenous molecule

synthesized from cysteine. H2S can be both a vasodilator and a vasoconstrictor according to its concentration (28); at high doses (1-10 µmol/kg) it can trigger erection in primates (29). The vasodilator activity is likely due to the stimulation of potassium channels regulated by ATP concentration, which in turn affect the intracellular concentration of Ca2+ (30). On the other hand, the vasoconstrictor activity probably derives from the interaction with NO and the consequent sequestration of the latter (28).
In summary, although drugs which are both safe and effective are already available for the management of ED, the development of novel therapeutic approaches goes on with the aim to improve therapy effectiveness, patient compliance and to generate more tailored therapies, also through the employment of vasodilator strategies which are independent from the NO/cGMP pathway.

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ORIGINAL PAPER

Prostate cancer and androgen deprivation: Optimal castration? Prospects and developments.

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Summary

Prostate cancer (PCa) therapy has always been connected with the problem of what optimal male castration is and how to achieve and control it.
Optimal medical castration should follow quite the same characteristics as surgical castration, then it should allow testosterone levels to be quickly and permanently reduced to levels ranging between 12 and 20 ng/dl.
It should also be pointed out that using luteinizing hormone-releasing hormone (LHRH) agonists does not result in immediate castration; castration occurs 2-4 weeks after the first injection.
Furthermore testosterone levels could also increase after subsequent injections if the depot formulation does not adequately cover the period between injections, as some LHRH receptors can remain free. This results in a new testosterone surge in conjunction with the following injections. Such episodes of increased testosterone levels in vicinity with injections are known as "miniflares". Yet, also persistently increased testosterone levels (> 50 ng/dl) might be shown, even under continuous treatment with LHRH analogues. Such increases are known as "late breakthrough escapes". A depot formulation of leuprolide acetate using a novel delivery system provides steady blood levels above the threshold of 0.1 mg/ml and completely suppresses pituitary gonadotropin secretion.

KEY WORDS: *Prostate Cancer; Androgen Deprivation, Castration.*

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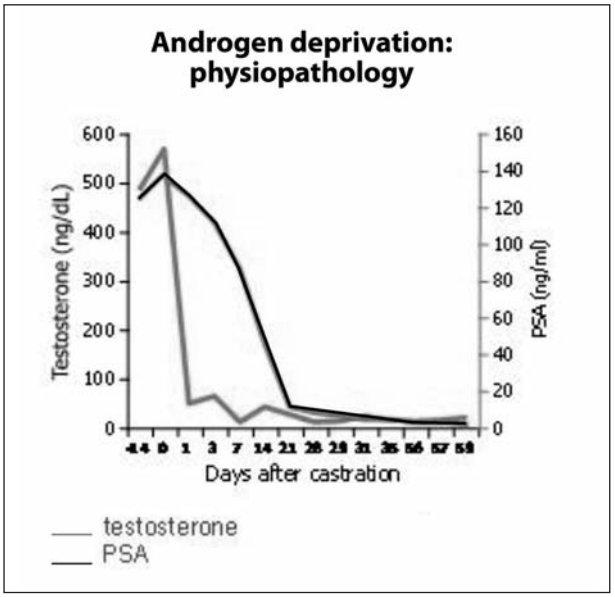
Prostate cancer (PCa) therapy has always been connected with the problem of what optimal male castration is and how to achieve and control it.
This problem has been considered important since prostate tissue was shown to be androgen-dependent; hence PCa therapy has always been aimed at androgen suppression, which used to be based on the administration of oestrogens or bilateral orchiectomy.
In 1941 *Huggins & Hodges* (1) assessed the effect of castration and hormone therapy on alkaline phosphatase levels in the blood. Hence they started to study the relationship between PCa and Androgen Deprivation (AD), although the ideal blood serum testosterone level required for effective and optimal castration was not defined.
Historically, surgical castration was thought to reduce total testosterone levels to < 50 ng/dl, and such value was consequently considered to be the therapeutic cut-off value for testosterone.
The importance of achieving low total serum testosterone levels was based on the fact that ineffective androgen suppression was associated with increased PCa mortality. Indeed, according to the whole literature, the total testosterone suppression level should be considered the surrogate endpoint for the assessment of the efficacy of hormone treatment.
The comparatively recent introduction of immunoassay methods has allowed testosterone levels < 15 ng/dl to be reported; as a result, the historic threshold has been questioned.
In 2000, *Oefelein et al.* showed that total testosterone levels in patients undergoing bilateral orchiectomy had a threshold value of 20 ng/dl.
Figure 1 and 2 show the physiopathology of androgen deprivation: drop in testosterone levels is accompanied by drop in PSA levels.
Surgical castration was believed to be the only and most effective PCa therapy, when *Schally at al.* managed to characterize the molecular structure of the luteinizing hormone-releasing hormone (LHRH) and surgical castration and/or medical antiandrogen or oestrogen therapy was gradually replaced by LHRH agonists. In particular, after a

Figure 1.



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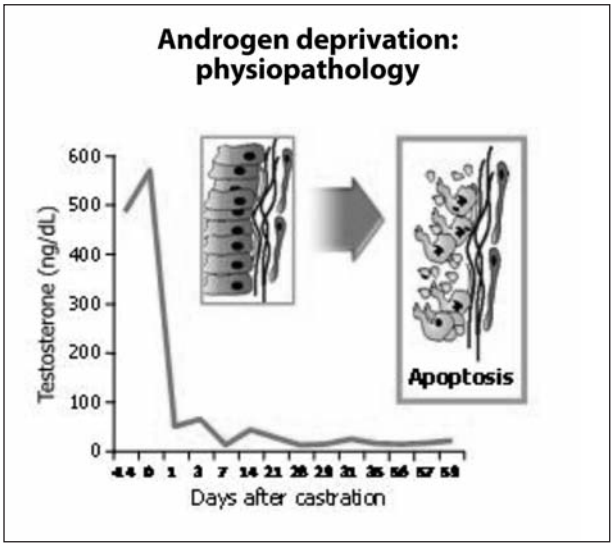
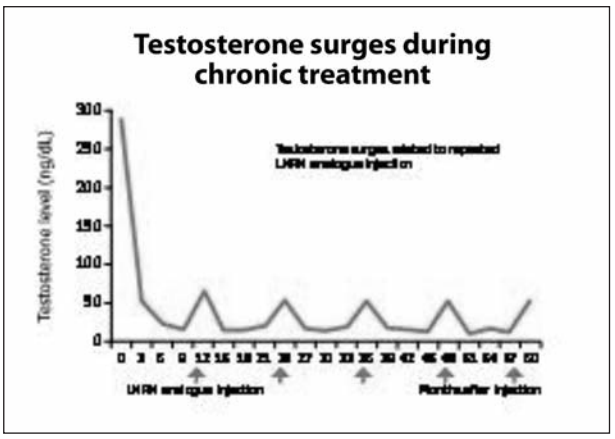


Figure 3.



therapy replacing surgical castration had been found, some objective parameters needed to be identified to determine optimal androgen deprivation. Moreover, clinical evidence of adrenal testosterone production had led to the concept of total androgen blockade (TAB). *Morote et al.* (2) conducted a study which – based on a multivariate analysis – showing that testosterone levels > 50 ng/dl were predictive of disease progression. In addition, it was suggested that regularly testing of testosterone levels in the blood and mataining critical castration values would make it possible to prevent disease progression and delay biochemical relapse – that is, the lower testosterone levels, the higher cancer-specific survival.

Hence it can be concluded that increased PSA levels associated with low testosterone levels are significant of nonresponsive disease, whereas high testosterone levels associated with low PSA levels are indicative of poor castration.

According to *Oefelein* (3), the gold standard of a good castration range is < 20 ng/dl testosterone; such value is reached by 94-98% of patients undergoing bilateral orchiectomy. However, can all LHRH analogues satisfy the above requirements?

This question can be answered by assessing and comparing pharmacology and clinical evidence.

No doubt all currently used analogues cause the so-called "*testosterone surge*", as related to initial pituitary stimulus which causes a temporary increase in testosterone levels; this evidence substantiates recent EAU guidelines (4) which recommend combination with an androgen agonist over the first two weeks' treatment with LHRH analogues.

It should also be pointed out that using LHRH agonists does not result in immediate castration; castration occurs 2-4 weeks after the first injection.

Furthermore testosterone levels could also increase after subsequent injections if the depot formulation does not adequately cover the period between injections, as some LHRH receptors can remain free. This results in a new testosterone surge in conjunction with the following injections. Such episodes of increased testosterone levels in vicinity with injections are known as "*miniflares*".

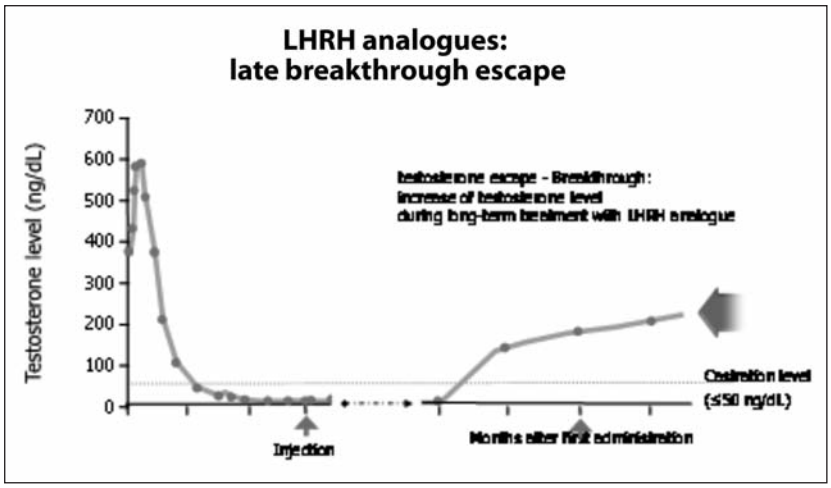
Yet, also persistently increased testosterone levels (> 50 ng/dl) might be shown, even under continuous treatment with LHRH analogues. Such increases are known as "*late breakthrough escapes*".

However, the above-mentioned phenomenon is not injection dependent; the increase in testosterone and PSA levels that is observed is due to the resistance to the drug. In any case this should not be classified as "*hormone resistance*" because it can disappear by replacing the analogue and/or increasing dosage.

To sum up: increased testosterone and PSA levels are indicative of late breakthrough escapes which can be managed by replacing therapy or the analogue; whereas low testosterone levels and high PSA levels are significant of hormone resistance.

It has to be pointed that therapy with LHRH analogues fails in 2-12% of patients, since they cannot decrease testosterone levels to < 50 ng/dl; likewise 13-46.4% of patients cannot reach testosterone levels < 20 ng/dl according to the new definition of castration, namely comparable to surgical castration.

Figure 4.



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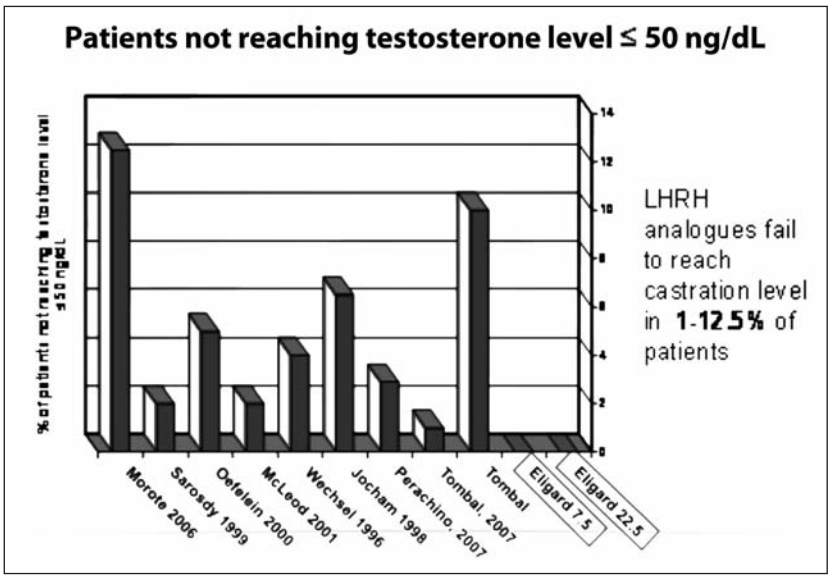

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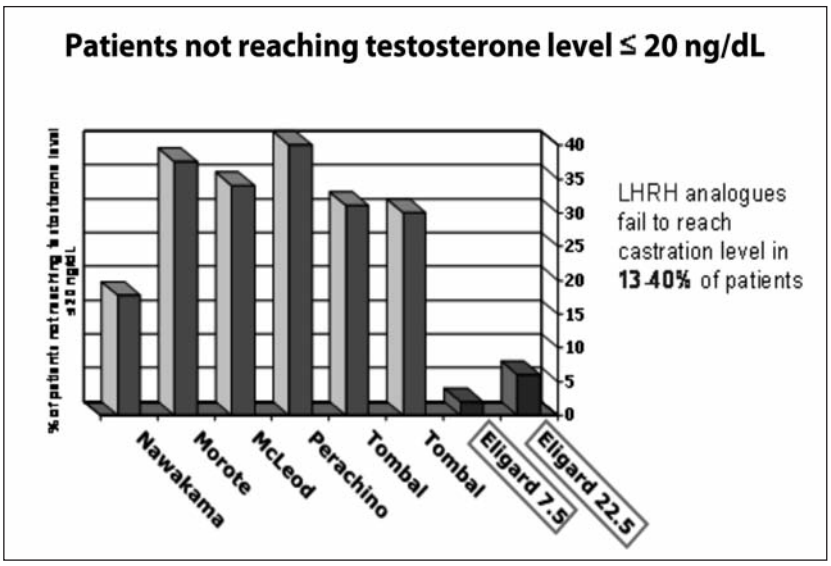


The Figures 5-9 summarize the above reported data and phenomena.

Optimal medical castration should follow quite the same characteristics as surgical castration, then it should allow testosterone levels to be quickly and permanently reduced to levels ranging between 12 and 20 ng/dl.

This goal was pursued through a new formulation which should, compared to standard LHRH analogues, be able to provide optimal testosterone control. This is possible by combining leuprorelin acetate with a biodegradable polymer (Atrigel - R); such formulation makes it possible to inject leuprorelin in a liquid form; once it has been injected, it solidifies, thereby forming a monosphere that allow a progressive release of the medicinal product with biodegradation of the polymer in a fixed period.

Dosage also plays a major role: in fact doses of 3.75 mg leuprolide may not be able to suppress gonadotropin secretion; in addition, in the presence of a large body mass, pharmacokinetics may not ensure satisfactory leuprorelin levels in the blood.

The FDA (Food and Drug Administration) found that a depot formulation of leuprolide acetate using this novel delivery system (Eligard - R) provides steady blood levels above the threshold of 0.1 mg/ml and completely suppresses pituitary gonadotropin secretion.

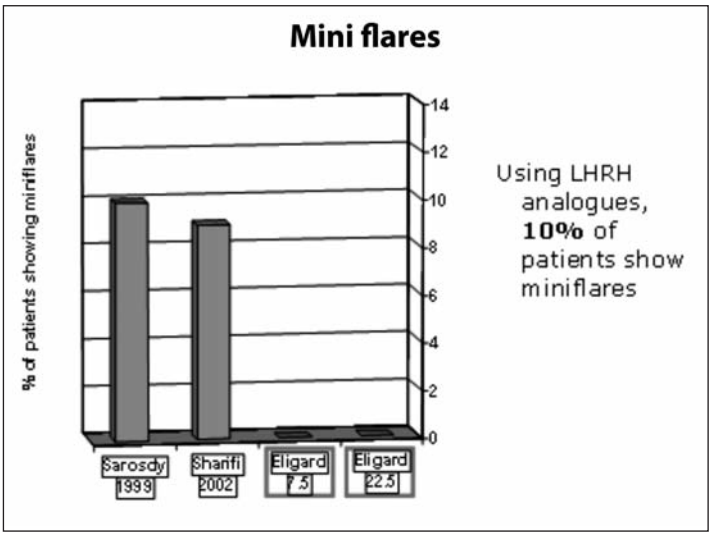
The following results are expected to be achieved in the future: introduction of a formulation for 6-month or 12-month administration, reduction of testosterone peaks in the blood between subsequent administrations, and finally improvement of quality of life (QL).

No doubt there is increasing evidence quality of life is a major parameter to be considered, especially in consideration of longer disease free interval and prolonged survival achieved by the improvements of therapy.

Berges, Schulmann and Boccafoschi (5-7) suggested that when testosterone levels are comparable to surgical castration, injection-related miniflares and breakthrough escapes occur more rarely, that PCa therapy should be increasingly aimed at customized treatment, and that most patients would like to become increasingly involved in the therapeutic/decision-making process.

Finally, in the future it would be useful to establish a quality assessment

Figure 7.



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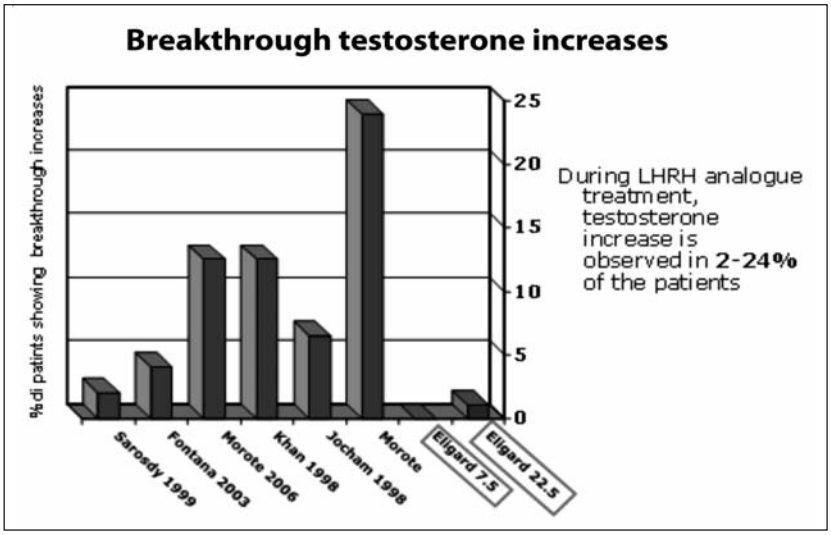

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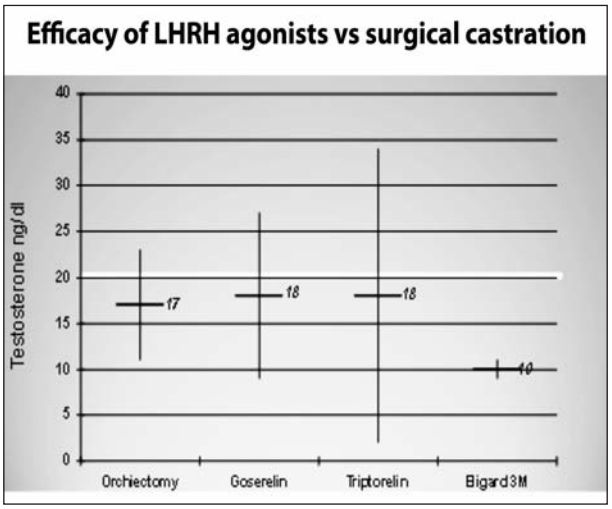

language for the follow-up period, including psychometric assessments, if necessary. There are many expectations about the future development of studies, at an advanced stage of experimentation, concerning the possibility to have molecules capable of controlling intracellular testosterone as well. Abiraterone seems to provide good prospects in this respect, since it can irreversibly inhibit cytochrome P (CYP17). The same goes for MDV3100, a nonsteroidal androgen receptor antagonist which blocks androgen from binding to the androgen receptor. Such drugs might be useful in hormone refractary patients.

Identification of the genetic polymorphisms of androgen receptors may also prove a major future prospect. Pharmacogenomics can make possible to determine time to progression, hormone refractoriness and/or the risk of undesirable side effects in individual patients; this would finally allow customized treatment.

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Manuscripts are accepted with the understanding that they have not been published or submitted for publication in any other journal.
Authors must submit the results of clinical and experimental studies conducted according to the *Helsinki Declaration* on clinical research and to the Ethical Code on animal research set forth by WHO (*WHO Chronicle 1985; 39:51*).
The Authors must obtain permission to reproduce figures, tables and text from previously published material.
Written permission must be obtained from the original copyright holder (generally the Publisher).

MANUSCRIPT PRESENTATION

Authors must submit the text (MAC and WINDOWS Microsoft Word are accepted) and illustrations by e-mail.
As an alternative manuscripts can be submitted by surface mail on disk with two hard copies of the manuscript and two sets of illustrations.
Manuscripts **must be written in English language** in accordance with the "*Uniform Requirements for Manuscripts submitted to biomedical journals" defined by The International Committee of Medical Journal Editors* (*http://www.ICMJE.org*). **Manuscripts in Italian language can be published after translation (expenses will be charged to the Authors)**. Manuscripts should be typed double spaced with wide margins.
They must be subdivided into the following sections:

TITLE PAGE

It must contain:
a) title;
b) a short (no more than 40 characters) running head title;
c) first, middle and last name of each Author without abbreviations;
d) University or Hospital, and Department of each Author;
e) **last name, address and e-mail of all the Authors**
f) corresponding Author
g) phone and/or fax number to facilitate communication;
h) acknowledgement of financial support;
i) list of abbreviations.

SUMMARY

The Authors must submit a long English summary (300 words, 2000 characters).
Subheadings are needed as follows:
Objective(s), Material and method(s), Result(s), Conclusion(s).
After the summary, three to ten key words must appear, taken from the standard Index Medicus terminology.

TEXT

For original articles concerning experimental or clinical studies and case reviews, the following standard scheme must be followed: Summary – Key Words - Introduction - Material and Methods - Results – Discussion - Conclusions - References - Tables - Legends - Figures.

SIZE OF MANUSCRIPTS

Literature reviews, Editorials and Original articles concerning experimental or clinical studies should not exceed 20 typewritten pages including figures, tables, and reference list. Case reports and notes on surgical technique shouid not exceed 10 type written pages (references are to be limited to 12). Letters to the editors should be not longer than 1000 words.

REFERENCES

The Author is responsible for the accuracy of the references.
References must be sorted in order of quotation and numbered with arabic digits between parentheses. Only the references quoted in the text can be listed. Journal titles must be abbreviated as in the Index Medicus. Only studies published on easily retrieved sources can be quoted. Unpublished studies cannot be quoted, however articles "in press" can be listed with the proper indication of the journal title, year and possibly volume. References
must be listed as follows:

JOURNAL ARTICLES

All Authors if there are six or fewer, otherwise the first three, followed by "et al.". Complete names for Work Groups or Committees. Complete title in the original language.
Title of the journal following Index Medicus rules. Year of publication; Volume number: First page.
Example: *Starzl T, Iwatsuki S, Shaw BW, et al. Left hepatic trisegmentectomy Surg Gynecol Obstet 1982; 155:21.*

BOOKS

Authors - Complete title in the original language. Edition number (if later than the first). City of publication: Publisher, Year of publication.
Example: *Bergel DIA. Cardiovascular dynamics. 2nd ed. London: Academic Press Inc., 1974.*

BOOK CHAPTERS

Authors of the chapters - Complete chapter title. In: Book Editor, complete Book Title, Edition number. City of publication: Publisher, Publication year: first page of chapter in the book.
Example: *Sagawa K. The use of central theory and system analysis. In: Bergel DH (Ed), Cardiovascular dynamics. 2nd ed. London: Academic Press Inc., 1964; 115.*

TABLES

Tables must be aimed to make comprehension of the written text easier. They must be numbered in Arabic digits and referred to in the text by progressive numbers. Every table must be accompanied by a brief title. The meaning of any abbreviations must be explained at the bottom of the table itself.
(If sent by surface mail tables must be clearly printed with every table typed on a separate sheet).

FIGURES

(graphics, algorithms, photographs, drawings)
Figures must be numbered and quoted in the text by number.
The meaning of all symbols, abbreviations or letters must be indicated. Histology photograph legends must include the enlargement ratio and the staining method. Legends must be collected in one or more separate pages.
(If sent by surface mail figures must be submitted in duplicate. On the back side of each figure the following data must appear: figure number, title of the paper, name of the first Author, an arrow pointing to the top of the figure).
Please follow these instructions when preparing files:
- Do not include any illustrations as part of your text file.
- Do not prepare any figures in Word as they are not workable.
- Line illustrations must be submitted at 600 DPI.
- Halftones and color photos should be submitted at a minimum of 300 DPI.
- Power Point files cannot be uploaded.
- Save art as either TIFF or EPS files.
- If at all possible please avoid transmitting electronic files in JPEG format. If this is unavoidable please be sure to save the JPEG at the highest quality available and at the correct resolution for the type of artwork it is
- Color art must be saved as CYMK, not RGB.
- PDF files for individual figures may be uploaded.

MANUSCRIPT REVIEW

Only manuscript written according to the above mentioned rules will be considered. All submitted manuscripts are evaluated by the Editorial Board and/or by two referees designated by the Editors. The Authors are informed in a time as short as possible on whether the paper has been accepted, rejected or if a revision
is deemed necessary.
The Editors reserve the right to make editorial and literary corrections with the goal of making the article clearer or more concise, without altering its contents. Submission of a manuscript implies acceptation of all above rules.

PROOFS

Authors are responsible for ensuring that all manuscripts are accurately typed before final submission. Galley proofs will be sent to the first Author. Proofs should be returned within seven days from receipt.

REPRINTS

A copy of the issue in which the article appears will be provided free of charge. Reprints are not provided. The cost to obtain the PDF file of the article is Euro 50.

UrOP Urologi Ospedalità Gestione Privata

6° Congresso Nazionale UrOP 2011

Jesolo, 12-14 maggio 2011

Giovedì 12 maggio 2011

10.00-12.30 **CORSO PER MEDICI**: **AGGIORNAMENTI DI ANDROLOGIA**
Direttori: R. Leonardi - S. Pecoraro

10.00-10.15 **Introduzione al corso: obiettivi e finalità** (R. Leonardi - S. Pecoraro)
10.15-10.30 **Il varicocele microchirurgico** (A. De Rienzo)
10.30-10.45 **Antiossidanti nelle OAT** (N. Spiezia)
10.45-11.00 **Testosterone: Mito, fatti e .... Misfatti** (S. Pecoraro)
11.00-11.15 **La chirurgia dell'eiaculazione** (L. Gallo)
11.15-11.30 **La diagnostica nella D.E.** (M. Valitutti)
11.30-11.45 **Ruolo degli IPDE-5 post chirurgia pelvica** (R. Leonardi)
11.45-12.00 **La terapia farmaco-fisica della IPP** (S. Brunori)
12.00-12.15 **IPP: Egydio vs. Nesbit** (L. Mavilla)
12.15-12.30 **Implantologia peniena** (M. Silvani)

15.30-18.00 **CORSO PER INFERMIERI**: **RICERCA INFERMIERISTICA ED EBN IN CAMMINO VERSO L'EVIDENCE BASED NURSING**
Coordinatore: Giovanni L. Pappagallo

15.30-16.10 **Dalle incertezze ai quesiti (P.I.C.O.)**
(Giovanni L. Pappagallo - Arianna Morosin)
16.10-16.50 **Dalla definizione degli outcomes alla ricerca bibliografica**
(Giovanni L. Pappagallo - Arianna Morosin)
16.50-17.30 **Dalla graduazione delle evidenze alla produzione di raccomandazioni per la pratica infermieristica**
(Giovanni L. Pappagallo - Arianna Morosin)
17.30-18.00 **Discussione** (Giovanni L. Pappagallo - Arianna Morosin)
Coordinati da C. A. Balanescu

Natural Orifice Surgery

Laparoscopia - Incontinenza urinaria - CA prostata

Giovedì 12 maggio 2011

14.00-14.30 Apertura del Congresso: **"Il lungo viaggio dal meato uretrale al calice superiore"** (G. Fiaccavento)

Saluto delle Autorità

14.30-16.00 **Uretra**
Provoker: G. Barbagli
Moderatori: C. Corsi, F. Galasso

BXO: L'inizio dei problemi (T. Garcovic)
Uretra come proteggerla (P. Emiliozzi)
Uretrotomia oggi: Opzioni terapeutiche e risultati a confronto
- Lama fredda (G. Fiaccavento)
- Laser (A. Picinotti)

16.00-16.30 Lettura ad invito: **Ruolo delle cellule staminali** (G. Bianchi)
Introduce: A. Tamai

16.30-17.00 Lettura IBSA: **Dalla cistite ricorrente alla cistite interstiziale** (D. De Vita)
Introduce: C. Boccafoschi

17.00-18.30 **Tavola rotonda: Cosa c'è di nuovo in laparoscopia**
Moderatori: G. Breda, V. Disanto
- Accessi e strumentario (L. Schips)
- Chirurgia robotica del rene (A. Porreca)
- Chirurgia robotica della pelvi (P. Pierini)

18.30-20.00 **Vescica: Tecnologie innovative NMIBC (Non Muscle Invasive Bladder Cancer). Confronto tra tecniche**
Provoker: V. Ricci Barbini
Moderatori: G. Loiero, A. Salvaggio

La strumentazione (M. Coscione)
Presidi diagnostici
- NBI (F. Pisanti)
- Hexvix (R. Fede)

Venerdì 13 maggio 2011

08.30-10.50 **Ostruzioni cervico uretrali e dintorni: tecnica e risultati a confronto**
Provoker: R. Giulianelli
Moderatori: G. Ferrari, M. Catanzaro
TUIP: quale e come?
- Monopolare (C. Nisticò)
- Bipolare (L. Albanesi)
- Laser (F. De Marco)

TURP
- Monopolare (G. Zarrelli)
- Bipolare (B. Gentile)
- Laser
  Laser Tulio (M. Schettini)
  Laser Holmio (I. Vavassori)
  Laser a Diodi (R. Leonardi)
  KTP (R. Oriti)

Minimal Invasive Treatment: TUNA (A. Tamai)

Stenosi Uretra Posteriore (V. Pansadoro)

La quarta porta: le terapie orali per la IPB
- Fitofarmaci (C. Ranno)
- Terapia di combinazione (L. Orestano)
- Le nuove molecole (P. Morello)

10.50-11.20 **Le neoplasie dell'alta via escretrice**
Moderatori: S. Bruschetta - G. Savoca

Quando è indicato un trattamento endourologico? (R. Zucconelli)

La strumentazione per l'accesso con i flessibili (G. Sepe)
- Retrace
- Flexor

Le neoplasie dell'uretere, del bacinetto e dei calici (I. Vavassori)

11.20-11.30 Lettura SIU (V. Mirone)
Introduce: V. Pansadoro

11.30-11.40 Pausa

11.40-12.00 **Linee guida nelle biopsie prostatiche. A che punto siamo?**
Introduce e modera: G.L. Pappagallo
- Biopsia Transrettale (G. Vincenti)
- Biopsia Transperineale (P. Emiliozzi)

12.00-12.40 **Faccia a faccia: CA prostata, caldo o freddo oppure ..... ?**
Moderatori: C. Nardi, F. Portoghese
- HI-FU (G. Comeri)
- Crioterapia (C. Morana)
- Terapia ormonale: quando? quale? come? (C. Boccafoschi)

12.40-12.50 **Terapia neoadiuvante nella chirurgia laparoscopica del Carcinoma prostatico** (G. Grosso)

12.50-13.00 Lettura SIA (F. Pirozzi Farina)
Introduce: S. Pecoraro

13.00-13.10 Lettura SIUD (S. Sandri)
Introduce: C. Boccafoschi

13.10-14.30 Snack Lunch

14.30-15.30 **Tavola rotonda: Sanità pubblica e sanità privata: Aspetti normativi e gestionali**. Con la partecipazione dei presidenti FISOPA, AIOP, UrOP e dei rappresentanti delle Istituzioni Sanitarie regionali e locali
Moderatori: C. Aragona, A. Dell'Adami

15.30-15.45 Lettura EAU (W. Artibani)
Introduce: G. Sepe

15.45-16.45 **Comunicazioni**
Moderatori: G. Ludovico, M. Gentile

Sabato 14 maggio 2011

08.30-09.30 **Incontinenza urinaria femminile e prolassi**
Provoker: F. Catanzaro
Moderatore: B. Gentile, M. Linciano
- Accesso vaginale (F. Forte)
- Accesso laparoscopico (A. Pansadoro)
- Prolasso vaginale e protesi (A. Dafiero)

09.30-10.00 Lettura GUONE (R. Bortolus)
Introduce: G. Fiaccavento

10.00-12.00 **Sessione Video**
Moderatori: M. Vermiglio, R. Cusumano

12.00-12.30 **Take home messages**
M. Cappa, R. Jungano, D. Tuzzolo

12.30-12.45 Compilazione questionario ECM

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